RESEARCH ARTICLE

Lower Plasma β-Amyloid 1-42 Levels in Amnestic Mild Cognitive Impairment Compared to Healthy Individuals

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Abstract

BACKGROUND: Amnestic mild cognitive impairment (aMCI) is strongly associated with an increased risk of progression to Alzheimer's disease (AD). In AD, cerebrospinal fluid (CSF) β-Amyloid 1-42 levels are known to decrease, a pattern which is also observed in aMCI. While in AD, apolipoprotein E (ApoE) ε4 allele is known to be a genetic risk factor, the role of ApoE ε4 allele in modulating plasma β-Amyloid 1-42 levels in aMCI remains unclear. Therefore, this study was performed to evaluate plasma β-Amyloid 1-42 levels in aMCI patients compared to cognitively healthy individuals and investigate its association with ApoE ε4 allele.

METHODS: A cross-sectional study involving 57 aMCI and 54 cognitively healthy control (HC) subjects was performed. Blood samples were taken from subjects from both groups for measurement of the plasma β-Amyloid 1-42 and ApoE ε4 allele. The plasma levels of β-Amyloid 1-42 were measured using an enzyme-linked immunosorbent assay (ELISA) methods, while the ApoE ε4 allele genotyping was conducted using polymerase chain reaction (PCR) techniques.

RESULTS: Plasma β-Amyloid 1-42 in individuals with aMCI (23.9 pg/mL) was significantly lower than that in HC (25.3 pg/mL) with cut-off value of 24.6 pg/mL (AUC: 70.8%; 95% CI: 61.1–80.5%; $p<0.001$) sensitivity of 64.8%, and specificity of 71.9%. There was no significant association between plasma β-Amyloid 1-42 and the ApoE ε4 allele. However, plasma β-Amyloid 1-42 in ε4 carriers were lower than in ε4 non-carriers.

CONCLUSION: Lower plasma β-Amyloid 1-42 levels were observed in aMCI patients compared to cognitively healthy individuals, suggesting its potential as a biomarker for identifying aMCI.

KEYWORDS: blood biomarkers, amyloid beta peptides, amnestic mild cognitive impairment (aMCI)

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Introduction

Mild cognitive impairment (MCI) is characterized as an intermediate phase between normal aging and dementia.(1) In 2004, the Key Symposium in Sweden released consensus criteria for MCI, broadening the concept to include more than just the memory domain.(2,3) MCI includes deficits in both memory and non-memory cognitive functions.(2,4) Patients with MCI are categorized into two subtypes: amnestic MCI (aMCI) and non-amnestic MCI (naMCI).(2,3) This classification can help predicting the type of

dementia MCI patients that are likely to develop.(1) However, due to the clinical variability and diversity of the underlying causes within the MCI group, there is currently no established treatment for MCI.(3) Several longitudinal studies have linked aMCI to a high risk of advancing to Alzheimer's disease (AD) dementia.(1,5,6) MCI offers important insights into the risk of developing dementia, presenting an opportunity for intervention to prevent the progression to dementia. However, not all aMCI cases will progress to AD dementia, and some may even revert to normal cognitive function.(1,5) It is plausible that cognitive deficits may result from various medical and psychiatric conditions rather than AD. Therefore, relying solely on clinical criteria for MCI may not be enough to identify aMCI patients who will progress to AD dementia.(1,5)

The National Institute on Aging-Alzheimer's Association (NIA-AA) working group has suggested updated criteria to better define MCI due to AD, incorporating pathological biomarkers that align with AD. Furthermore, diagnostic criteria for AD recommend the use of cerebrospinal fluid (CSF) biomarkers to assess the etiology and prognosis in individuals with MCI.(1,5,7,8) These updated criteria are anticipated to enhance the accuracy of MCI diagnosis. However, the use of amyloid CSF biomarkers, in particular, is not easily accessible in most primary healthcare settings, as it is more invasive and costly, limiting its practical use.(1,5)

Therefore, it is necessary to evaluate the potential of plasma biomarkers, which is not invasive, in distinguishing aMCI patients from healthy elderly subjects. Recent developments in blood-based assays have shown that circulated β-Amyloid 1-42 levels can be reliably measured and are linked to β-Amyloid 1-42 levels in the brain. It was indicated that plasma levels of β-Amyloid 1-42 and other target proteins are altered in patients with AD and those in the prodromal stage of AD, such as MCI.(1,5) Several studies have indicated that plasma β-Amyloid 1-42 levels is reduced levels of these plasma biomarkers have been observed in patients with AD and MCI, and they can help predict the presence of AD and MCI, as well as the progression from normal cognition to impairment.(9-14) Reduced plasma β-Amyloid 1-42 has also been linked to poorer cognitive function.(9,15-21)

Other than β-Amyloid 1-42, apolipoprotein E (ApoE) ε4 allele is known to be a genetic risk factor in AD that might be able to distinguish between aMCI and cognitively healthy individuals. Another factor that may influence the relationship between plasma β-Amyloid 1-42 and other outcomes is genetic variation, particularly ApoE.(22) However, the role of ApoE ε4 allele in modulating plasma β-Amyloid 1-42 levels has not been fully explored. This gap underscores the importance of further investigation into plasma biomarkers and genetic factors in the context of MCI. Additionally, since carriers of the ApoE ε4 genotype begin accumulating β-Amyloid 1-42 at an earlier age than non-carriers (6), indicating that β-Amyloid 1-42 accumulation is a primary cause of "sporadic AD". Therefore, this study was performed to evaluate plasma β-Amyloid 1-42 levels in aMCI patients compared to cognitively healthy individuals and investigate its association with ApoE ε4 allele.

Methods

Study Design and Participants Recruitment

A cross-sectional and was conducted at the National Brain Center Hospital, Jakarta, Indonesia from February to June 2024. Patients with a significant complaint of their cognition as well as a significant deficit in memory without significant decline in daily activity were classified as aMCI group. Participants without any cognitive complaint or deficit were classified as the healthy control (HC) group. The inclusion criteria for subjects were: elderly aged >50 years old; had adequate vision, hearing, and physical conditions to participate in the evaluations; had at least a high school education (for the global cognitive assessment); clinically stable, with or without pharmacological treatment prior to the study. Meanwhile the exclusion criteria were: had any neurological disorder that may impact cognition; had psychiatric conditions affecting cognitive function; had alcohol abuse or use of substances that could interfere with cognitive assessment; and naMCI patients.

All subjects underwent a cognitive and clinical assessment. Blood samples were also taken from subjects for the measurement of plasma levels of β-Amyloid 1-42, and ApoE genotyping. All participants provided informed consent to take part in the study. The study protocol was approved by the Institutional Review Board of the National Brain Center Hospital (Approval No. DP.04.03/D. XXIII.9/014/2024).

Clinical and Cognitive Assessments

Clinical assessments were performed following the guidelines set by the NIA-AA working group. A cognitive interview, including an evaluation of global cognitive function, was conducted using the Indonesian version of the Montreal Cognitive Assessment (MoCA-Ina). The maximum score was 30, with a score of ≥26 considered

normal. The MoCA cut-off score of 26 was based on various international studies.(23) The aMCI was defined as a score below 26 (adjusted for age and education), which may indicate issues with memory or recall. Demographic data, such as age, sex, education level, and risk factors, were also collected.

Measurement of Plasma β-Amyloid Level

To avoid epitope masking that might hinder the recognition of up to 50% of these amyloid peptides in immunoassays, subjects were asked to fast before the blood was withdrawn for the examination. Fasting blood samples were used to measure the plasma levels of β-Amyloid 1-42 using an enzyme-linked immunosorbent assay (ELISA) kit (Catalog No. EQ 6521-9601; EUROIMMUN, Lübeck, Germany). The microplate wells was coated with capture antibodies specific for β-Amyloid 1-42, and incubated with plasma samples. After incubated, the wells were washed to remove unbound components, and enzyme-conjugated detection antibodies was added. A substrate solution was also added to produce a colorimetric reaction proportional to the β-Amyloid 1-42 concentration. After the reaction stopped, the optical density (OD) was measured at the specified wavelength using the RT-6500 Microplate Reader (Rayto, Shenzhen, China). All the procedure adhered strictly to the manufacturer's protocol. Calibration curves and quality controls were used to quantify the β-Amyloid 1-42 levels in plasma samples accurately.

ApoE ε4 Allele Genotyping

Genomic DNA was extracted from peripheral blood samples and analyzed for ApoE genotyping using a real-time polymerase chain reaction (PCR) allelic discrimination assay, which was performed with TaqMan™ SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, MA, USA). Two single nucleotide polymorphisms (SNPs), rs429358 (g.7903T>C) and rs7412 (g.8041C>T) that define the three ApoE alleles (ε2, ε3, and ε4) were targeted. Sequence-specific probes were labeled with FAM for the wild-type allele and VIC for the mutant allele. These probes were hybridized to the target SNP regions, and fluorescence was detected during the PCR amplification process using a real-time PCR using the Roche LightCycler LC 96 system (Roche, Basel, Switzerland) to determine ApoE alleles. The differential binding of the probes to their respective SNPs enables precise identification of each allele, allowing for the determination of the ApoE diplotype, and thw ApoE isoforms (ApoE-ε2, ApoE-ε3, or ApoE-ε4) was accurately distinguished. In this study, only ApoE-ε4 was measured.

Statistical Analysis

Participant characteristics were described using descriptive statistics. Continuous variables were presented as means±standard deviations (SD), and group differences were analyzed using Student's t-test. Categorical variables were presented as frequencies and proportions, with comparisons made using the Chi-square test. Stratified analysis was performed to examine which subgroups (age, sex, education, and ApoE allele) were associated with plasma β-Amyloid 1-42 levels, with a significance threshold of $p<0.05$. All analyses were conducted using R Statistical Software v4.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Participants' Characteristics

A total of 111 participants were recruited for the study, 57 (51.4%) of them were aMCI patients and 54 (48.6%) were HC. In general, there were no statistically significant differences in the characteristics between the aMCI group and the HC group. Only plasma β-Amyloid 1-42 level was statistically significant with aMCI group having lower levels than HC group (Figure 1, Table 1).

Cut-off Points for aMCI Plasma Biomarkers

Due to more participants in aMCI group than HC group, aMCI was used as the reference group instead of HC in the ROC analysis. Figure 2 showed the receiver operating characteristics (ROC) curve of the biomarkers analyzed with area under the curve (AUC) of 0.708 (95% CI: 0.61–0.805). The optimal cut-off point using Youden index was determined as 24.6 pg/mL with sensitivity of 64.8%, specificity of 71.9%, positive predictive value (PPV) of

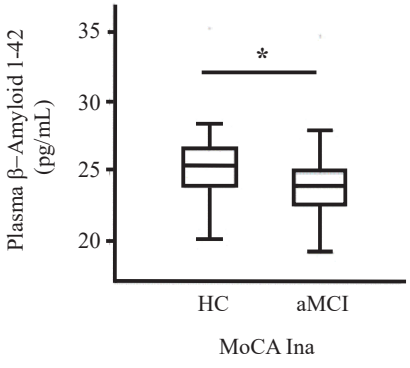

Figure 1. Distribution of plasma β-Amyloid 1-42 levels in HC and aMCI groups. Group differences was analyzed using Student's t-test, *$p<0.05$ is considered significant.

Table 1. Subjects' characteristics.

Variable	HC	aMCI	*p*- value
Number of subjects, n (%)	54 (48.6)	57 (51.4)	
Age (year), mean±SD	65.4±5.8	65.1±9.5	0.823
Age groups, n (%)			
<60	8 (14.8)	13 (22.8)	0.556
60-69	33 (61.1)	31 (54.4)	
≥70	13 (24.1)	13 (22.8)	
Gender, n (%)			
Male	25 (46.3)	30 (52.6)	0.633
Female	29 (53.7)	27 (47.4)	
Education, n (%)			
High School	14 (25.9)	24 (42.1)	0.055
AD, BD	12 (22.2)	16 (28.1)	
MD, PhD, Postdoc	28 (51.9)	17 (29.8)	
BMI, n (%)			
Normal	12 (22.2)	17 (29.8)	0.659
Overweight	15 (27.8)	14 (24.6)	
Obese	27 (50.0)	26 (45.6)	
Smoker, n (%)			
No	48 (88.9)	50 (87.7)	1.000
Yes	6 (11.1)	7 (12.3)	
Alcohol, n (%)			
No	49 (90.7)	55 (96.5)	0.392
Yes	5 (9.3)	2 (3.5)	
Hypertension, n (%)			
No	28 (51.9)	34 (59.6)	0.525
Yes	26 (48.1)	23 (40.4)	
Dyslipidaemia, n (%)			
No	27 (50.0)	25 (43.9)	0.647
Yes	27 (50.0)	32 (56.1)	
DM, n (%)			
No	44 (81.5)	43 (75.4)	0.588
Yes	10 (18.5)	14 (24.6)	
Family history of Dementia, n (%)			
No	52 (96.3)	52 (91.2)	0.479
Yes	2 (3.7)	5 (8.8)	
Allele ApoE, n (%)			
ApoE ε4	10 (18.5)	18 (31.6)	0.172
Non ApoE ε4	44 (81.5)	39 (68.4)	
Plasma β-Amyloid 1-42 level (pg/mL), mean±SD	25.3±2.4	23.9±2.4	0.002*

Differences between groups in continuous variables were analyzed using Student's t-test, while for catagorical variables were analyzed using the Chi-square test.

68.6%, negative predictive value (NPV) of 68.3%, positive likelihood ratio (LR) of 2.31, and negative LR of 0.49 for distinguishing between aMCI patients and HC. Table 2 presented the performance of using 24.6 pg/mL as cut-off point to differentiate HC and aMCI as well as the positive and negative LRs.

Based on Youden index and tested performance using the estimated optimal cut-off level, with aMCI as reference and HC as target listed the sensitivity, specificity, PPV, NPV, likelihood ratio (LR) was calculated. Table 3 presented the comparison of predicted diagnosis based on optimal cut-off level against the clinical diagnosis. The proportion of

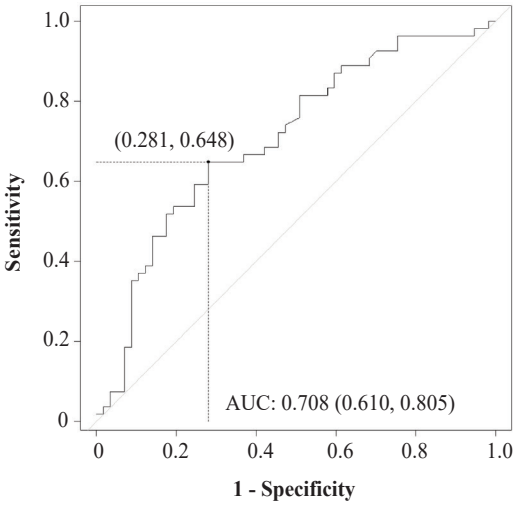

Figure 2. ROC curve of plasma Amyloid-β1–42 level (pg/mL) and aMCI occurance. ROC analysis was used to assess the ability of plasma Amyloid β1-42 levels to differentiate between aMCI and HC. The optimal cut-off value was 24.6 pg/mL as determined by the highest Youden Index, representing the best balance between sensitivity and specificity.

patients with plasma β-Amyloid 1-42 below 24.6 pg/mL was the cut-off point in each group.

Adjusted and Stratified Analysis

In terms of odds to be diagnosed for aMCI, higher plasma β-Amyloid 1-42 was significantly associated with lower odds (odds ratio (OR): 0.75; 95% CI: 0.61–0.90; *p*=0.004). Even when we adjusted for other demographic and clinical factors, increasing levels of plasma β-Amyloid 1-42 was still significantly associated with lower odds for aMCI (adjusted OR: 0.73; 95% CI: 0.58–0.9; *p*=0.005) (Table 4).

Further stratification analysis revealed that within age groups, plasma β-Amyloid 1-42 was significantly different in participants aged 70 years and older. Within female participants, the plasma β-Amyloid 1-42 was significantly different but not within male participants. When observed at the education level, the plasma β-Amyloid 1-42 was only significantly different in high education level (MD, PhD, or PostDoc graduated). While the plasma β-Amyloid 1-42 only significantly different among participants without ApoE ε4 (Table 5).

ApoE Genotype

Figure 3 presented the ApoE genotyping results with each graph displaying the amplification curves for different genotypes, based on the fluorescent signals from the FAM and VIC probes used in the PCR assay. The crossover point, where fluorescence becomes detectable, helps identify

Table 2. Performance of plasma β-Amyloid 1-42 with 24.6 pg/mL as cut-off point.

	Based on Youden index	
	Percentage (%)	**95% CI**
Sensitivity	64.8	50.6–77.3
Specificity	71.9	58.5–83.0
PPV	68.6	54.6–80.2
NPV	68.3	54.6–80.4
Positive LR	2.31	1.46–3.66
Negative LR	0.49	0.33–0.73

allele-specific patterns. In the homozygous wild type (FAM), amplification occurs with the FAM probe and not with the VIC probe, indicating that both alleles are wild-type. In the homozygous mutant (VIC), amplification occurs with the VIC probe and not with the FAM probe, indicating that both alleles are mutant. In the heterozygous (FAM and VIC), amplification occurs with both probes, confirming the presence of both wild-type and mutant alleles.

Discussion

In this study, plasma β-Amyloid 1-42 levels were significantly lower in aMCI subjects compared to HC subjects, with a cut-off point of 24.6 pg/mL. These findings support the potential utility of plasma β-Amyloid 1-42 as a non-invasive biomarker for detecting aMCI. However, the cut-off values we established for β-Amyloid 1-42 were lower than those reported in previous studies.(24) These discrepancies may arise from various factors, such as sample size differences, different assay techniques, or demographic differences in the study populations, as our participants were relatively younger (mean age of 65.4 and 65.1 years for aMCI and HC, respectively) compared to other studies. Furthermore, the criteria for selecting HC subjects may differ, leading to potential variability in the β-Amyloid 1-42 levels observed. To mitigate such variability, consistent inclusion criteria

Table 3. Subjects distribution based on cut-off points for plasma β-Amyloid 1-42 levels to differentiate HC and aMCI.

β-Amyloid 1-42	HC	aMCI	Total
≥24.6 pg/mL	34 (63%)	16 (28.1%)	50
<24.6 pg/mL	20 (37%)	41 (71.9%)	61
Total	54	57	111

The proportion of patients with plasma β-Amyloid 1-42 <24.6 pg/mL cut-off point in each group.

Table 4. OR of plasma β-Amyloid 1–42 level after adjusted by other factors.

Variable	OR (Univariable)	OR (Multivariable)
Age groups		
<60	-	-
60-69	0.58 (0.20-1.56, p=0.28)	1.05 (0.30-3.67, p=0.93)
≥70	0.62 (0.19-1.96, p=0.41)	1.11 (0.26-4.75, p=0.88)
Gender		
Male	-	-
Female	0.78 (0.37-1.63, p=0.50)	0.79 (0.30-2.12, p=0.63)
Education		
High School	-	-
AD, BD	0.78 (0.28-2.12, p=0.62)	0.58 (0.16-2.01, p=0.39)
MD, PhD, Postdoc	0.35 (0.14-0.85, p=0.02*)	0.26 (0.08-0.74, p=0.01*)
BMI		
Normal	-	-
Overweight	0.66 (0.23-1.85, p=0.43)	0.62 (0.16-2.30, p=0.47)
Obese	0.68 (0.27-1.69, p=0.40)	0.61 (0.20-1.79, p=0.36)
Smoker		
No	-	-
Yes	1.12 (0.35-3.71, p=0.84)	0.94 (0.20-4.68, p=0.93)
Alcohol		
No	-	-
Yes	0.36 (0.05-1.74, p=0.23)	0.31 (0.03-2.05, p=0.24)
Hypertension		
No	-	-
Yes	0.73 (0.34-1.54, p=0.40)	0.81 (0.31-2.09, p=0.66)
Dyslipidemia		
No	-	-
Yes	1.28 (0.61-2.72, p=0.51)	1.40 (0.58-3.46, p=0.45)
DM		
No	-	-
Yes	1.43 (0.58-3.66, p=0.44)	1.49 (0.49-4.75, p=0.48)
Family history of Dementia		
No	-	-
Yes	2.50 (0.51-18.01, p=0.28)	3.54 (0.47-38.78, p=0.24)
Allele ApoE		
ApoE ε4	-	-
Non ApoE ε4	0.49 (0.20-1.17, p=0.11)	0.47 (0.15-1.37, p=0.17)
Plasma Amyloid-β1–42 level (pg/mL)	0.75 (0.61-0.90, p=0.00*)	0.73 (0.58-0.90, p=0.00*)

Logistic regression was used to identify factors associated with plasma β-Amyloid 1-42 levels. Simple logistic regression was conducted for univariable analysis in each factor. Multiple logistic regression was conducted for multivariable analysis in all factors. Hosmer and Lemeshow test was performed to test goodness and fit of the multiple logistic regression (p=0.24). *p<0.05 is considered significant.

across all subjects were employed, which helped reduce heterogeneity in the HC group.

The results of this study were consistent with other research indicating that β-Amyloid 1-42 levels are reduced in individuals with aMCI. Previous studies have reported that a decrease in plasma β-Amyloid 1-42 is an early marker of amyloid deposition in the brain, a hallmark of AD. Reduced plasma β-Amyloid 1-42 levels were associated with a higher risk of conversion from aMCI to AD.(16) Our findings support the idea that β-Amyloid 1-42 in plasma could be a useful biomarker for detecting early-stage cognitive decline, such as in aMCI, which has been suggested to be an intermediate stage between normal aging and AD. However, it is essential to note that plasma β-Amyloid 1-42 alone may not be sufficient for definitive diagnosis, and its utility could be enhanced when combined with other biomarkers

Table 5. Plasma Amyloid-β1–42 level after stratified by other factors.

Variable	Plasma Amyloid-β1–42 Level (pg/mL)		*p*-value
	HC	aMCI	
Age groups			
<60	24.0±1.7	23.2±2.0	0.344
60-69	25.3±2.6	24.2±2.8	0.095
≥70	26.2±1.6	24.0±1.4	0.001*
Sex			
Male	25.0±2.1	23.9±2.0	0.060
Female	25.6±2.6	23.9±2.7	0.017*
Education			
High School	24.8±2.2	24.7±2.8	0.881
AD, BD	25.7±3.7	23.4±2.4	0.060
MD, PhD, Postdoc	25.5±1.7	23.3±1.3	0.000*
Allele ApoE			
ApoE ε4	24.8±1.6	23.6±1.8	0.093
Non ApoE ε4	25.5±2.5	24.1±2.6	0.014*

Plasma Amyloid-β1–42 level differences between healthy control and amnestic mild cognitive impairment were analyzed using Student's t-test.

or clinical assessments. For instance, other studies have reported a cutoff range for plasma β-Amyloid 1-42 between 40–80 pg/mL.(22,25,26) This variation highlights the complexity of using plasma β-Amyloid 1-42 as a reliable biomarker across different settings and underscores the need for standardized protocols in future research.

A key finding of our study was that the frequency of the ApoE ε4 allele was higher in aMCI patients compared to cognitively healthy elderly individuals. The ApoE ε4 allele is well-established as a major genetic risk factor for AD, particularly late-onset AD. Previous studies have shown that the ApoE ε4 allele is associated with increased amyloid plaque deposition and an earlier age of onset for AD.(27,28) Carriers of the ApoE ε4 allele showed increased β-Amyloid deposition in the brain, even in the absence of clinical symptoms of AD.(27) Similarly, in current study, we observed that a higher proportion of aMCI patients carried the ApoE ε4 allele, which may suggest an underlying genetic predisposition for amyloid accumulation and cognitive decline. However, in this study, there was no statistically significant correlation between plasma β-Amyloid 1-42 levels and the presence of the ApoE ε4 allele in aMCI patients. This result contrasts with previous studies linking the ApoE ε4 allele with lower levels of β-Amyloid 1-42 in both plasma and CSF.(29) The lack of significant correlation in our study may be attributed to several factors, including the relatively small sample size, the stage of disease (aMCI *vs.* AD), and the cross-sectional nature of the study. It is possible that in the earlier stages of cognitive decline, such as in aMCI, the relationship between ApoE ε4 and β-Amyloid 1-42 levels is not as pronounced as in later stages of AD.

The relationship between the ApoE ε4 allele and β-Amyloid 1-42 levels is complex and not yet fully understood. ApoE is involved in lipid metabolism and the clearance of amyloid plaques in the brain, and the ApoE ε4 allele has been shown to impair the ability to clear β-Amyloid. This leads to an accumulation of β-Amyloid plaques in the brain, which is a characteristic feature of AD pathology. The ApoE ε4 allele may also influence the processing of β-Amyloid precursor protein (APP), which affects the production of β-Amyloid peptides, particularly β-Amyloid 1-42, the most neurotoxic form of β-Amyloid. This may explain why ApoE ε4 carriers often show lower plasma levels of β-Amyloid 1-42, as amyloid plaques are deposited in the brain rather than remaining in circulation. Additionally, ApoE ε4 has been shown to modulate the inflammatory response in the brain, which could further contribute to β-Amyloid accumulation. Microglial activation in response to amyloid plaques is known to exacerbate the neurodegenerative process, and ApoE ε4 carriers may have an enhanced inflammatory response, which accelerates the onset and progression of AD.

The findings underscore the need for further research to elucidate the complex interactions between ApoE ε4, β-Amyloid 1-42, and the progression from aMCI to AD. Longitudinal studies with larger sample sizes and more diverse populations are needed to validate these results and better understand the role of ApoE and β-Amyloid 1-42 in the pathophysiology of cognitive impairment and AD. This study is unique in its focus on the relationship between plasma β-Amyloid 1-42 levels and the ApoE ε4 allele specifically in individuals with aMCI, rather than in late-stage AD. While previous studies have extensively explored the role of β-Amyloid 1-42 and ApoE in AD, fewer have examined their relationship in the aMCI population, which is crucial for early detection and intervention. Despite its strengths, this study has several limitations. First, the relatively small sample size limits the generalizability of our findings. A larger sample size would increase the statistical power of the study and provide a more robust analysis of the relationship between plasma β-Amyloid 1-42 and the ApoE ε4 allele in aMCI. Second, the cross-sectional design of the study precludes any conclusions about causality or the progression of aMCI to AD. Longitudinal studies are needed to track the changes in β-Amyloid 1-42 levels over

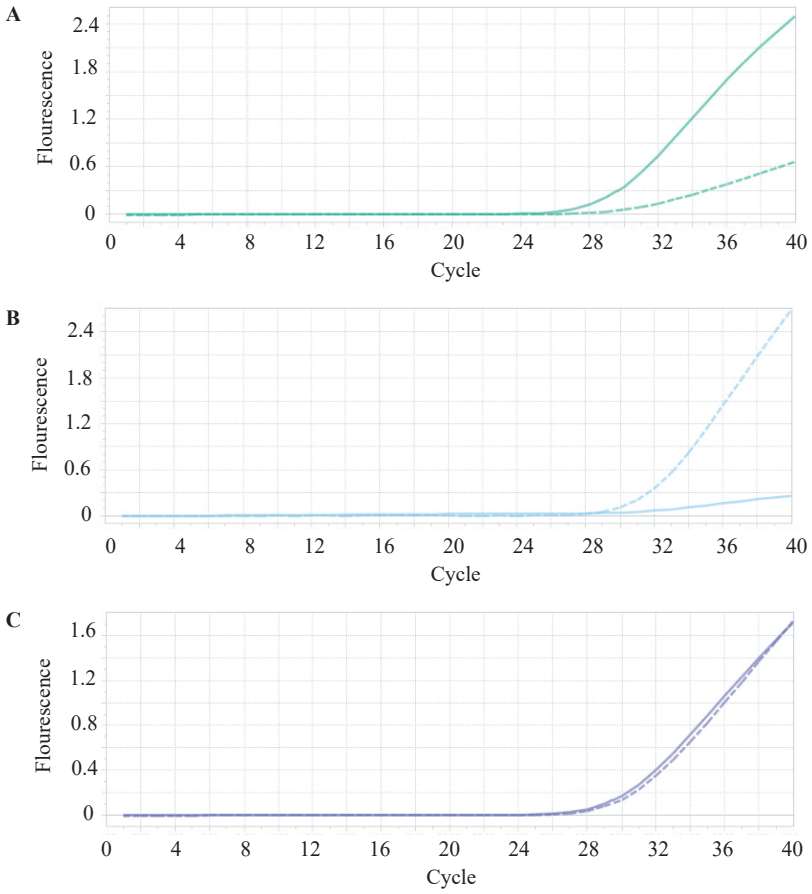

Figure 3. ApoE genotyping results, each graph shows the amplification curve for the different genotypes based on the fluorescent signals for FAM (wild-type, straight line) and VIC (mutant, dashed line) probes used in the PCR assay. A: The Homozygous Wild Type (FAM) shows amplification with a FAM probe and no amplification with VIC, indicating that both alleles are wild type. B: The Homozygous Mutant (VIC) shows amplification with the VIC probe and no amplification with FAM, indicating both alleles are mutant. C: The Heterozygous (FAM and VIC) shows amplification with both probes, confirming the presence of both wild type and mutant alleles.

time and their potential role in predicting the conversion from aMCI to AD. Furthermore, this study relied on plasma β-Amyloid 1-42 levels as a biomarker, which may not fully capture the complex pathophysiology of cognitive decline. Future research could benefit from incorporating additional biomarkers, such as tau protein or neurofilament light chain (NFL), as well as advanced neuroimaging techniques like positron emission tomography (PET) scans, to provide a more comprehensive understanding of the mechanisms underlying cognitive impairment. Another limitation is the potential for biases in our study, such as selection bias in the recruitment of HC subjects and confounding factors like comorbid conditions or medication use, which may have influenced the results. Furthermore, the relatively homogeneous nature of our study population, with participants mainly from a single region, limits the generalizability of the findings to more diverse populations. Future studies should aim to include larger, more diverse cohorts to explore the role of plasma β-Amyloid 1-42 in aMCI across different demographic groups and settings. Further research with a larger sample size and a longitudinal design is needed to better understand the relationship between plasma β-Amyloid 1-42, ApoE genotype, and the progression of aMCI to AD.

Conclusion

Lower plasma β-Amyloid 1-42 levels were observed in aMCI patients compared to cognitively healthy individuals, suggesting its potential as a biomarker for identifying aMCI.

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Authors Contribution

All authors were involved in concepting and planning of the research. C performed the data acquisition/collection. C and HBN calculated the data and performed the analysis.

C, HBN and RD drafted the manuscript and designed the figures. All authors aided in interpreting the results and took parts in giving critical revision of the manuscript.

References

1. Park JE, Choi KY, Kim BC, Choi SM, Song MK, Lee JJ, *et al.* Cerebrospinal fluid biomarkers for the diagnosis of prodromal alzheimer's disease in amnestic mild cognitive impairment. Dement Geriatr Cogn Dis Extra. 2019; 9(1): 100–13.
2. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004; 256(3): 183–94.
3. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, *et al.* Mild cognitive impairment – beyond controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004; 256(3): 240–6.
4. Petersen RC, Roberts RO, Knopman DS, Boeve BF, Geda YE, Ivnik RJ, *et al.* Mild cognitive impairment: Ten years later. Arch Neurol. 2009; 66(12): 1447–55.
5. Risacher SL, Fandos N, Romero J, Sherriff I, Pesini P, Saykin AJ, *et al.* Plasma amyloid beta levels are associated with cerebral amyloid and tau deposition. Alzheimers Dement. 2019; 11: 510–9.
6. Saido TC. Metabolism of amyloid β peptide and pathogenesis of Alzheimer's disease. Proc Jpn Acad Ser B Phys Biol Sci. 2013; 89(7): 321–39.
7. Meiliana A, Wijaya A. The search for biomarkers in alzheimer's disease. Indones Biomed J. 2010; 2(1): 4–25.
8. Meiliana A, Dewi NM, Wijaya A. New insight in the molecular mechanisms of neurodegenerative disease. Indones Biomed J. 2018l 10(1): 16–24.
9. Ait-ghezala G, Abdullah L, Volmar CH, Paris D, Luis CA, Quadros A, *et al.* Diagnostic utility of APOE, soluble CD40, CD40L, and Abeta1-40 levels in plasma in alzheimer's disease. Cytokine. 2008; 44(2): 283–7.
10. Lopez OL, Kuller LH, Mehta PD, Becker JT, Gach HM, Sweet RA, *et al.* Plasma amyloid levels and the risk of AD in normal subjects in the cardiovascular health study. Neurology. 2008; 70(19): 1664–71.
11. Luis CA, Abdullah L, Paris D, Quadros A, Mullan M, Mouzon B, *et al.* Serum beta-amyloid correlates with neuropsychological impairment. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn. 2009; 16(2): 203–18.
12. Pesini P, Perez-Grijalba V, Monleon I, Boada M, Tarraga L, Martinez-Lage P, *et al.* Reliable measurements of the beta-amyloid pool in blood could help in the early diagnosis of AD. Int J Alzheimers Dis. 2012; 2012: 604141. doi: 10.1155/2012/604141.
13. Ruiz A, Pesini P, Espinosa A, Perez-Grijalba V, Valero S, Sotolongo-Grau O, *et al.* Blood amyloid beta levels in healthy, mild cognitive impairment and alzheimer's disease individuals: Replication of diastolic blood pressure correlations and analysis of critical covariates. PLoS One. 2013; 8(11): e81334. doi: 10.1371/journal.pone.0081334.
14. Wang MJ, Yi S, Han JY, Park SY, Jang JW, Chun IK, *et al.* Oligomeric forms of amyloid-beta protein in plasma as a potential blood-based biomarker for Alzheimer's disease. Alzheimers Res Ther. 2017; 9(1): 98. doi: 10.1186/s13195-017-0324-0.
15. Graff-Radford NR, Crook JE, Lucas J, Boeve BF, Knopman DS, Ivnik RJ, *et al.* Association of low plasma Abeta42/Abeta40 ratios with increased imminent risk for mild cognitive impairment and alzheimer disease. Arch Neurol. 2007; 64(3): 354–62.
16. Hanon O, Vidal JS, Lehmann S, Bombois S, Allinquant B, Treluyer JM, *et al.* Plasma amyloid levels within the Alzheimer's process and correlations with central biomarkers. Alzheimers Dement. 2018; 14(7): 858–68.
17. Luis CA, Abdullah L, Paris D, Quadros A, Mullan M, Mouzon B, *et al.* Serum beta-amyloid correlates with neuropsychological impairment. Neuropsychol Dev Cogn B Aging Neuropsychol Cogn. 2009; 16(2): 203–18.
18. Leung R, Proitsi P, Simmons A, Lunnon K, Guntert A, Kronenberg D, *et al*. Inflammatory proteins in plasma are associated with severity of Alzheimer's disease. PLoS One. 2013; 8(6): e64971. doi: 10.1371/journal.pone.0064971.
19. Sattlecker M, Khondoker M, Proitsi P, Williams S, Soininen H, Kloszewska I, *et al.* Longitudinal protein changes in blood plasma associated with the rate of cognitive decline in alzheimer's disease. J Alzheimers Dis. 2016; 49(4): 1105–14.
20. Yaffe K, Weston A, Graff-Radford NR, Satterfield S, Simonsick EM, Younkin SG, *et al.* Association of plasma beta-amyloid level and cognitive reserve with subsequent cognitive decline. JAMA. 2011; 305(3): 261–6.
21. Albani D, Marizzoni M, Ferrari C, Fusco F, Boeri L, Raimondi I, *et al.* Plasma Abeta42 as biomarker of prodromal Alzheimer's disease progression in patients with amnestic mild cognitive impairment: evidence from the PharmaCog/E-ADNI study. J Alzheimers Dis 2019; 69(1): 37–48.
22. Yang YH, Huang LC, Hsieh SW, Huang LJ. Dynamic blood concentrations of Aβ1-40 and Aβ1-42 in alzheimer's disease. Front Cell Dev Biol. 2020; 8: 768. doi: 10.3389/fcell.2020.00768.
23. Prasetyo BT, Lumempouw SF, Ramli Y, Herqutanto. Nilai Normal Montreal Cognitive Assesment versi Indonesia (MoCA-Ina). Jakarta: Program Studi Ilmu Bedah Saraf, Fakultas Kedokteran Universitas Indonesia; 2011
24. Palmqvist S, Insel PS, Stomrud E, Janelidze S, Zetterberg H, Brix B, *et al.* Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in alzheimer's disease. EMBO Mol Med. 2019; 11(12): e11170. doi: 10.15252/emmm.201911170.
25. Figurski MJ, Waligórska T, Toledo J, Vanderstichele H, Korecka M, Lee VM, *et al.* Alzheimer's disease neuroimaging initiative. Improved protocol for measurement of plasma β-amyloid in longitudinal evaluation of Alzheimer's Disease Neuroimaging Initiative Study patients. Alzheimers Dement. 2012; 8(4): 250–60.
26. Manafikhi R, Haik MB, Lahdo R, AlQuobaili F. Plasma amyloid β levels in Alzheimer's disease and cognitively normal controls in Syrian population. Med J Islam Repub Iran. 2021; 35: 19. doi: 10.47176/mjiri.35.19.
27. Di Battista AM, Heinsinger NM, Rebeck GW. Alzheimer's disease genetic risk factor APOE-ε4 also affects normal brain function. Curr Alzheimer Res. 2016; 13(11): 1200–7.
28. Serrano-Pozo A, Das S, Hyman BT. APOE and alzheimer's disease: Advances in genetics, pathophysiology, and therapeutic approaches. Lancet Neurol. 2021; 20(1): 68-80.
29. Castellano JM, Kim J, Stewart FR, Jiang H, DeMattos RB, Patterson BW, *et al.* Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. Sci Transl Med. 2011; 3(89): 89ra57. doi: 10.1126/scitranslmed.3002156.