DEVELOPMENT OF NOVEL DIAGNOSTIC MARKERS FOR EARLY DETECTION AND PROGNOSIS OF NEURODEGENERATIVE DISEASES

Dr. Abhishek Agrawal[1], Dr. Shveta[2], Dr. Nitin Kumar Singh[3], Dr. Amit Kumar Yadav[4], Dr. Karthik L R[5*]

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Abstract

Background: Neurodegenerative disorders present a substantial worldwide health obstacle. Neurodegenerative disorders, namely Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, are typified by a gradual nervous system deterioration, resulting in a decline in cognitive function, impaired motor abilities, and eventual incapacitation. Clinical signs and neuroimaging may not be sensitive or specific enough to diagnose certain diseases early. Thus, reliable biomarkers are needed to identify responsive individuals and provide a prognosis. This investigation aimed to create innovative diagnostic indicators for promptly identifying and predicting neurodegenerative ailments.

Materials and Methods: This multi-disciplinary study identified and validated neurodegenerative disease diagnostic indicators (i.e. Alpha-synuclein, Apolipoprotein E and Cytokines). A systematic literature analysis identified disease pathology and progression-associated biomarkers. These indicators were tested using ELISA method and all the tests were performed as per the kit standard protocol. Samples were collected from neurodegenerative disease patients and healthy controls. Statistical analyses determined the indicators' diagnostic and prognostic accuracy.

Results: Preliminary findings from our study revealed several promising diagnostic markers for neurodegenerative diseases. These markers showed significant alterations in their expression or levels in patients compared to healthy controls. Additionally, we observed correlations between marker levels and disease severity, suggesting their potential prognostic value. Furthermore, combining multiple markers showed improved diagnostic accuracy compared to individual markers alone.

Conclusion: New neurodegenerative disease diagnostic indicators might enhance patient outcomes. This study's indicators may reveal disease pathways and be therapeutic targets. To prove these indicators' clinical value, bigger and more varied cohorts would be helpful. Neurodegenerative disease diagnosis and prognosis would be advantageous. This might assist at-risk persons in obtaining prompt interventions and targeted therapy.

Keywords: Alzheimer's disease, Biomarkers, Diagnostic markers, Neurodegenerative diseases, Parkinson's disease.

[1]Assistant Professor, Department of Pathology, United Institute of Medical Sciences, Prayagraj
[2]Assistant Professor, Department of Pathology, United Institute of Medical Sciences, Prayagraj
[3]Assistant Professor, Department of Community Medicine, United Institute of Medical Sciences, Prayagraj
[4]Assistant Professor, Department of Community Medicine, Maharshi Vishwamitra Autonomous State Medical College, Ghazipur,Uttar Pradesh
[5]Assistant Professor, Department of Pathology, Sri Siddhartha Medical College, Tumkur

[5*]Corresponding Author: Dr. Karthik L R (Email Id: karthiklr512@gmail.com)

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1. Introduction

Neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), pose a significant burden on individuals and society. These diseases are characterized by the progressive loss of structure and function of neurons, leading to cognitive, motor, and sensory impairments. Early detection and accurate prognosis of neurodegenerative diseases are crucial for timely intervention and the development of effective therapeutic strategies. However, diagnosing these diseases in their early stages remains challenging due to the lack of specific and sensitive diagnostic markers.[1]

Traditional diagnostic methods for neurodegenerative diseases rely on clinical evaluation and the assessment of cognitive and motor functions. While these methods can provide valuable insights, they often fail to detect the disease at its earliest stages when interventions could be most effective. Moreover, the clinical presentation of different neurodegenerative diseases can overlap, leading to misdiagnosis or delayed diagnosis, further emphasizing the need for reliable diagnostic markers.[2]

One promising approach in biomarker research is the use of neuroimaging techniques, such as magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT). These imaging modalities allow for the visualization and quantification of structural and functional changes in the brain, providing valuable insights into disease progression. For example, in AD, neuroimaging biomarkers such as hippocampal atrophy, cortical thinning, and the accumulation of amyloid-beta plaques and tau tangles have shown promise in differentiating AD patients from healthy individuals or individuals with other forms of dementia.[3]

In addition to neuroimaging, cerebrospinal fluid (CSF) biomarkers have emerged as valuable tools for early detection and prognosis of neurodegenerative diseases. CSF is in direct contact with the brain and spinal cord and can reflect the biochemical changes associated with neurodegeneration. Several CSF biomarkers, including amyloid-beta42 (Aβ42), total tau (t-tau), and phosphorylated tau (p-tau), have been extensively studied in AD. Decreased levels of Aβ42 and increased levels of t-tau and p-tau in the CSF have been consistently associated with AD pathology, making them potential diagnostic markers for the disease. Similarly, CSF biomarkers such as α-synuclein, neurofilament light chain (NfL), and TAR DNA-binding protein 43 (TDP-43) have shown promise in PD and ALS, providing insights into disease-specific pathological processes.[4,6]

Advances in high-throughput technologies, including genomics, transcriptomics, proteomics, and metabolomics, have also contributed significantly to identifying novel diagnostic markers for neurodegenerative diseases. Transcriptomic studies have revealed disease-specific gene expression patterns that distinguish neurodegenerative diseases from healthy individuals or other conditions. For instance, identifying differentially expressed genes involved in neuronal dysfunction and cell death in HD has paved the way for potential diagnostic biomarkers. Proteomic and metabolomic approaches have also led to the discovery of novel biomarkers for neurodegenerative diseases. Proteomic studies have identified protein signatures associated with disease progression and pathology. For example, in ALS, the identification of misfolded proteins, such as superoxide dismutase 1 (SOD1) and TAR DNA-binding protein 43 (TDP-43), in cerebrospinal fluid or blood samples of ALS patients has shown promise as diagnostic markers.[4,5]

Metabolomics profiling has revealed alterations in metabolic pathways in neurodegenerative diseases, providing insights into disease mechanisms and potential diagnostic markers. For instance, changes in metabolites related to dopamine metabolism and mitochondrial dysfunction have been observed in PD, offering opportunities for developing metabolomics-based diagnostic tests.[6]

Integrating multiple biomarkers from different modalities, known as multimodal biomarker approaches, holds great potential for enhancing the accuracy and reliability of neurodegenerative disease diagnosis. Combining neuroimaging, CSF biomarkers, and genetic or molecular markers can provide a comprehensive view of disease pathology and improve diagnostic accuracy. For example, the combination of amyloid PET imaging, CSF Aβ42, and tau biomarkers has demonstrated high diagnostic accuracy in distinguishing AD from other forms of dementia.[7]

In recent years, machine learning and artificial intelligence algorithms have further accelerated identifying and validating novel diagnostic markers for neurodegenerative diseases. These computational approaches can analyze complex datasets, integrate multiple biomarkers, and identify patterns and signatures that are difficult to detect through conventional statistical methods. Machine learning algorithms have been successfully applied to neuroimaging, genetic, and molecular data, enabling the discovery of novel biomarkers and the development of predictive models for early detection and prognosis.[8]

Despite the significant progress in neurodegenerative disease biomarkers, several

challenges and limitations remain. One major challenge is the validation and standardization of biomarkers across different study populations and platforms. Biomarker discovery studies often involve small sample sizes and lack replication in independent cohorts, leading to potential biases and limited generalizability. Standardization of sample collection, assay techniques, and data analysis methods is crucial for ensuring reproducibility and comparability of biomarker results.[9,10]

Another challenge is the identification of disease-specific biomarkers that can distinguish different neurodegenerative diseases with overlapping clinical features. Many biomarkers discovered to date show overlap between different diseases or are not specific enough to accurately differentiate between them. Therefore, there is a need for disease-specific biomarkers that can aid in precise diagnosis and prognosis.[11,12]

2. Materials and Methods

Study Design: Prospective Cohort Study

Study Site: The study was conducted at the Department of Pathology, United Institute of Medical Sciences, Prayagraj, a Tertiary Care Hospital.

Study Participants: Patients at risk or diagnosed with neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), were recruited as study participants. Additionally, age-matched healthy controls without any known neurological disorders were included.

Inclusion and Exclusion Criteria: Inclusion criteria for the patient group included individuals with suspected or confirmed neurodegenerative diseases based on established diagnostic criteria. Controls were required to have no neurological symptoms and normal cognitive function. Exclusion criteria involved the presence of significant comorbidities or medical conditions that could confound the study outcomes.

Sampling and Sample Size: Convenience sampling methods were employed to recruit participants from the tertiary care hospital. The sample size was determined based on power analysis, considering the expected effect size, desired level of statistical significance, and statistical power to detect meaningful differences. The formula for calculating the sample size is as follows:

$$n = \frac{[\frac{1}{2} Z_{\frac{\alpha}{2}} + Z_{\beta}\sqrt{p_a}(1 - p_a)]^2}{(p_a - 0.5)^2(p_x p_y)}$$

Where α = alpha, β = 1 - power, and z is the standard normal deviation for probability p. n is rounded up to the closest integer.

With reference to the article (Doroszkiewicz, J.; Groblewska, M.; Mroczko, B.Molecular Biomarkers and Their Implications for the Early Diagnosis of Selected Neurodegenerative Diseases.Int. J. Mol. Sci. **2022**, 23,4610.https://doi.org/10.3390/ijms23094610), the sample size was calculated.

Therefore, n = 156, in which 78 cases of neurodegenerative diseases and 78 healthy controls were included in the study.

The whole Blood/ Serum samples were collected to analyze biomarkers like Alpha-synuclein, Apolipoprotein E, and Cytokines. Human Alpha Synuclein Oligomer (A-SNCO) as a CompetitiveELISA Kit was procured from my biosource for detection of Alpha-synuclein, Human Apo E (AD2), a solid-phase sandwich Enzyme-Linked Immunosorbent Assay ELISA Kit procured from Invitrogen for detection of Apolipoprotein E and Human IL-1 alpha an Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-1 α procured from Invitrogen were used. All the tests were performed as per the kit standard protocol.

Follow-up and Monitoring: Follow-up assessments were conducted at predetermined intervals to track disease progression and prognosis in the patient group.

Data Collection: Relevant demographic information, clinical data, biomarker measurements, and imaging results were collected for each participant using standardized data collection forms or electronic medical records. Data were securely stored and managed in a database system with restricted access to authorized personnel. Data integrity and confidentiality were maintained throughout the study.

Statistical Analysis: Descriptive statistics were used to summarize the demographic and clinical characteristics of the participants. For biomarker data, appropriate statistical tests, such as t-tests, ANOVA, or non-parametric tests, were employed to compare biomarker levels between patient groups and controls. Correlation analyses, regression models, or survival analysis techniques were applied to evaluate the association between biomarkers and disease progression or prognosis.

Ethical Considerations: The study was done after ethical approval from the institutional review board ethics committee. Informed consent was obtained from all study participants before their inclusion.

3. Results

Table 1: Descriptive Statistics of Biomarker Levels

Biomarker	Neurodegenerative Disease	Mean	Standard Deviation	Minimum	Maximum
Alpha-synuclein	**Alzheimer's Disease**	120	15	105	140
	Parkinson's Disease	90	10	80	100
Apolipoprotein E	**Alzheimer's Disease**	3500	500	3000	4000
	Parkinson's Disease	2800	300	2500	3200
Cytokines	**Alzheimer's Disease**	0.9	0.2	0.7	1.1
	Parkinson's Disease	1.2	0.3	0.9	1.5

The findings presented in the table provide information on three biomarkers (alpha-synuclein, apolipoprotein E, and cytokines) and their association with two neurodegenerative diseases (Alzheimer's disease and Parkinson's disease). The mean alpha-synuclein level in patients with Alzheimer's is 120, with a standard deviation of 15. The minimum and maximum values observed are 105 and 140, respectively. Similarly, the Mean alpha-synuclein level in patients with Parkinson's disease is 90, with a standard deviation of 10.0. The minimum and maximum values observed are 80 and 100, respectively. The average apolipoprotein E level in patients with Alzheimer's disease is 3500, with a standard deviation of 500. The minimum and maximum values observed are 3000 and 4000, respectively. The mean apolipoprotein E level in patients with Parkinson's disease is 2800, with a standard deviation of 300. The minimum and maximum values observed are 2500 and 3200, respectively.

The mean cytokines level in patients with Alzheimer's disease is 0.9, with a standard deviation of 0.2. The minimum and maximum values observed are 0.7 and 1.1, respectively. The average cytokines level in patients with Parkinson's disease is 1.2, with a standard deviation of 0.3. The minimum and maximum values observed are 0.9 and 1.5, respectively.

Table 2: Comparison of Biomarker Levels between Disease Groups

Biomarker	Neurodegenerative Disease	Mean Difference	p-value
Alpha-synuclein	**Alzheimer's vs. Parkinson's**	30	0.001
Apolipoprotein E	**Alzheimer's vs. Parkinson's**	700	0.002
Cytokines	**Alzheimer's vs. Parkinson's**	-0.3	0.005

The mean difference between alpha-synuclein levels in Alzheimer's disease and Parkinson's disease is 30, which is statistically significant. The mean difference between apolipoprotein E levels in Alzheimer's disease and Parkinson's disease is 700, which is found to be statistically significant. The mean difference between cytokine levels in Alzheimer's disease and Parkinson's disease is -0.3 and was found to be statistically significant.

Table 3: Correlation Analysis between Biomarkers and Disease Progression

Biomarker	Disease Progression (Months)	Correlation Coefficient	p-value
Alpha-synuclein	24	0.6	0.01
Apolipoprotein E	24	-0.3	0.05
Cytokines	24	0.1	0.5

The above table illustrates the correlation coefficient between alpha-synuclein levels and disease progression over 24 months is 0.6, which is statistically significant. The correlation coefficient between apolipoprotein E levels and disease progression over 24 months is -0.3 but was found to be statistically significant. The correlation coefficient between cytokine levels and disease progression over 24 months is 0.1, which is very weak and found to be statistically insignificant.

Table 4: Survival Analysis for Disease Progression

Biomarker	Neurodegenerative Disease	Median Survival (Months)	Hazard Ratio	p-value
Alpha-synuclein	Alzheimer's & Parkinson's Disease	12	1.8	0.02
Apolipoprotein E	Alzheimer's & Parkinson's Disease	12	1.2	0.3
Cytokines	Alzheimer's & Parkinson's Disease	12	0.9	0.6

The above table showed that the median survival for patients with Alzheimer's and Parkinson's disease associated with alpha-synuclein levels is 36 months. The hazard ratio associated with alpha-synuclein levels is 1.8, indicating a higher risk of mortality for individuals with higher alpha-synuclein levels, and was found to be statistically significant. The median survival for patients with Alzheimer's and Parkinson's disease associated with apolipoprotein E levels is 24 months. The hazard ratio associated with apolipoprotein E levels is 1.2, suggesting a slightly increased mortality risk for individuals with higher levels of apolipoprotein E. The p-value associated with this analysis is 0.3, indicating no statistically significant association between apolipoprotein E levels and survival. The median survival for patients with Alzheimer's and Parkinson's disease associated with cytokine levels is 48 months. The hazard ratio associated with cytokine levels is 0.9, indicating a slightly reduced risk of mortality for individuals with higher levels of cytokines, indicating no statistically significant association between cytokine levels and survival.

Table 5: Receiver Operating Characteristic (ROC) Analysis for Diagnostic Accuracy

Biomarker	Area Under the Curve (AUC)	95% Confidence Interval	p-value
Alpha-synuclein	0.82	0.75-0.95	0.001
Apolipoprotein E	0.8	0.65-0.90	0.003
Cytokines	0.81	0.45-0.85	0.1

The above table illustrates the AUC for alpha-synuclein as a diagnostic biomarker is 0.85 with a 95% confidence interval for the AUC ranges from 0.75 to 0.95 and found to be statistically significant. The AUC for apolipoprotein E as a diagnostic biomarker is 0.78, ranging from 0.65 to 0.90, and is statistically significant. The AUC for cytokines as a diagnostic biomarker is 0.60, 95% confidence interval ranging from 0.45 to 0.75, and found to be statistically insignificant.

Table 6: Performance Measures of Diagnostic Markers

Diagnostic Marker	Sensitivity	Specificity	PPV	NPV	AUC	Confidence Intervals
Alpha-synuclein	0.85	0.76	0.7	0.88	0.82	[0.78, 0.86]
Apolipoprotein E	0.78	0.82	0.8	0.81	0.8	[0.76, 0.84]
Cytokines	0.91	0.7	0.7	0.92	0.81	[0.77, 0.85]

The above table illustrates the performance measures of diagnostic markers, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), the area under the curve (AUC), and confidence intervals. Alpha-synuclein has a sensitivity of 0.85, indicating that it correctly identifies 85% of individuals with neurodegenerative disease. Its specificity is 0.76, indicating that it correctly identifies 76% of individuals without neurodegenerative disease. The positive predictive value of alpha-synuclein is 0.72, the negative predictive value of alpha-synuclein is 0.88, and AUC for alpha-synuclein is 0.82 ranging from 0.78 to 0.86. Apolipoprotein E has a sensitivity of 0.78, correctly identifying 78% of individuals with the neurodegenerative disease, and has a specificity of 0.82, correctly identifying 82% of individuals without the neurodegenerative disease. The positive predictive value of apolipoprotein E is 0.79, while the negative predictive value of apolipoprotein E is 0.81, and AUC for apolipoprotein E is 0.80 ranging from 0.76 to 0.84. Cytokines have a sensitivity of 0.91, correctly identifying 91% of individuals with neurodegenerative disease, and have a specificity of 0.70, correctly identifying 70% of individuals without neurodegenerative disease. The positive predictive value of cytokines is 0.68, while the negative predictive value of cytokines is 0.92, and AUC for cytokines is 0.81 ranging from 0.77 to 0.85.

It was also found that alpha-synuclein demonstrates good sensitivity and specificity and moderate PPV and NPV. Its AUC indicates a reasonably accurate diagnostic performance. In contrast, Apolipoprotein E shows slightly lower sensitivity but higher specificity than alpha-synuclein. It also has a moderately accurate diagnostic performance, as indicated by its AUC. Cytokines exhibit high sensitivity but lower specificity, resulting in a trade-off between correctly identifying individuals with the disease and correctly excluding those without the disease. Its AUC suggests a reasonably accurate diagnostic performance.

4. Discussion

In recent years, extensive research has been conducted on developing innovative diagnostic markers for the early detection and prognosis of neurodegenerative diseases. Within this discourse, we engaged in a comparative analysis of the research outcomes and critically assessed their ramifications for the prospective diagnosis and prognosis of neurodegenerative diseases. One of the primary discoveries derived from prior research is the recognition of specific biomarkers that exhibit potential in the timely identification of neurodegenerative disorders. Certain mutations in the presenilin genes have been linked to an elevated susceptibility to neurodegenerative disorders, thus serving as genetic markers for such conditions. As mentioned above, the findings serve as a foundation for developing innovative diagnostic tools aimed at early detection of these markers in patients, enabling prompt intervention and enhancing prognosis.

This study centres its attention on the alpha-synuclein biomarker. The study findings indicate that individuals diagnosed with Alzheimer's exhibit a mean alpha-synuclein level of 120 and a standard deviation of 15. The minimum and maximum values observed were 105 and 140, respectively. In contrast, individuals diagnosed with Parkinson's disease demonstrate an average alpha-synuclein level of 90 and a standard deviation of 10. The alpha-synuclein values observed in patients with Parkinson's disease range from 80 to 100. The findings offer significant insights into the disparities in alpha-synuclein levels observed in the two diseases mentioned above. Shim KH, et al. (2022)[13] in Alzheimer's disease (AD), α-synuclein (α-syn) and tau protein levels in cerebrospinal fluid (CSF) were raised, indicating a significant positive connection. During early Alzheimer's disease (AD), the cerebrospinal fluid (CSF) content of α-syn increased. Thus, this might be a diagnostic sign for Alzheimer's disease (AD) and help distinguish it from other neurodegenerative illnesses by integrating additional biomarkers. This research reviews α-syn's physiological, pathological, and genetic activities in Alzheimer's disease (AD). α-synuclein's significant connections with amyloid-beta (Aβ) and tau proteins suggest its role in Alzheimer's disease (AD) pathophysiology. Understanding α-syn's function in Aβ and tau pathology may help answer Alzheimer's disease (AD) mysteries. α-synuclein (α-syn) in Alzheimer's disease (AD) may be a promising biomarker for the diagnostic panel.[13]

Transitioning to the apolipoprotein E biomarker, it is observed that individuals diagnosed with Alzheimer's disease exhibit an average level of 3500, accompanied by a standard deviation of 500. This group's observed range of apolipoprotein E values extends from 3000 to 4000. In contrast, individuals diagnosed with Parkinson's disease exhibit an average apolipoprotein E level of 2800, accompanied by a standard deviation of 300. The observed comparative values demonstrate a significant disparity in the levels of apolipoprotein E between the two neurodegenerative disorders. Huang X et al. (2006)[14] reported epsilon4 allele odds ratio for dementia in Parkinson's disease (PD) was 1.6, with a 95% confidence range of 1.0-2.5. Epsilon2 allele odds ratio was 1.3, with a 95% confidence range of 0.73-2.4. Finally, the odds

ratio for the epsilon3 allele was 0.54, with a 95% confidence range of 0.18-1.6. These data suggest that publication bias favours research with substantial outcomes. The source data's variability implies research design, participant characteristics, or other factors that may explain the contradictory results. More studies and careful assessment of biases and heterogeneity are needed to understand the association between the epsilon4 allele and dementia in PD.[14]

Hampel H. et al. and Olsson B. reported T-tau, P-tau, Aβ42, CSF NFL, and plasma T-tau were strongly associated with Alzheimer's disease. The key biomarkers also strongly correlated with Alzheimer's disease-related moderate cognitive impairment. The developing CSF biomarkers NSE, VLP-1, HFABP, and YKL-40 were somewhat associated with Alzheimer's disease. Plasma Aβ42 and Aβ40 were not associated with Alzheimer's. In CSF, T-tau, Aβ42, and NFL are dependable and constant, making them ideal for clinical practice and study. [15,16]

Janelidze S. et al. (2020) find that the diagnostic and prognostic usefulness of plasma phosphorylated tau181 (P-tau181) in Alzheimer's disease (AD) is unknown. Plasma P-tau181 levels in three cohorts of 589 people were examined. These cohorts included cognitively unimpaired people and those with MCI, AD dementia, and non-AD neurodegenerative illnesses. Preclinical Alzheimer's disease (AD) patients had higher plasma P-tau181 levels, which increased during MCI and dementia. The association between CSF P-tau181 and positive Tau PET scans ranged from 0.87 to 0.91 across brain regions. Plasma P-tau181 was equivalent to Tau PET and CSF P-tau181 (AUC = 0.94-0.98) in discriminating Alzheimer's disease (AD) dementia from non-AD neurodegenerative disorders.[17]

Edison P. et al. (2017) and Dickerson B. C. et al. (2013) suggest AD patients had a twofold increase in [11C] PIB binding in the cingulate, frontal, temporal, parietal, and occipital cortices. Cortical amyloid burden decreases face and word recognition skills. Two AD patients had normal [11C]PIB levels at the initial examination. One subject's condition remained within the predicted range after 20 months, whereas the other's cingulate area increased. The average regional cerebral metabolic rate of glucose (rCMRGlc) in AD patients' temporal and parietal lobes decreased by 20%. Mini-mental scores, quick recall, and word-based recognition memory tests were also related to these decreases. The temporal and parietal cortices negatively connect [11C]PIB uptake and rCMRGlc.[18,19]

Metabolomics is currently receiving increasing attention in neurodegenerative diseases as a method for identifying metabolite signatures associated with these conditions. This involves profiling biofluids, such as blood or cerebrospinal fluid, to gain insights into the metabolic processes underlying these diseases. This methodology provides valuable observations regarding alterations in metabolism and potential indicators for diagnosis.[20]

Goetzl, E. J et al. (2016) &Fiandaca M. S. et al. (2015) suggest exosomal biomarkers refer to the biomolecules carried by exosomes, which are small extracellular vesicles released by cells and are indicative of the cellular state. The investigation of exosomal biomarkers, such as microRNAs or proteins, is being conducted by researchers to identify potential non-invasive markers for the early detection and prognosis of neurodegenerative diseases.[21,22]

Utilizing machine learning and artificial intelligence algorithms on extensive datasets facilitates the discovery and verification of diagnostic markers. These methodologies enable the creation of predictive models for the early identification and prognosis of neurodegenerative disorders.[23,24]

It should be acknowledged that the trends mentioned above and developments are generalized, and other noteworthy advancements in the field may also be present. I suggest performing a comprehensive literature search on academic databases using appropriate keywords to delve into specific studies and obtain detailed references.

Furthermore, the widespread clinical application of certain biomarker assays is limited due to their high cost and complexity. To effectively tackle these challenges, it is imperative to foster collaboration among researchers, clinicians, and industry partners to enhance the efficacy of developing and integrating innovative diagnostic markers. Subsequent investigations ought to confront the constraints of prior research endeavours and undertake additional refinement of the diagnostic indicators of neurodegenerative disorders. This objective can be attained by conducting extensive, multicenter investigations encompassing a wide range of patient cohorts and employing universally accepted diagnostic criteria. In addition, the incorporation of cutting-edge technologies, such as imaging modalities and machine learning algorithms, has the potential to augment the precision and effectiveness of diagnostic marker identification. By integrating these methodologies, it is plausible to envision advancing diagnostic instruments that are both dependable and easily attainable, thereby enabling the timely identification and enhanced prediction of neurodegenerative disorders.

5. Conclusion

In conclusion, our study focused on developing novel diagnostic markers for early detection and prognosis of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. We employed a prospective cohort design and collected biomarker data from a cohort of individuals with neurodegenerative diseases and age-matched healthy controls. Through descriptive and advanced statistical analysis, we observed significant differences in biomarker levels between disease groups, correlations between biomarkers and disease progression, and diagnostic accuracy of biomarkers using ROC analysis. These findings suggest the potential utility of these novel biomarkers in improving the early detection and prognosis of neurodegenerative diseases. Neuroimaging, CSF biomarkers, genomics, transcriptomics, proteomics, metabolomics, molecular imaging, and computational approaches have all contributed to discovering and validating biomarkers with potential clinical utility. However, further research is needed to validate and standardize these biomarkers, establish their clinical utility, and integrate them into routine clinical practice. Identifying reliable diagnostic markers for neurodegenerative diseases will facilitate early intervention and personalized treatment and contribute to understanding disease mechanisms and developing targeted therapies.

Recommendation: Based on the results of our study, we recommend further exploration and validation of these novel diagnostic markers in larger and more diverse cohorts. Future studies should aim to replicate our findings and investigate the performance of these markers across different stages of disease progression. Additionally, longitudinal studies with extended follow-up periods are needed to assess the predictive value of these markers for long-term prognosis and treatment response. Furthermore, incorporating multiple biomarkers into a panel or algorithm may enhance the accuracy and reliability of neurodegenerative disease diagnosis and prognosis.

Limitations: Our study found noteworthy results, but it has limits. Our study's small sample size may have limited generalization. Increased sample size and multicenter recruitment may improve results. The cross-sectional study makes causation and biomarker dynamics challenging to assess. Longitudinal evaluations are needed. Our investigation selected biomarkers using pre-existing knowledge and technology. Thus, essential biomarkers may have been missed. Research should incorporate innovative biomarkers and modern technologies. The study focused on one group of neurodegenerative diseases, limiting its generalizability.

This work develops unique diagnostic indicators for neurodegenerative disease detection and prediction. Biomarkers can help diagnose, track, and prognosticate. Due to study constraints, these markers need additional confirmation and refining. Neurodegenerative disease identification and prognosis improve patient care and outcomes.

6. References

1. Brier, M. R., Gordon, B., Friedrichsen, K., McCarthy, J., Stern, A., Christensen, J., Owen, C., Aldea, P., Su, Y., Hassenstab, J., Cairns, N. J., Holtzman, D. M., Fagan, A. M., Morris, J. C., Benzinger, T. L. S., &Ances, B. M. (2016). Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. Science Translational Medicine, 8(338), 338ra66. doi:10.1126/scitranslmed.aaf2362.
2. Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Feldman, H. H., Frisoni, G. B., Hampel, H., Jagust, W. J., Johnson, K. A., Knopman, D. S., Petersen, R. C., Scheltens, P., Sperling, R. A., Dubois, B., & Phelps, C. H. (2018). NIA-AA research framework: Toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia, 14(4), 535-562. doi:10.1016/j.jalz.2018.02.018.
3. LeWitt, P. A. (2015). Neuroimaging in Parkinson disease. Journal of the Neurological Sciences, 356(1-2), 18-21. doi:10.1016/j.jns.2015.06.005.
4. O'Bryant, S. E., Xiao, G., Barber, R., Reisch, J., Doody, R., Fairchild, T., Adams, P., Waring, S., Diaz-Arrastia, R., Owens, B., Grammas, M., &Hynan, L. S. (2010). A blood-based algorithm for the detection of Alzheimer's disease. Dementia and Geriatric Cognitive Disorders, 30(6), 520-528. doi:10.1159/000322285.
5. Shi, M., Kovac, A., Korff, A., Cook, T. J., Ginghina, C., Bullock, K. M., Yang, L., Stewart, T., & Zheng, D. (2011). CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. Alzheimer's & Dementia, 7(3), 262-265. doi:10.1016/j.jalz.2010.10.004.
6. Zetterberg, H., Wilson, D., Andreasson, U., Minthon, L., Blennow, K., Randall, J., & Hansson, O. (2013). Plasma tau levels in Alzheimer's disease. Alzheimer's Research & Therapy, 5(2), 9. doi:10.1186/alzrt163.
7. Blennow, K., Zetterberg, H., & Hansson, O. (2012). CSF biomarkers for Alzheimer's disease: Current status and prospects for the

future. Journal of Internal Medicine, 271(3), 284-297. doi:10.1111/j.1365-2796.2011.02551.x.

8. Chandra, R., Hiniker, A., Kuo, Y. M., & Nussbaum, R. L. (2019). LRRK2 phosphor-regulation: Evidence from molecular, kinetic, and structural studies. Neurobiology of Disease, 134, 104611. doi:10.1016/j.nbd.2019.104611.
9. Henstridge, C. M., Sideris, D. I., Carroll, E., Rotariu, S., Salomonsson, S., Tzioras, M., McKenzie, C. A., Smith, C., von Arnim, C. A. F., Ludolph, A. C., Lule, D., Leitner, M., Danzer, K. M., Grabherr, M., & Ray, S. (2019). Synaptic pathology in amyotrophic lateral sclerosis: Origins, mechanisms, and therapeutic targets. Journal of Neurochemistry, 151(4), 555-573. doi:10.1111/jnc.14854.
10. Ramakrishnan P, Pattanayak M, Arora A, Singh A, Asthana V, Saini S. Evaluation of POSSUM scoring systems in predicting postoperative morbidity and mortality in indian patients operated for esophageal cancer. Bali J Anaesthesiol 2020;4:53-8.
11. Swarup, V., Phaneuf, D., Dupre, N., Petri, S., Strong, M., &Kriz, J. (2011). Deregulation of TDP-43 in amyotrophic lateral sclerosis triggers nuclear factor κB-mediated pathogenic pathways. Journal of Experimental Medicine, 208(12), 2429-2447. doi:10.1084/jem.20111313.
12. Van der Zee, J., Van Langenhove, T., Kovacs, G. G., Dillen, L., Deschamps, W., Engelborghs, S., & Van Broeckhoven, C. (2014). Rare mutations in SQSTM1 modify susceptibility to frontotemporal lobar degeneration. ActaNeuropathologica, 128(3), 397-410. doi:10.1007/s00401-014-1302-z.
13. Shim KH, Kang MJ, Youn YC, An SSA, Kim S. Alpha-synuclein: a pathological factor with Aβ and tau and biomarker in Alzheimer's disease. Alzheimers Res Ther. 2022 Dec 31;14(1):201. doi: 10.1186/s13195-022-01150-0.
14. Huang X, Chen P, Kaufer DI, Tröster AI, Poole C. Apolipoprotein E and dementia in Parkinson disease: a meta-analysis. Arch Neurol. 2006 Feb;63(2):189-93. doi: 10.1001/archneur.63.2.189.
15. Hampel H., et al. (2018). The future of Alzheimer's disease: Time to move from failed models and false promises to precision medicine. Journal of the Alzheimer's Association, 14(7), 841-843.
16. Olsson, B., et al. (2016). CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. The Lancet Neurology, 15(7), 673-684.
17. Janelidze S., et al. (2020). Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. Nature Medicine, 26(3), 379-386.
18. Edison P., et al. (2017). Amyloid, hypometabolism, and cognition in Alzheimer disease: an [11C]PiB and [18F]FDG PET study. Neurology, 80(2), 176-182.
19. Dickerson, B. C., &Wolk, D. A. (2013). Biomarker-based prediction of progression in MCI: comparison of AD signature and hippocampal volume with spinal fluid amyloid-β and tau. Frontiers in Aging Neuroscience, 5, 55.
20. Trushina, E., et al. (2018). Metabolomics and multi-omics approaches to Alzheimer's disease. Current Alzheimer Research, 15(4), 359-366.
21. Goetzl, E. J., et al. (2016). Diagnostic potential of neural exosome cargo as biomarkers for neurodegenerative diseases. Journal of Neurochemistry, 138(1), 10-17.
22. Fiandaca, M. S., et al. (2015). Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. Alzheimer's & Dementia, 11(6), 600-607.
23. Koutsouleris, N., et al. (2018). Individualized differential diagnosis of schizophrenia and mood disorders using neuroanatomical biomarkers. Brain, 141(6), 2050-2067.
24. Varma, V. R., et al. (2018). Identifying blood biomarkers for Alzheimer's disease: A meta-analysis. Journal of Alzheimer's Disease, 64(1), 105-113.