SHORT COMMUNICATION

Comparison of potential plasma biomarkers in Alzheimer's disease and neurodegenerative dementias in Pakistani population

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Abstract

This study aimed to compare the mean plasma levels of Amyloid β42, Phosphorylated Tau and Neurofilament Light chain in patients diagnosed with Alzheimer's Clinical Syndrome (ACS), and other neurodegenerative dementias to find affordable and less-invasive means of diagnosing Alzheimer's disease (AD) early in its course.

Blood samples of 36 subjects presenting with cognitive decline to the neurology OPDs of Dow and Civil hospitals, Karachi, were centrifuged, and plasma was stored at -80°C. Before analysis, it was thawed at 4°C and protein levels were measured through ELISA.

Two-thirds of the patients were females but age distribution across both the groups was not significantly different (p=0.21). No difference was observed in the mean plasma concentrations of Aβ42, P-Tau, and NFL between the two groups (p-values 0.78 and 0.27 and 0.09 respectively).

Our study suggests that despite being promising in CSF, Aβ42, P-Tau, and NFL cannot differentiate between different neurodegenerative dementias when measured in plasma.

Keywords: Biomarkers, Alzheimer disease, Amyloid beta peptides, Tau proteins, Neurofilament proteins.

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Introduction

The high degree of similarity in the clinical presentation of different neurodegenerative dementias (ND) makes their differential diagnosis challenging.[1] Although atrophy patterns of the brain are used to discern between different NDs, they do not allow early diagnosis.

With the rising prevalence of dementia in the low and low-middle income countries, correct and timely diagnosis of Alzheimer's disease (AD) has become an increasing need. Pathological hallmarks of the disease include Amyloid-β plaques and neurofibrillary tangles made up of hyperphosphorylated tau.[2] The research framework proposed by National Institute of Aging - Alzheimer's Association (NIA-AA), in 2018, suggests establishing diagnosis of AD on the basis of PET or CSF biomarkers for Amyloid-β and Tau.[3] However, the invasiveness of lumbar puncture, and the high cost and scarce availability of PET prevent their wide scale use in clinics.[4] According to the World Alzheimer Report 2021, 90% patients with dementia in low and middle income countries remain undiagnosed, the major reason for which is reported to be lack of access to reliable diagnostic tests.[5]

Despite considerable global research on biomarker diagnosis of AD, diagnosis in Pakistan is still being made solely on clinical grounds, and a substantial gap in AD research exists between Pakistan and the developed world. In an effort to open the arena of research around biochemical diagnosis of Alzheimer's disease in Pakistan, the plasma levels of three proteins—Amyloid-β42, P-tau and NFL—was investigated as suggested in the NIA-AA research framework in terms of their ability to differentiate between different neurodegenerative dementias.[3]

Patients/Methods and Results

The study recruited individuals with cognitive impairment presenting at Civil Hospital, Dow University Hospital, and Abdul Qadeer Khan Institute of Behavioural Sciences, Karachi, Pakistan, from May to October 2021 through convenience sampling.

Sample size was calculated through PASS version 11 software with test for One-Sample Sensitivity and Specificity with 95% CI, 80% power of the test, taking sensitivity of NFL at 84%[6] with margin of error at 5%, specificity of NFL at 78%[6] with margin of error 5% and Alzheimer's disease prevalence of 6.14% (5.7 million / 92.78 million).[7] As the available total estimated population size of patients with Alzheimer's Clinical Syndrome within one year is 30, using Finite Population Correction (FPC) factor

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the calculated sample size came out to be 28 patients.

Adults more than 50 years of age who presented with cognitive decline to the OPDs and were diagnosed with neurodegenerative dementia by consultant neuro-physician were considered eligible for the study. Cognitive decline was defined as a score less than 24 on the Mini-Mental State Examination (MMSE) both for the English and Urdu versions.[8] Neurocognitive disorder was diagnosed using the Diagnostic and Statistical Manual-5 (DSM-5).

Patients were excluded if they had a history of severe brain injury, psychiatric disease, TIA/CVA within three months of cognitive decline or patients with sudden onset cognitive decline. Participants with normal pressure hydrocephalus, brain tumour, vitamin B12 deficiency, or thyroid disorders were also excluded from the study.

The participants were stratified into two groups. The first group comprised those who were diagnosed with probable or possible AD based on DSM-5 criteria, referred to as having Alzheimer's Clinical Syndrome (ACS group) as suggested in NIA-AA 2018 research framework;[9] these were further stratified into mild and severe cognitive impairment groups (MCI and SCI). Patients with other types of neurodegenerative dementias such as Lewy body dementia were grouped separately (ND group).

Ethical approval was obtained from the Institutional Review Board of Dow University of Health Sciences (Ref: IRB-1999/DUHS/Approval/2021/418). All procedures were in alignment with the code of ethics given by the World Medical Association (Declaration of Helsinki). Informed written consent was obtained from all patients or their attendants as applicable and anonymity of data was ensured.

Demographic and lifestyle details of the subjects were obtained through a questionnaire filled by the attendant. History of depressive episodes was enquired from the attendant by asking about any unusual crying or similar behavioural episodes. Evidence for dyslipidaemia was obtained through lipid profile of the patients; derangement in at least two categories of lipid profile was taken as dyslipidaemia. History of physical activity was self-reported by the attendants and was asked in terms of average number of hours per day the patient was engaged in some physical activity. Observing standard operating procedures of venipuncture, 5cc blood was drawn from all subjects into tubes containing EDTA as anticoagulant. All samples were collected between 9 am and 12 noon to avoid any diurnal variation in protein levels and were transported to the lab in ice. Centrifugation was performed at 3000rpm for 15 minutes at 4°C within two hours of collecting the sample. The collected plasma was aliquoted into three polypropylene tubes and stored at -80°C pending biochemical analysis.

Plasma Aβ42, P-tau, and NFL were measured through ELISA performed on Thermoscientific Spectrophotometer Multiskan™ Sky (Model number 51119700) and absorbances were recorded at 450nm. Commercially available kits CEA946Hu (Human Aβ1-42) purchased from USCN Business Co. Ltd, E4844Hu (Human Microtubule associated protein Tau) purchased from Bioassay Technology Laboratory, and SEE038Hu (Human NFL) purchased from USCN Business Co. Ltd. were used for the three proteins and ELISA was performed using kit instructions. Protein concentrations in each sample were calculated using standard curves.

Data was analysed using IBM SPSS version 26.0. Chi square

Table-1: Demographic and health status data of study population divided across different groups (n=36).

Parameters	n (%)	ND (n=8)	ACS (n=28)		*p*-value
			MCI	SCI	
Mean Age (years)		65.63 (±5.8)	68.11 (±11.7)		0.7
Mean MMSE		14.75 (±9.7)	14.8 (±8.4)		
Gender					0.21
Female	24 (67)	5 (63)	6 (21)	13 (46)	
Male	12 (33)	3 (38)	6 (21)	3 (11)	
Ethnicity					0.72
Urdu speaking	21 (58)	5 (63)	6 (21)	10 (36)	
Sindhi	8 (22)	1 (13)	4 (14)	3 (11)	
Panjabi	2 (6)	1 (13)	0	1 (4)	
Pashtun	4 (11)	1 (13)	2 (7)	1 (4)	
Baloch	1 (3)	0	0	1 (4)	
Education					0.29
No formal education	11 (31)	1(13)	3(11)	7 (25)	
Primary	6 (17)	1(13)	2 (7)	3 (11)	
High school	3 (8)	0	2(7)	1 (4)	
Graduation	16 (44)	6(75)	5 (18)	5 (18)	
Mid-life physical activity					1
5+ hours	7 (19)	1(13)	3 (11)	3 (11)	
3-5 hours	11 (31)	3(38)	3(11)	5 (18)	
2-3 hours	15 (42)	4(50)	5 (18)	6 (21)	
1-2 hours	3 (8)	0	1(4)	2 (7)	
Diabetes	11 (31)	3(38)	3 (11)	5 (18)	0.67
Hypertension	19 (53)	5(63)	4 (14)	10 (36)	0.69
Hyperlipidaemia	8 (22)	1(13)	3 (11)	4 (14)	0.65
Depression	21 (58)	4(50)	8 (29)	9 (32)	0.69
Family h/o dementia	8 (22)	1(13)	3 (11)	4 (14)	0.65
Smoking	12 (33)	2(25)	2 (7)	8 (29)	0.69

MCI category includes ACS patients with MMSE score between 18 and 24. SCI category includes ACS patients with MMSE score less than 18. Some categories were merged to satisfy conditions for Chi squared test.

Table-2: Mean plasma concentrations of Aβ42, NFL and P-tau in ACS and ND groups.

Plasma protein	ACS group (pg/ml) Mean ± SD	ND group (pg/ml) Mean ± SD	*p*-value
Aβ42	33.33±18.00	37.99±25.30	0.78
NFL	213.9±78.5	179.6±123.2	0.09
P-Tau	265.48± 66.68	216.23± 90.15	0.27

test was used to compare categorical variables while mean plasma concentrations of proteins in the two groups were compared using Mann Whitney U test. Level of significance was taken as 0.05. ND group was kept as reference in receiver operating characteristics (ROC) analysis and cut-off concentrations were computed for the three proteins.

Demographic details of the study participants are shown in Table 1. The study group consisted of 36 patients, 8 (23%) patients were in the ND group, while the remaining 28 (77%) patients were diagnosed with ACS by the consultant physician. This corresponds with that reported in literature where AD accounts for 60-70% cases of neurodegenerative dementias.[1] Comparison of ages in the two groups revealed no significant difference (p=0.70). Females dominated the study cohort (n=24, 67%), but gender distribution across both groups was statistically similar (p=0.21). Both groups were statistically homogenous in terms of their demographic, comorbid, and lifestyle profiles.

The mean plasma concentration of Aβ42 was slightly lower in the ACS group as compared to the ND group, but the difference was not statistically significant (p=0.78). Plasma NFL and P-Tau levels followed a similar trend, as they both were slightly raised in ACS patients than in patients with other neurodegenerative dementias, but the raise was not statistically significant (p=0.09 and 0.27, respectively). The mean plasma concentrations of the three proteins have been provided in Table 2. No correlation was observed between the mean plasma levels of proteins under investigation and cognitive status of the patient as reflected by the MMSE score.

ROC analysis revealed that Aβ42 (AUC 0.53; 95% CI 0.28-0.79; p=0.76), NFL (AUC 0.70; 95% CI 0.44-0.95; p=0.08) and P-Tau (AUC 0.64; 95% CI 0.44-0.84; p=0.25) did not reveal promising results for differentiating ACS from other neurodegenerative dementias.

Conclusion

Despite being promising in CSF, Aβ42, P-Tau, and NFL cannot differentiate between different neurodegenerative dementias when measured in plasma. Further larger studies employing more sensitive quantification techniques might suggest more encouraging outcome.

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Conflict of Interest: None.

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