Original Article

Evaluation of Plasma Amyloid Peptides $A\beta_{1-40}$ and $A\beta_{1-42}$ as Diagnostic Biomarker of Alzheimer's Disease, its Association with Different Grades of Clinical Severity and 18F-Fluorodeoxyglucose Positron Emission Tomography Z score in the Indian Population: A Case-Control Study

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Abstract

Background: We estimated plasma amyloid-peptides levels ($A\beta_{1-42}$ and $A\beta_{1-40}$) as diagnostic biomarker of Alzheimer's disease (AD) and evaluated its association with clinical severity and 18F-fluorodeoxyglucose positron emission tomography (18F-FDG PET) Z score of the different brain regions in the Indian population. **Patients and Methods:** A case-control study was conducted. Diagnostic and statistical manual-IV, Dubois, and NIA-AA criteria were used for the diagnosis of AD. The plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ concentration and 18F-FDG PET Z score were estimated for different brain regions. **Results:** Forty-seven cognitive impairment patients (AD = 29, mild cognitive impairment = 18) and 33 age-matched controls were enrolled. Plasma $A\beta_{1-42}$ level was significantly higher in the AD group compared to controls (P = 0.046) and a cut-off >5.7 ng/mL has a specificity of 96.9%, sensitivity of 27.6%, positive predictive value 88.9%, and negative predictive value 60.4% for differentiating AD patients from controls. Significant correlation was seen between $A\beta_{1-40}/A\beta_{1-42}$ ratio and 18F-FDG PET Z score in the bilateral-parietal, temporal, frontal-association area, and posterior-cingulate areas. **Conclusion:** As a diagnostic biomarker of AD, plasma $A\beta_{1-42}$ level showed good specificity but low sensitivity in the Indian population.

Keywords: *Alzheimer's disease, A^{β}_{1-40}, A^{β}_{1-42}, diagnostic biomarker, mild cognitive impairment, 18F-fluorodeoxyglucose positron emission tomography*

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in older patients (>60–65 years) and accounts for 4.9% of deaths among elderly people in the USA.[1] Global prevalence was about 25 million in 2010 which is anticipated to be doubled by 2030 because of increased life expectancy. AD is predicted to affect one in 85 people globally by 2050.[2,3] For populations above 65 years, the prevalence of AD in Asian countries varies from 6.44% in South India, 4.86% in Shanghai (China), and 3.92% in Sri Lanka.[4]

Despite such a significant effect of AD on the human race and decades of research devoted to finding a cure for this dementing illness, little has been achieved in terms of cure or reduction in the rate of its progression. This is partly related to an inherent problem in that the pathogenic process in AD starts years before clinical onset and drugs are likely to be most effective if started in preclinical phase or in the early stage of mild cognitive impairment (MCI) or AD. To know the effects of the intervention, one should be able to diagnose MCI with certainty and to determine which MCI patients are going to progress to Alzheimer's disease and also the rate of disease progression.

There are various imaging and laboratory biomarkers (decreased cerebrospinal fluid [CSF] $A\beta_{1-42}$, increased CSF tau, decreased 18F-Fluorodeoxyglucose [18F-FDG] uptake on cerebral cortices positron emission tomography [PET], amyloid PET imaging, and measures of brain atrophy on magnetic resonance [MR]), which can assist in the diagnosis of AD. However, these are either invasive (CSF), expensive, and not readily available.[5,6]

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Recently, significant attention had been given to the role of plasma biomarkers in the early diagnosis of AD as well as in its differentiation from other forms of dementia. The most commonly used plasma biomarkers include serum amyloid peptides. Because plasma sampling is simpler and less invasive than lumbar puncture, it is well suited to use in old age patients or when multiple measures are required, such as in clinical trials. However, the published data on plasma Aβ levels in AD is conflicting. One study indicated that low or decreasing plasma Aβ42 levels and Aβ42/Aβ40 ratio were related to cognitive decline during the follow-up.[7] A high variation in the prevalence and progress of AD among different geographic regions is noted, which can be an indicator of the difference in the pathogenesis of AD among different geographic regions (e.g., variation in the incidence of different AD causing mutations in different population, variation in cultural and dietary factors, and prevalence of different forms of inherited patterns). Again, amyloid-beta negative Alzheimer's disease is also a known entity. However, till now no study has evaluated the association between plasma amyloid-beta level, clinical dementia stages, and 18F-FDG PET Z score in the Indian population. This is the first study to evaluate the same in the Indian population. Thus, we planned the current study to determine the role of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels in the diagnosis of Alzheimer's disease.

Patients and Methods

The current cross-sectional study was conducted in the Department of Pharmacology and Neurology at apex care and teaching hospital in northern India. The study was started after getting approval from the institutional ethics committee (Histo/15/IEMEC/37) and written informed consent from all participants. The patients were recruited from 2014 to 2015. During this period, the patients with dementia were screened for inclusion in the study. The diagnosis of dementia was made based on the diagnostic and statistical manual (DSM)-IV criteria.[8] Patients with dementia were then evaluated in detail to determine the exact etiology of dementia. All these patients underwent detailed hematological (complete hemogram including erythrocyte sedimentation rate and c reactive protein) and biochemical (blood sugars, renal and liver function tests, thyroid function tests, serum electrolytes, calcium, and phosphorus) investigations. All these patients also underwent electrocardiogram and echocardiogram, serum venereal disease research laboratory, and testing for human immunodeficiency and hepatitis viruses. Neuroimaging (MR imaging) and 18F-FDG PET imaging were done in a few of these patients. Other investigations including chest X-ray, ultrasonography of abdomen, vasculitis profile, thyroid peroxidase antibodies, toxicology profile, serum Vitamin B12 levels, electroencephalography, and CSF analysis were performed wherever indicated. The patients who were diagnosed to be suffering from AD based on Dubos criteria[9] and MCI[10] were included in the study. The procedure for the selection of cases is depicted in Figure 1.

The inclusion and exclusion criteria for the study groups are given below:

Inclusion criteria for cases

1. Age >50 years
2. DSM IV criteria for dementia
3. Diagnosis of AD and MCI based on Dubois and NIA-AA criteria, respectively
4. Study informant available
5. Adequate vision and hearing for neuropsychological testing
6. Normal appropriate laboratory tests
7. Willing to give written informed consent and for follow-up.

Exclusion criteria

1. Neurological diseases were other than AD, MCI
2. Central nervous system infection or focal neurological lesions of clinical significance
3. Medical diseases or psychiatric disorders (like depression) could interfere with study participation.

Once included, all these participants were further subjected to detailed clinical history and examinations as well as neuropsychological battery was administered by the trained neuropsychologist.

Various neuropsychological tests which were conducted on all the patients are given below:

1. Mini-mental status examination (MMSE)[11]
2. Postgraduate institute memory scale[12]
3. Alzheimer's disease assessment scale– cognitive (COG)[13,14]
4. Verbal fluency[15,16]
   a. Controlled oral word test (phonic)
   b. Animal naming test (categorical)

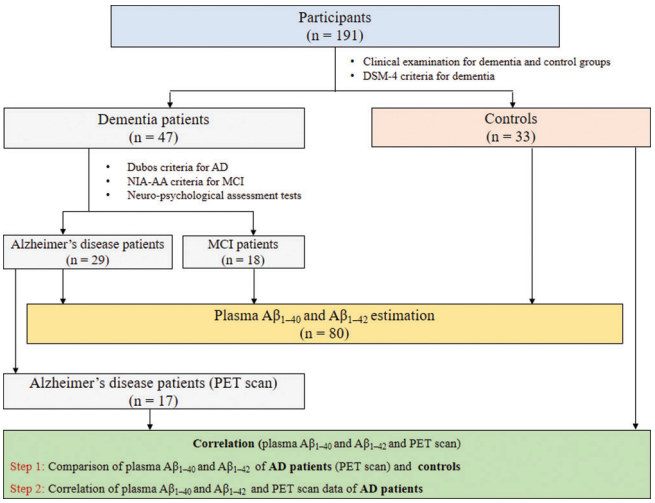

Figure 1: Study flow chart and patient enrollment

5. Alzheimer's disease cooperative study – Activity of daily living inventory[17]
6. Quality of life–AD[18]
7. Clinical dementia rating scale.[19,20]

AD patients were further categorized according to MMSE score into mild,[21-27] moderate,[11-20] and severe (≤10).[21]

Plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ estimation

For plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels estimation, 3 ml of venous blood was drawn from all participants at the time of enrollment. Plasma was separated according to standard procedure and stored until further use. Plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels were detected by enzyme-linked immunosorbent assay kits manufactured by QAYEE-BIO Company. Plasma amyloid peptides were compared with age- and sex-matched healthy controls. The control group consisted of the most patient spouse, attendants or close relatives of cases as well as institutional staff persons.

18F-fluorodeoxyglucose positron emission tomography scan

Regional images of the brain were acquired 45–60 min after the IV injection of 150–180 MBq of 18F-FDG using a standard protocol. Normalized metabolism score (Z score) in different brain areas was estimated using automated software (cortex ID V.1.04, GE Healthcare, Wisconsin, USA). In cortex ID v. 1.04, the patient's data are subtracted from age-matched normal population data and a difference of more than 2 standard deviation (SD) (Z score >2) in a cortical area denotes significant hypometabolism as compared to the healthy population.

Statistical analysis

Statistical analysis was performed by the Statistical Package for the Social Sciences version 22 (IBM corporation, Newyork, version 22). The continuous data were analyzed by independent *t*-test or one-way analysis of variance with Bonferroni *post hoc* analysis. Dichotomized data were analyzed by Chi-square test or Fisher exact test whichever was applicable. Receiver operative curve analysis of plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ levels was done in MedCalc software to determine the sensitivity and specificity. The two-tailed $P < 0.05$ with 95% confidence interval was considered statistically significant. Z score from PET scan data was calculated and correlation study was performed between plasma amyloid peptides (individual values and ratios) with the Z score for the AD and MCI groups.

Results

The current study included 47 cases of cognitive impairment (AD-29; MCI-18) and 33 controls after the screening of 191 participants. The mean (±SD) age was 69.8 (±9.9) years in the AD group, 68.7 (±7.07) years in the MCI group, and 60.9 (±9.05) years in the control group. Men constituted 14 (48.3%) of participants in the AD group, 15 (83.3%) in the patients of the MCI group, and 23 (70%) in the control group [Table 1]. Among AD patients, 7 (24.1%) patients had mild, 15 (15.8%) had moderate, and 7 (24.1%) patients had severe dementia. The mean age was significantly lower in controls to AD and MCI patients. Regarding associated medical diseases, hypertension was seen in 10 patients in the AD group with a mean duration of 10 years, 11 patients in the MCI group with a mean duration of 14.7 years, and seven patients in the control group with a mean duration of 8.1 years. Seven patients in the AD group had diabetes mellitus with a mean duration of 8.1 years, 5 in the MCI group had diabetes mellitus with a mean duration of 15 years, and 4 in the control group had diabetes with a mean duration of 6.75 years. These, as well as other demographic data, are reported in Table 1. All the patients underwent detailed laboratory investigations as mentioned in the patients and methods section. In comparison, all the investigations were comparable between AD and MCI patients.

Neuropsychological assessment tests of study groups

In the current study, all the patients and controls underwent detailed neuropsychological assessment [Table 2]. AD patients were further subdivided into three groups on the basis of MMSE scores; a) Mild (MMSE: 26–21) $n = 7$; b) moderate (MMSE 20–11) $n = 15$; and c) severe (MMSE ≤10) $n = 7$. In the AD group, the mean MMSE score in mean ± SD was 15.1 ± 5.67 and for MCI patients was 25.5 ± 2.68 [Table 2].

Plasma biomarkers

The mean plasma value of $A\beta_{1-42}$ was 2.3 ± 1.56 ng/mL in AD patients, 1.6 ± 0.35 ng/mL in the MCI patients, and 1.65 ± 0.62 ng/mL in the control group. When compared plasma $A\beta_{1-42}$ was found significantly high in AD patients as compared to the control group [Table 3]. The plasma amyloid peptides estimation was evaluated in all 80 participants. The plasma value of $A\beta_{1-40}$ in mean ± SD was 1.51 ± 1.75 ng/mL in the AD group, 1.26 ± 1.54 ng/mL in the MCI group, and 0.98 ± 0.66 ng/Ml in the control group. Although a trend of increasing of $A\beta_{1-40}$ level was seen in the AD and MCI groups compared to the control, on statistical analysis, the difference was found statistically insignificant [Table 3]. In the current study, we also measured the ratio of plasma levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ such as $A\beta_{1-40}/A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ and compared the values among all the groups. We did not find any statistically significant difference for any of these measures among all three study groups [Table 3]. We further analyzed the sensitivity and specificity of plasma $A\beta_{1-42}$ levels for differentiating AD patients from controls. It was found that plasma $A\beta_{1-42}$ >5.7 ng/mL has a specificity of 96.9% for differentiating AD patients from controls, though the sensitivity was only 27.6%. Positive and negative predictive values of $A\beta_{1-42}$ >5.7 ng/mL for the diagnosis of AD were 88.9% and 60.4% [Supplementary Table 1].

Table 1: Demographic profile of Alzheimer disease, mild cognitive impairment and control groups

Parameter	AD (*n*=29)	MCI (*n*=18)	Controls (*n*=33)	*P**	*P*¶	*P*§
Age in years (mean±SD)	69.8±9.90	68.7±7.07	60.9±9.05	1.000	0.001	0.013
Men, *n* (%)	14 (48.3)	15 (83.3)	23 (69.7)	0.029	0.120	0.335
Mean duration of illness in years (mean±SD)	3.5±2.39	3.1±2.30	-	0.536	-	-
Education status						
Illiterate, *n* (%)	7 (24.1)	1 (5.6)	1 (3)	0.113	0.023	0.811
Primary school (up to 5th standard), *n* (%)	4 (13.8)	2 (11.1)	7 (21.2)			
Middle school (6th-9th standard), *n* (%)	10 (34.5)	4 (22.2)	7 (21.2)			
High school and higher education (≥10th standard), *n* (%)	8 (27.6)	11 (61.1)	18 (54.5)			
Hypertension, *n* (%)	11 (37.9)	12 (66.7)	7 (21.2)	0.055	0.147	0.001
Diabetes mellitus, *n* (%)	7 (24.1)	6 (33.3)	4 (12.1)	0.520	0.319	0.136
Coronary artery disease, *n* (%)	2 (6.9)	2 (11.1)	1 (3)	0.631	0.595	0.281
Alcohol, *n* (%)	4 (13.8)	4 (22.2)	5 (15.1)	0.691	1.000	0.702
Smoking, *n* (%)	3 (10.3)	0 (0)	2 (6.0)	0.275	0.657	0.534

**P*-value between AD and MCI groups, ¶*P*-value between control and AD group, §*P*-value between control and MCI group. AD: Alzheimer disease, MCI: Mild cognitive impairment, SD: Standard deviation

Table 2: Neuropsychological assessment Alzheimer disease and mild cognitive impairment groups

Neuro-psychological tests	AD (*n*=29)		MCI (*n*=18)		*P**
	Mean±SD	*n*	Mean±SD	*n*	
MMSE	15.10±5.68	29	25.56±2.68	18	0.000
PGIMS total score	39.00±15.68	20	58.13±16.06	15	0.001
Remote memory	3.60±1.85	20	4.8±1.70	15	0.058
Recent memory	3.00±1.81	20	3.93±1.39	15	0.105
Mental balance	3.10±2.79	20	6.53±2.44	15	0.001
Attention and concentration	6.20±2.09	20	8.133±2.29	15	0.014
Delayed recall	4.20±2.78	20	6.33±2.19	15	0.020
Immediate recall	5.21±2.57	19	6.00±2.98	15	0.413
Verbal retention for similar pair	3.05±1.71	19	3.73±1.33	15	0.216
Verbal retention for dissimilar pair	5.52±3.01	19	6.40±4.08	15	0.478
Visual retention	1.10±1.82	19	5.33±3.87	15	0.000
Recognition	5.00±2.43	19	6.93±2.94	15	0.044
Verbal fluency test					
Animal naming test	5.72±2.80	18	9.07±3.63	14	0.006
COWA test	3.39±2.35	18	6.04±2.30	14	0.003
Quality of Life					
Patient	33.89±6.71	18	34.50±5.98	14	0.791
Care giver	30.89±6.22	18	33.29±4.60	14	0.237
ADAS-score	16.12±4.69	18	9.37±4.86	13	0.001
ADCS-ADL score	50.29±11.52	17	62.23±9.26	13	0.005
Clinical dementia rating scale	0.97±0.42	19	0.607±0.21	14	0.006

**P*-value between AD and MCI groups. Statistical analysis has done by independent *t*-test and $P<0.05$ has considered as significant. AD: Alzheimer disease, MCI: Mild cognitive impairment, SD: Standard deviation, COWA Test: Controlled oral word association test, ADAS-score: AD assessment scale-score, ADCS-ADL score: AD cooperative study-activities of daily living score, MMSE: Mini-mental status examination, PGIMS: Postgraduate institute memory scale

In the current study, AD patients were further subdivided into three subgroups on the basis of MMSE score (Mild = 7, moderate = 15, and severe = 7). Plasma value of $A\beta_{1-40}$ was 1.04±0.41 ng/mL in the mild AD group, 1.63±1.83 ng/mL in a moderate AD group, and 1.71±2.43 ng/mL in the severe AD group. The mean plasma value of $A\beta_{1-42}$ was 2.16±0.88 ng/mL in the mild AD group, 2.27±1.78 ng/mL in a moderate AD group, and 2.5.

1±1.78 ng/mL in the severe AD group. We did not find any significant difference in both plasma amyloid peptides in AD subgroups. However, we identified an incremental trend in both amyloid peptides as severity increases in AD patients [Supplementary Table 2].

Correlation analysis with plasma amyloid peptides and 18F-fluorodeoxyglucose positron emission tomography Z score

Twenty-nine patients who had undergone 18F-FDG PET scan during the workup of cognitive impairment were identified. Out of these, two had mild, 11 had moderate, and 4 had severe AD, while in MCI group 12 were gone through PET scan overall, in AD patients hypometabolism was observed in bilateral parietal and temporal lobes including precuneus and cingulate and mildly reduced in the frontal cortex while in the MCI group some patients showed mildly reduced in the bilateral temporoparietal cortex and cingulate gyrus and some not shown any definitive evidence of hypo/hypermetabolism in the entire brain. Mean plasma amyloid peptides of all 17 AD patients and were compared to the control group (n = 33) and significant difference in $A\beta_{1-42}$ was found (P = 0.03) [Supplementary Table 3]. Similarly, mean plasma amyloid peptides of 12 MCI patients were compared with 33 controls [Supplementary Table 4].

A significant correlation was found between $A\beta_{1-40}$ and Z score in the left temporal association area and between $A\beta_{1-40}/A\beta_{1-42}$ ratio and Z score for bilateral parietal association areas, median parietal areas, temporal association areas, frontal association areas, posterior cingulate areas, right median frontal area, and average cerebral and global score [Table 4]. As most of the MCI patients belonged amnestic mild cognitive impairment category so we clubbed all 12 MCI patients with 17 AD patients and correlation was performed as discussed above. We found a significant correlation of $A\beta_{1-40}$ with PET Z score in the left parietal association area, left temporal association area, left posterior cingulate area, and left median parietal area [Supplementary Table 5].

Discussion

The treatment of AD continues to be far from satisfactory. This is partially related to the fact that by the time AD is diagnosed clinically, the pathological process is already in the advanced stage. Furthermore, to test the efficacy of the new intervention, it is imperative that it is applied at a stage when the pathological process has just begun. In other words, to test the efficacy of a new intervention, one needs to diagnose presymptomatic AD with reasonable certainty. Current investigational modalities (radiological imaging, nuclear imaging, and various CSF biomarkers [Aβ peptides,

Table 3: Comparison of plasma amyloid peptides among all three groups

Parameter	Mean±SD			*P**	*P*¶	*P*$
	AD (*n*=29)	MCI (*n*=18)	Control (*n*=33)			
Plasma values of Aβ1-40 (ng/ml)	1.51±1.75	1.26±1.58	0.98±0.66	1.000	0.31	1.000
Plasma values of Aβ1-42 (ng/ml)	2.30±1.57	1.61±0.35	1.65±0.62	0.087	0.046	1.000
Ratio of plasma Aβ1-40/Aβ1-42	0.61±0.26	0.80±0.99	0.59±0.19	0.645	1.000	0.480
Ratio of plasma Aβ1-42/Aβ1-40	1.92±0.85	1.87±0.81	1.89±0.74	1.000	1.000	1.000

*P-value between AD and MCI groups, ¶P-value between control and AD group, $$P$-value between control and MCI group. The P value is significant between AD and control groups. $A\beta_{1-40}$: Amyloid $beta_{1-40}$, $A\beta_{1-42}$: Amyloid $beta_{1-42}$, AD: Alzheimer disease, MCI: Mild cognitive impairment, SD: Standard deviation

Table 4: Correlation of amyloid peptides and fluorodeoxyglucose positron emission tomography *Z* scores

Brain areas	Plasma $A\beta_{1-40}$ (*P*)		Plasma $A\beta_{1-42}$ (*P*)		Plasma $A\beta_{1-40}/A\beta_{1-42}$ (*P*)		Plasma $A\beta_{1-42}/A\beta_{1-40}$ (*P*)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Right parietal association area	0.313	0.221	0.007	0.978	0.640	0.006	−0.620	0.008
Left parietal association area	0.444	0.074	0.136	0.602	0.730	0.001	−0.679	0.003
Right temporal association area	0.325	0.203	0.035	0.895	0.687	0.002	−0.714	0.001
Left temporal association area	0.520	0.032	0.282	0.272	0.728	0.001	−0.725	0.001
Right frontal association area	0.291	0.257	0.005	0.984	0.578	0.015	−0.576	0.016
Left front association area	0.339	0.183	0.119	0.648	0.501	0.041	−0.391	0.121
Right posterior cingulate area	0.319	0.217	0.040	0.878	0.550	0.022	−0.488	0.047
Left posterior cingulate area	0.453	0.068	0.197	0.449	0.589	0.013	−0.382	0.130
Right anterior cingulate area	0.292	0.256	0.113	0.666	0.371	0.142	−0.257	0.319
Left anterior cingulate area	0.259	0.315	0.129	0.662	0.281	0.274	−0.102	0.696
Right median frontal area	0.087	0.740	−0.212	0.414	0.498	0.042	−0.588	0.013
Left median frontal area	0.161	0.537	−0.048	0.855	0.401	0.111	−0.346	0.173
Right median parietal area	0.256	0.322	−0.032	0.903	0.536	0.027	−0.541	0.025
Left median parietal area	0.433	0.082	0.116	0.656	0.763	<0.001	−0.694	0.002
Average cerebral score	0.326	0.201	0.021	0.935	0.669	0.003	−0.662	0.004
Average global score	0.295	0.251	−0.016	0.951	0.632	0.006	−0.656	0.004

Correlation study was done by spearman correlation coefficient. $A\beta_{1-40}$: Amyloid $beta_{1-40}$, $A\beta_{1-42}$: Amyloid $beta_{1-42}$

p-tau, t-tau, and mmp-9]) which are being used for this purpose, are either too costly or invasive and difficult to applied widely mostly in peripheral hospitals. Thus, in the present study, we tried to assess the role of two amyloid peptides of plasma in the diagnosis of AD. Currently, the CSF level of these biomarkers has been included in the research diagnostic criteria offered by the National Institute on Aging and Alzheimer's Association, and the international working group. Recently, a "Biological definition" of AD has been suggested with the A/T/N classification which used biomarker of β-amyloid pathology (A), tau (T), and neurodegenerative markers (N).[22]

The published data on the role of plasma amyloid peptides level in AD are conflicting. Previous studies suggested that during early-stage AD, there is a gradual rise in plasma levels of amyloid peptides but as the disease process progress, their level gradually decreases and finally becomes normal so much so that once AD is clinical evidence, plasma levels of $A\beta_{1-42}$ levels are comparable to healthy controls.[23] A study concluded that decreasing levels of $A\beta_{1-42}$ in serial measurements may be associated more with cognitive decline than the plasma amyloid-beta peptides and indicate the development of AD[7] while numerous large studies have consistently reported that a lower $A\beta_{1-42}/A\beta_{1-40}$ ratio in plasma is associated with a higher risk of dementia.[24]

In the present study, plasma $A\beta_{1-42}$ levels were found significantly higher in AD patients as compared to controls. However, other measures such as plasma $A\beta_{1-40}$ and $A\beta_{1-40}/A\beta_{1-42}$ ratio did not show any significant differences between the three groups. Plasma $A\beta_{1-42}$ levels of 5.7 ng/mL had a sensitivity of 27.6% and specificity of 97% in differentiating AD from control with insignificant *P* value which could be due to large variability in patients and control group's age and study with larger sample size is recommended.

18F-FDG PET is a common molecular imaging technique which used as a biomarker. Basically, it measures the intracellular glucose metabolism and used in various applications in neuroscience including in the study of dementia where it has been used from the past three decades. 18F-FDG PET has become the most sensitive and specific imaging modality for the diagnosis of AD and nowadays it is considered an imaging biomarker for AD before the onset of dementia and in clinical trials.[25] The quantitative analysis of brain hypometabolism shown in 18F-FDG is done by Z score. A positive Z score >2 represents a significant reduction in metabolic activity comparable to the normal reference data. AD patients show hypometabolism in bilateral temporal lobes (middle and inferior temporal gyri), bilateral limbic system (parahippocampal gyrus and posterior cingulate gyrus), bilateral parietal lobe, and bilateral lateral parietal cortex.[26] Womack *et al*., have found temporoparietal hypometabolism was more sensitive (sensitivity, 93.6% $P = 0.003$), but posterior cingulate hypometabolism was more specific (specificity, 71.4% $P = 0.01$) for diagnosing AD.[27,28]

In the present study, we found a moderate positive correlation of amyloid peptide ratio ($A\beta_{1-40}/A\beta_{1-42}$) to a Z score of PET in commonly affected brain areas indicating higher $A\beta_{1-40}/A\beta_{1-42}$ ratios in patients with hypometabolism on PET scan. This promising finding needs to be evaluated in larger studies.

Our studies have several limitations. The main limitation is the smaller sample size. Other is the control groups were not fully matched to cases with respect to age and gender distribution. Furthermore, we could not do serial measurements of plasma amyloid peptides in patients with dementia.

Conclusion

The results of our study reveal relatively low sensitivity of plasma amyloid-beta peptides for differentiating AD from healthy controls. Future studies involving larger sample size and longitudinal measurement of plasma levels of various amyloid peptides will help in better characterization of the role of various biomarkers in differentiating AD from healthy controls. The identification of AD disease in the early phase is still a major challenge, so the combined plasma amyloid and FDG PET approach might be helpful in the early detection of pathological changes in older age individuals.

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Conflicts of interest

There are no conflicts of interest.

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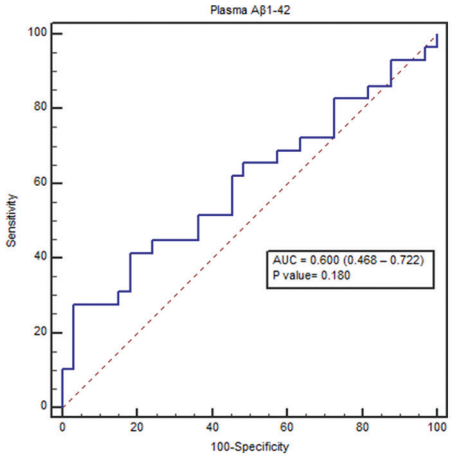
Supplementary Table 1: Sensitivity/specificity/positive and negative predictive value of plasma amyloid beta$_{1-42}$ in differentiating Alzheimer disease patients from controls

Biomarkers	Value (ng/ml)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	*P*
Plasma Aβ$_{1-42}$	>5.72	27.59 (12.7-47.2)	96.97 (84.2-99.9)	88.9 (51.8-99.7)	60.4 (46.0-73.5)	0.18
Youden Index	0.2456	AUC=0.600 (0.468-0.722)				

PPV: Positive predictive value, NPV: Negative predictive value, CI: Confidence interval, AUC: Area under the curve, Aβ$_{1-42}$: Amyloid beta1-42



Supplementary Table 2: Levels of plasma amyloid peptides in Alzheimer disease subgroups as defined by mini-mental status examination

Amyloid peptides	AD subgroups based on MMSE score			*P**
	Mild AD (MMSE: 26-21)	Moderate AD (MMSE: 20-11)	Severe AD (MMSE: ≤10)	
AD patient, *n* (%)	7 (24)	15 (52)	7 (24)	
Plasma values of Aβ$_{1-40}$ (ng/ml), mean±SD	1.04±0.41	1.64±1.83	1.72±2.43	0.725
Plasma values of Aβ$_{1-42}$ (ng/ml), mean±SD	2.16±0.88	2.27±1.78	2.50±1.78	0.919
Ratio of plasma Aβ$_{1-40}$/Aβ$_{1-42}$, mean±SD	0.50±0.14	0.67±0.24	0.61±0.35	0.370
Ratio of plasma Aβ$_{1-42}$/Aβ$_{1-40}$, mean±SD	2.14±0.62	1.66±0.53	2.25±1.42	0.244

*Statistical analysis has done by "one-way ANOVA." MMSE: Mini-mental status examination, AD: Alzheimer disease, Aβ$_{1-40}$: Amyloid beta$_{1-40}$, Aβ$_{1-42}$: Amyloid beta$_{1-42}$, SD: Standard deviation

Supplementary Table 3: Comparative analysis of amyloid beta$_{1-40}$ and amyloid beta$_{1-42}$ of 17 Alzheimer disease patients who have undergone positron emission tomography scan with 33 controls

Parameter	Mean±SD		*P**
	AD (*n*=17)	Control (*n*=33)	
Plasma values of Aβ$_{1-40}$ (ng/ml)	1.91±2.20	0.98±0.66	0.030
Plasma values of Aβ$_{1-42}$ (ng/ml)	2.60±1.87	1.65±0.62	0.010
Ratio of plasma Aβ$_{1-40}$/Aβ$_{1-42}$	0.66±0.30	0.59±0.19	0.371
Ratio of plasma Aβ$_{1-42}$/Aβ$_{1-40}$	1.85±0.86	1.89±0.74	0.876

**P*-value between AD and control groups. SD: Standard deviation, AD: Alzheimer disease, Aβ$_{1-40}$: Amyloid beta$_{1-40}$, Aβ$_{1-42}$: Amyloid beta$_{1-42}$

Supplementary Table 4: Comparative analysis of amyloid beta$_{1-40}$ and amyloid beta$_{1-42}$ of 12 mild cognitive impairment patients who have undergone positron emission tomography scan

Parameter	Mean±SD		*P**
	MCI (*n*=12)	Control (*n*=33)	
Plasma values of Aβ$_{1-40}$ (ng/ml)	0.96±0.42	0.98±0.6593	0.885
Plasma values of Aβ$_{1-42}$ (ng/ml)	1.63±0.28	1.65±0.62	0.916
Ratio of plasma Aβ$_{1-40}$/Aβ$_{1-42}$	0.59±0.23	0.59±0.19	0.992
Ratio of plasma Aβ$_{1-42}$/Aβ$_{1-40}$	1.92±0.71	1.89±0.74	0.903

MCI: Mild cognitive impairment, SD: Standard deviation, Aβ$_{1-40}$: Amyloid beta$_{1-40}$, Aβ$_{1-42}$: Amyloid beta$_{1-42}$

Supplementary Table 5: Correlation of plasma level of amyloid peptides and grading of normalized fluorodeoxyglucose positron emission tomography scores in all 29 dementia patients (Alzheimer disease 17 and mild cognitive impairment 12)

Brain areas	Plasma Aβ$_{1-40}$ (*P*)		Plasma Aβ$_{1-42}$ (*P*)		Plasma Aβ1-40/Aβ$_{1-42}$ (*P*)		Plasma Aβ1-42/Aβ$_{1-40}$ (*P*)	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Right parietal association area	0.347	0.065	0.155	0.423	0.455	0.013	-0.388	0.038
Left parietal association area	0.422	0.022	0.250	0.1	0.494	0.006	-0.402	0.031
Right temporal association area	0.348	0.064	0.168	0.383	0.487	0.007	-0.444	0.016
Left temporal association area	0.450	0.014	0.327	0.084	0.460	0.012	-0.372	0.047
Right frontal association area	0.309	0.102	0.119	0.540	0.448	0.015	-0.387	0.038
Left front association area	0.350	0.063	0.230	0.231	0.378	0.043	-0.248	0.195
Right posterior cingulate area	0.321	0.089	0.128	0.507	0.452	0.014	-0.366	0.051
Left posterior cingulate area	0.407	0.028	0.265	0.164	0.454	0.013	-0.293	0.122
Right anterior cingulate area	0.255	0.182	0.144	0.457	0.227	0.146	-0.177	0.359
Left anterior cingulate area	0.215	0.262	0.141	0.465	0.200	0.298	-0.055	0.777
Right median frontal area	0.128	0.507	-0.045	0.818	0.321	0.089	-0.313	0.098
Left median frontal area	0.183	0.342	0.094	0.629	0.236	0.217	-0.144	0.456
Right median parietal area	0.299	0.115	0.105	0.589	0.421	0.023	-0.376	0.044
Left median parietal area	0.420	0.023	0.216	0.261	0.551	0.002	-0.446	0.015
Average cerebral score	0.316	0.095	0.152	0.430	0.431	0.020	-0.360	0.055
Average global score	0.285	0.134	0.135	0.486	0.379	0.042	-0.325	0.085

Correlation study was done by spearman correlation coefficient. Aβ$_{1-40}$: Amyloid beta$_{1-40}$, Aβ$_{1-42}$: Amyloid beta$_{1-42}$