DOI: https://doi.org/10.54393/pjhs.v5i09.2153

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (P): 2790-9352, (E): 2790-9344
Volume 5, Issue 9 (September 2024)

Original Article

OPEN ACCESS

Biomarker Profiles in Serum and CSF for Early Diagnosis of Selected Neuro-degenerative Diseases

Muhammad Anique[1*], Masooma Talib[2], Amna Ihsan[3], Iqra Anwar[2], Ambreen Zeeshan[4] and Naveed Ahsan[5]

[1]Department of Pathology, Bhitai Medical and Dental College, Mirpurkhas, Pakistan
[2]Department of Biochemistry, Watim Medical and Dental College, Rawalpindi, Pakistan
[3]Department of Biochemistry, King Edward Medical University, Lahore, Pakistan
[4]Department of Biochemistry, Fatima Memorial College of Medicine and Dentistry, Lahore, Pakistan
[5]Department of Biochemistry, Bhitai Medical and Dental College, Mirpurkhas, Pakistan

ARTICLE INFO

Keywords:
Alzheimer's Disease, Biomarkers, Cerebrospinal Fluid, Neurodegenerative Disorders

How to Cite:
Anique, M., Talib, M., Ihsan, A., Anwar, I., Zeeshan, A., & Ahsan, N. (2024). Biomarker Profiles in Serum and CSF for Early Diagnosis of Selected Neuro-degenerative Diseases: Serum and CSF for Early Diagnosis of Neurodegenerative Diseases. Pakistan Journal of Health Sciences (Lahore), 5(09). https://doi.org/10.54393/pjhs.v5i09.2153

***Corresponding Author:**
Muhammad Anique
Department of Pathology, Bhitai Medical and Dental College, Mirpurkhas, Pakistan
aniquemuhammad@icloud.com

Received Date: 16th August, 2024
Acceptance Date: 27th September, 2024
Published Date: 30th September, 2024

ABSTRACT

Biomarker research and justification for neurodegenerative illnesses have seen enormous efforts over the last ten years. Bio-fluid-based biomarkers have been believed to provide a better and easier approach to detecting biomarkers for diagnosing nervous system pathologies. **Objectives:** To evaluate the diagnostic potential of certain biomarkers in serum and cerebrospinal fluid to diagnose Alzheimer's disease, Parkinson's disease, and Huntington's disease at an initial stage. **Methods:** 280 participants were taken and distributed into four groups, comprising, 70 patients with early-stage Alzheimer's disease, 70 with early-stage Parkinson's disease, 70 with early-stage Huntington's disease, and 70 age-matched healthy controls. Blood and cerebrospinal fluid samples were drawn and medical history was taken from the patients. Serum and cerebrospinal fluid levels of amyloid-beta (Aβ42), total tau (t-tau), phosphorylated tau (p-tau), alpha-synuclein, huntingtin protein, and neuro-filament light chain were evaluated using enzyme-linked immunosorbent assays. **Results:** Alzheimer's disease patients showed reduced serum Aβ42 (80.4 ± 15.6 pg/mL) and elevated t-tau (140.5 ± 18.2 pg/mL). Parkinson's disease patients had raised serum alpha-synuclein (12.5 ± 2.3 ng/mL) and neuro-filament light chain. Huntington's disease patients showed significant increases in serum huntingtin protein (8.2 ± 2.0 ng/mL). These profiles indicate efficacy in early diagnosis. **Conclusions:** It was concluded that Aβ42 and tau effectively detect Alzheimer's disease, while Parkinson's disease patients can be effectively diagnosed with Serum and cerebrospinal fluid levels of the neuro-filament light chain. Similarly, huntingtin protein and neuro-filament light chain are sensitive enough to detect Huntington's disease at its early stages.

INTRODUCTION

Neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) are among the major public health concerns due to their progressive nature. These neurodegenerative diseases are known to destroy the cognitive and motor functions of our body. Alzheimer's disease is one of the most common and persuasive causes of dementia in older age individuals, affecting over 50,000,000 individuals globally [1]. Alzheimer's disease is recognized by the intracellular formation of neurofibrillary masses and the extracellular accumulation of amyloid-β (Aβ) peptide fibrils. Its complex pathophysiology and multiple aetiology remain unclear [2]. Since there is currently no treatment for Alzheimer's disease, the high failure rate in clinical trials may stem from inclusion criteria, research design, or attempts to treat the disease after it has progressed [3]. Parkinson's disease is a complicated neurological condition that is mainly caused by the decrease of dopamine-releasing neurons present in the brain area (substantia nigra), ultimately resulting in the progressive loss of motor function [4]. As of right now, the sole clinical criteria used for Parkinson's disease diagnosis are the presence of neuro-motor symptoms, which have limited accuracy in the initial phases of the disease [5]. It is

DOI: https://doi.org/10.54393/pjhs.v5i09.2153

thought that neurodegeneration is neither severe nor widespread during this early stage, providing an ideal opportunity for new medicines and treatments that modify the disease. However, more factors would be needed in addition to the clinical criteria for the diagnosis of early Parkinson's disease [6]. Huntington's disease is an autosomal dominant condition resulting from a Cytosine, Adenine, and Guanine (CAG) tri-nucleotide replication extension in the gene named Huntington's disease (HTT). This genetic mutation ultimately causes the creation of a non-functional protein, known as huntingtin-protein, which gathers within the nerve cells, causing neurodegeneration primarily in the striatum but also affecting other brain parts. Huntington's disease is established through a combination of motor, cognitive, and psychiatric symptoms, with a premanifest stage that offers a critical prospect for early therapeutic intervention. There are no treatments available at the moment that can cure Huntington's disease but its progression can be slowed down [7]. However, the identification of novel biomarkers linked to the early stages of the disease is crucial since molecular changes occur well in advance of the emergence of neurodegenerative symptoms [8]. Although biomarkers for neurodegenerative disease diagnosis have been thoroughly studied, more research is still necessary to determine their clinical applicability [9]. Several imaging techniques have been created to offer detailed insights into the anatomy and operation of the brain [10]. However, because of their high costs and infrastructure requirements, these approaches are difficult to deploy in a clinical context and have limited application in routine early diagnosis cases. Detectable molecular biomarkers in bodily fluids are thought to be the best way to support clinical diagnosis. Because CSF is thought to act as the brain's surrogate, it is the recommended bio-fluid for biomarker research about neurodegenerative illnesses. Even though CSF is widely used in Parkinson's disease biomarker research, there are still few validated biomarkers that can be used in a clinical context [11]. Serum, plasma, and cerebrospinal fluid (CSF) are considered the most authentic and easily available body fluids to detect various metabolites or toxins to detect a disease at an early stage. While CSF biomarkers such as Aβ1-42, total tau, and phosphorylated tau have shown utility in Alzheimer's disease, and α-synuclein and neuro-filament light chain (NfL) are being explored for Parkinson's disease and Huntington's disease, the invasiveness of CSF collection limits its widespread clinical application. Therefore, identifying non-invasive, cost-effective blood-based biomarkers is a critical research priority.

This study aims to establish some diagnostic parameters that use blood to detect these factors for the detection of Alzheimer's disease, Parkinson's disease, and Huntington's disease at an early stage. By leveraging the differential expression of specific biomarkers linked with these disorders, we propose that these parameters will significantly improve diagnostic precision and feasibility in clinical settings.

METHODS

This comparative cross-sectional study included 280 participants: 70 with early-stage Alzheimer's disease, 70 with early-stage Parkinson's disease, 70 with early-stage Huntington's disease, and 70 age-matched healthy controls. Participants were recruited from Bhitai Medical and Dental College, Mirpurkhas, Sindh, from April 2023 to September 2023. The duration of the study was six months and a convenience sampling technique was used. Written consent was taken from all the patients before the study, and it was started after approval from the institutional ethics committee of Bhitai Medical and Dental College, Mirpurkhas, Sindh (Ref. No. BDMC/R&D/ERC/2023-15). The sample size was calculated on the basis of 80% statistical power and a significance level of ≤0.05. The following formula for sample size calculation in diagnostic studies was used:

$$n = \frac{Z^2 \times P \times (1 - P)}{d^2}$$

Where Z is the Z-value (1.96 for 95% confidence), P is the expected prevalence 2.21%, and d is the desired precision (5%). The inclusion criteria for this study involved participants aged 40-75 years who were clinically suspected for the diagnosis of initial-stage Alzheimer's disease, Parkinson's disease, and Huntington's disease based on established clinical criteria, as well as similar age group healthy controls without any history of neurodegenerative disorders. Participants were required to provide informed consent and have the capacity to undergo blood sampling and, where applicable, lumbar puncture for CSF collection. The patients were excluded from the study with a history of any known psychiatric condition, any cardiovascular or systemic ailment, active infections, or those on medications known to affect the central nervous system. Blood samples were drawn from all selected patients and centrifuged at 2000 RPM for 10 minutes, to obtain serum. CSF was drawn by a lumbar puncture. All the samples taken in this way were preserved at -80°C until further analysis. Serum and CSF levels of amyloid-beta (Aβ42), total tau (t-tau), phosphorylated tau (p-tau), alpha-synuclein, huntingtin protein, and NfL were measured using enzyme-linked immunosorbent assays (ELISAs). The samples were sent to the lab with the tag numbers without mentioning the names of the patients to ensure blinding. Statistical analyses were carried out using SPSS-27. Differences among the groups were assessed by one-way analysis of variance (ANOVA) followed by Turkeys' post-hoc test to find significant differences among the groups.

DOI: https://doi.org/10.54393/pjhs.v5i09.2153

RESULTS

Serum levels of Aβ42 were significantly decreased in Alzheimer's disease patients, while t-tau and p-tau levels were significantly raised compared to the controls. In Parkinson's disease patients, serum levels of alpha-synuclein and NfL were significantly raised. NfL levels were also raised in Huntington's disease Huntington's disease. In Huntington's disease patients, serum huntingtin protein levels were significantly higher, compared to all the other groups (Table 1).

Table 1: Serum and Plasma Biomarker Levels in Study Groups

Biomarker	Healthy Controls (n=70)	Alzheimer's Disease (n=70)	Parkinson's Disease (n=70)	Huntington's Disease (n=70)	p-value
Serum Aβ42 (pg/mL)	150.5 ± 20.3	80.4 ± 15.6**	145.8 ± 18.4	148.7 ± 17.3	<0.001
Serum t-tau (pg/mL)	75.2 ± 10.4	140.5 ± 18.2**	80.2 ± 12.1	78.6 ± 11.9	<0.001
Serum p-tau (pg/mL)	32.4 ± 5.8	90.7 ± 14.3**	33.2 ± 6.2	35.0 ± 6.0	<0.001
Plasma α-synuclein (ng/mL)	4.6 ± 1.0	4.8 ± 1.1	12.5 ± 2.3**	5.0 ± 1.2	<0.001
Plasma NfL (pg/mL)	22.7 ± 5.5	30.5 ± 6.4**	35.8 ± 7.2**	60.2 ± 10.5**	<0.001
Serum huntingtin protien (ng/mL)	1.5 ± 0.5	1.6 ± 0.6	1.4 ± 0.4	8.2 ± 2.0**	<0.001

Values are shown as mean ± standard deviation (SD). ** shows a statistically significant difference compared to healthy controls (p<0.001) in the post-hoc analysis.

Similarly, the analysis of cerebrospinal fluid (CSF) biomarkers provided further differentiation between the groups. In Alzheimer's disease patients, CSF levels of Aβ42 were significantly reduced, while t-tau and p-tau levels were significantly elevated. These biomarkers are strongly associated with Alzheimer's disease pathology, with lower Aβ42 and higher tau proteins reflecting the presence of amyloid plaques and neurofibrillary masses in the brain. In Parkinson's disease patients, CSF alpha-synuclein levels were significantly higher, consistent with the accumulation of this protein in the brain, supporting its use as a biomarker for early Parkinson's disease detection. NfL levels in CSF were moderately increased, indicating ongoing neurodegenerative processes in Parkinson's disease. In Huntington's disease patients, CSF levels of huntingtin protein were significantly elevated, providing a clear distinction between Alzheimer's disease, Parkinson's disease, and healthy controls. This specific increase in huntingtin protein, along with elevated NfL levels, underscores its utility as a diagnostic biomarker for Huntington's disease (Table 2).

Table 2: Cerebrospinal Fluid Biomarker Levels in Study Groups

Biomarker	Healthy Controls (n=70)	Alzheimer's Disease (n=70)	Parkinson's Disease (n=70)	Huntington's Disease (n=70)	p-value
CSF Aβ42 (pg/mL)	800.2 ± 95.5	350.4 ± 60.8**	780.1 ± 92.3	790.2 ± 88.7	<0.001
CSF t-tau (pg/mL)	70.2 ± 12.6	150.3 ± 20.2**	75.5 ± 10.8	72.3 ± 11.1	<0.001
CSF p-tau (pg/mL)	20.5 ± 4.0	60.7 ± 9.3**	21.0 ± 4.3	22.4 ± 4.2	<0.001
CSF α-synuclein (ng/mL)	3.0 ± 0.8	3.2 ± 0.9	15.0 ± 2.7**	3.3 ± 0.8	<0.001
CSF NfL (pg/mL)	30.2 ± 7.5	40.4 ± 8.2**	45.8 ± 9.0 **	75.2 ± 13.5**	<0.001
CSF huntingtin protein (ng/mL)	0.8 ± 0.3	0.9 ± 0.4	0.7 ± 0.2	4.2 ± 1.5**	<0.001

Values are shown as mean ± SD. ** presents a statistical significance level compared to healthy controls (p<0.001) in the post-hoc analysis.

DISCUSSION

The results of the current study highlight the potential of specific serum and CSF biomarkers in the early detection of Alzheimer's disease, Parkinson's disease, and Huntington's disease. These results are consistent with existing literature, which highlights the importance of biomarkers in identifying neurodegenerative diseases at an early stage, potentially before significant clinical symptoms emerge [12, 13]. In Alzheimer's disease, the observed decrease in serum and CSF levels of Aβ42 and the increase in tau proteins (both total and phosphorylated) align with the well-documented pathological processes underlying the condition [14, 15]. Hampel *et al.*, [2], Michno *et al.*, [16], and Xu *et al.*, [17] have shown that Aβ42 aggregates into amyloid plaques in the brain, a hallmark of Alzheimer's disease, leading to a reduction in its levels in the CSF. Similarly, Moore *et al.*, described the accumulation of hyper-phosphorylated tau, which forms neuro-fibrillary tangles, another key feature of Alzheimer's disease pathology [18]. The correlation between decreased CSF Aβ42 and elevated tau levels has been extensively validated in previous studies by Campbell *et al.*, [19], Grangeon *et al.*, [20] supporting their use as reliable biomarkers for early Alzheimer's disease diagnosis. For Parkinson's disease, the significant increase in alpha-synuclein levels in both serum and CSF is consistent with its role in the development of Lowy bodies. Ganguly *et al.*, [21] have demonstrated that alpha-synuclein accumulates in the brain, with its ultimate build-up in the CSF, making it a critical biomarker for Parkinson's disease. Furthermore, the observed rise in NfL levels in Parkinson's disease patients suggests axonal degeneration, a hallmark of the disease's neurodegenerative nature. These findings are in line with the existing research, as noted by Chen *et al.*, [6] which reports elevated alpha-synuclein and NfL levels in the CSF in most Parkinson's disease cases. The results of our study indicate that huntingtin protein levels are significantly elevated in the serum and CSF of patients in the Huntington's disease group. The results of a study by Caron *et al.*, [22] support these findings, having reported increased huntingtin protein levels in the CSF of advanced-stage Huntington's disease patients. Similarly, the

DOI: https://doi.org/10.54393/pjhs.v5i09.2153

significant elevation of NfL levels in Huntington's disease patients in our study confirms the initiation of neuronal damage, a typical feature of the disease. The observed differences in biomarker levels across Alzheimer's disease, Parkinson's disease, and Huntington's disease patients underscore the importance of these biomarkers in serum and CSF for diagnosing these neurodegenerative diseases at an early stage. it is recommended that routine screening of these biomarkers in both serum and CSF be considered for individuals at risk of neurodegenerative diseases to facilitate early diagnosis and intervention. Further large-scale studies should be conducted to validate the use of these biomarkers in clinical practice, potentially leading to the development of more standardized diagnostic protocols.

CONCLUSIONS

It was concluded that the study establishes the potential of specific serum and CSF biomarkers for the initial detection of Alzheimer's disease, Parkinson's disease, and Huntington's disease. Aβ42, NfL, t-tau and p-tau are useful biomarkers for early diagnosis of Alzheimer's disease, α-synuclein and NfL for Parkinson's disease whereas NfL and huntingtin protein can be assessed for early detection of Huntington's disease.

Authors Contribution

Conceptualization: MA
Methodology: MA, MT, AI
Formal analysis: IA, AZ, NA
Writing review and editing: MA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Vogt AC, Jennings GT, Mohsen MO, Vogel M, Bachmann MF. Alzheimer's Disease: A Brief History of Immunotherapies Targeting Amyloid B. International Journal of Molecular Sciences. 2023 Feb; 24(4): 3895. doi: 10.3390/ijms24043895.

[2] Hampel H, Hardy J, Blennow K, Chen C, Perry G, Kim SH *et al.* The Amyloid-B Pathway in Alzheimer's Disease. Molecular Psychiatry. 2021 Oct; 26(10): 5481-503. doi: 10.1038/s41380-021-01249-0.1

[3] Matuszyk MM, Garwood CJ, Ferraiuolo L, Simpson JE, Staniforth RA, Wharton SB. Biological and Methodological Complexities of Beta-Amyloid Peptide: Implications for Alzheimer's Disease Research. Journal of Neurochemistry. 2022 Feb; 160(4): 434-53. doi: 10.1111/jnc.15538.

[4] Shah AA, Kashyap K, Gupta A. Parkinson's Disease: An Introduction to the Causes and Management Strategies. International Journal of Pharmaceutical Research (09752366). 2020 Oct; 12(4). doi: 10.31838/ijpr/2020.12.04.698.

[5] Shankar J, Geetha KM, Wilson B. Potential Applications of Nanomedicine for Treating Parkinson's Disease. Journal of Drug Delivery Science and Technology. 2021 Dec; 66: 102793. doi: 10.1016/j.jddst.2021.102793.

[6] Chen JH, Chan L, Chung CC, Bamodu OA, Hong CT. Blood Neurofilament Light Chain in Parkinson's Disease: Comparability Between Parkinson's Progression Markers Initiative (Ppmi) And Asian Cohorts. Journal of Clinical Medicine. 2021 Oct; 10(21): 5085. doi: 10.3390/jcm10215085.

[7] Bourn D and Bourn D. Autosomal Dominant Inheritance and Huntington Disease. Diagnostic Genetic Testing: Core Concepts and the Wider Context for Human DNA Analysis. 2022: 23-35. doi: 10.1007/978-3-030-85510-9_2.

[8] Doroszkiewicz J, Groblewska M, Mroczko B. Molecular Biomarkers and Their Implications for the Early Diagnosis of Selected Neurodegenerative Diseases. International Journal of Molecular Sciences. 2022 Apr; 23(9): 4610. doi: 10.3390/ijms23094610.

[9] Klyucherev TO, Olszewski P, Shalimova AA, Chubarev VN, Tarasov VV, Attwood MM *et al.* Advances in the Development of New Biomarkers for Alzheimer's Disease. Translational Neurodegeneration. 2022 Apr; 11(1): 25. doi: 10.1186/s40035-022-00296-z.

[10] Koníčková D, Menšíková K, Tučková L, Hényková E, Strnad M, Friedecký D *et al.* Biomarkers of Neurodegenerative Diseases: Biology, Taxonomy, Clinical Relevance, and Current Research Status. Biomedicines. v2022 Jul; 10(7): 1760. doi: 10.3390/biomedicines10071760.

[11] Varesi A, Carrara A, Pires VG, Floris V, Pierella E, Savioli G *et al.* Blood-Based Biomarkers for Alzheimer's Disease Diagnosis and Progression: An Overview. Cells. 2022 Apr; 11(8): 1367. doi: 10.3390/cells11081367.

[12] Ehrenberg AJ, Khatun A, Coomans E, Betts MJ, Capraro F, Thijssen EH *et al.* Relevance of Biomarkers Across Different Neurodegenerative Diseases. Alzheimer's Research & Therapy. 2020 Dec; 12: 1-1. doi: 10.1186/s13195-020-00637-y.

[13] Hansson O. Biomarkers for Neurodegenerative Diseases. Nature Medicine. 2021 Jun; 27(6): 954-63.

[14] Baldacci F, Mazzucchi S, Della Vecchia A, Giampietri L, Giannini N, Koronyo-Hamaoui M *et al.* The Path to

DOI: https://doi.org/10.54393/pjhs.v5i09.2153

Biomarker-Based Diagnostic Criteria for the Spectrum of Neurodegenerative Diseases. Expert Review of Molecular Diagnostics. 2020 Apr; 20(4): 421-41. doi: 10.1038/s41591-021-01382-x.

[15] Pathak N, Vimal SK, Tandon I, Agrawal L, Hongyi C, Bhattacharyya S. Neurodegenerative Disorders of Alzheimer, Parkinsonism, Amyotrophic Lateral Sclerosis and Multiple Sclerosis: An Early Diagnostic Approach for Precision Treatment. Metabolic Brain Disease. 2021 Nov; 37: 1-38. doi: 10.1007/s11011-021-00800-w.

[16] Michno W, Blennow K, Zetterberg H, Brinkmalm G. Refining The Amyloid B Peptide and Oligomer Fingerprint Ambiguities in Alzheimer's Disease: Mass Spectrometric Molecular Characterization in Brain, Cerebrospinal Fluid, Blood, and Plasma. Journal of Neurochemistry. 2021 Oct; 159(2): 234-57. doi: 10.1111/jnc.15466.

[17] Xu C, Zhao L, Dong C. A Review of Application of Aβ 42/40 Ratio in Diagnosis and Prognosis of Alzheimer's Disease. Journal of Alzheimer's Disease. 2022 Jan; 90(2): 495-512. doi: 10.3233/JAD-220673.

[18] Moore KB, Hung TJ, Fortin JS. Hyper-phosphorylated tau (p-tau) and Drug Discovery in the Context of Alzheimer's Disease and Related Tauopathies. Drug Discovery Today. 2023 Mar; 28(3): 103487. doi: 10.1016/j.drudis.2023.103487.

[19] Campbell MR, Ashrafzadeh-Kian S, Petersen RC, Mielke MM, Syrjanen JA, van Harten AC *et al.* P-tau/aβ42 and aβ42/40 Ratios in CSF Are Equally Predictive of Amyloid Pet Status. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 2021 May; 13(1): e12190. doi: 10.1002/dad2.12190.

[20] Grangeon L, Paquet C, Guey S, Zarea A, Martinaud O, Rotharmel M *et al.* Cerebrospinal Fluid Profile of Tau, Phosphorylated Tau, Aβ 42, and Aβ 40 in Probable Cerebral Amyloid Angiopathy. Journal of Alzheimer's Disease. 2022 Jan; 87(2): 791-802. doi: 10.3233/JAD-215208.

[21] Ganguly U, Singh S, Pal S, Prasad S, Agrawal BK, Saini RV *et al.* Alpha-Synuclein as A Biomarker of Parkinson's Disease: Good, But Not Good Enough. Frontiers in Aging Neuroscience. 2021 Jul; 13: 702639. doi: 10.3389/fnagi.2021.702639.

[22] Caron NS, Haqqani AS, Sandhu A, Aly AE, Findlay Black H, Bone JN *et al.* Cerebrospinal Fluid Biomarkers for Assessing Huntington Disease Onset and Severity. Brain Communications. 2022 Dec; 4(6): fcac309. doi: 10.1093/braincomms/fcac309.