FACTA UNIVERSITATIS
Series **Medicine and Biology** Vol. 25, No 2, 2023, pp. 37–41

UDC 616.894:615
https://doi.org/10.22190/FUMB231012006B

Original Scientific Article

THE INFLUENCE OF THE APOEε4 ALLELE ON PRO-INFLAMMATORY CYTOKINE LEVELS IN THE CEREBROSPINAL FLUID OF PATIENTS WITH ALZHEIMER'S DISEASE

Jelena Bašić[1], Vuk Milošević[2], Branka Đorđević[1], Nikola Stefanović[1], Vladana Stojiljković[1], Tatjana Jevtović Stoimenov[1], Ivana Stojanović[1]

[1]Department of Biochemistry, Faculty of Medicine, University of Niš, Niš, Serbia
[2]Clinic of Neurology, University Clinical Center Niš, Faculty of Medicine, University of Niš, Niš, Serbia

Abstract. *Single nucleotide polymorphisms (SNPs) rs429358 and rs7412, the most commonly investigated variants in the apolipoprotein E gene (APOE) are crucial for APOEε4 carrier status determination. However, their association with inflammatory cytokine levels in patients with dementia due to Alzheimer's disease (AD) remains unclear. This study aimed to investigate the influence of the APOEε4 allele on pro-inflammatory cytokine levels in the cerebrospinal fluid of patients with AD dementia. The research was conducted on 36 patients with probable dementia due to AD. APOE rs429358 and rs7412 were analyzed using the Real-Time PCR method with allele-specific TaqMan assays, followed by the analysis of APOEε4 allele carrier status. Patients carrying at least one APOEε4 allele were assigned as APOEε4+. Core biomarkers (Aβ42/40 ratio, t-Tau, p-Tau levels), as well as pro-inflammatory cytokine (Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)) levels, were determined in the patient's cerebrospinal fluid (CSF) using Enzyme-linked Immunosorbent Assay (ELISA). Seventeen patients (47.22%) were assigned as APOEε4+. CSF TNF-α levels were significantly higher in APOEε4+ AD dementia patients in comparison to APOEε4– patients ($p<0.001$), while no significant differences in IL-1β levels between these two groups were obtained. Correlation analysis showed that TNF-α negatively correlated with the Aβ42/40 ratio ($p=0.033$), while positive correlation with t-Tau and p-Tau was observed ($p=0.001$, $p=0.015$, respectively). These findings highlight the potential significance of TNF-α in the context of APOEε4 positivity and its implications in AD pathology.*

Key words: *Apolipoprotein E ε4, Alzheimer's disease, IL-1β, TNF-α*

Introduction

Dementia is a condition marked by declined cognitive abilities, alterations in personality, and behavioral changes, causing disruptions in everyday tasks. Alzheimer's disease (AD), the predominant cause of dementia, accounts for 60-70% of all dementia cases [1]. According to the 2019 World Health Organization (WHO) Global Burden of Disease (GBD) Study, Serbia had approximately 130,000 reported cases of dementia. Projections suggest that this number is expected to rise to around 180,000 cases by the year 2050 [2]. The most prevalent type of AD is the late-onset form (LOAD), which is linked to genetic variations in the apolipoprotein E gene (*APOE*) [3]. The pathogenesis of AD involves three main factors: proteinopathy, neurodegeneration, and neuroinflammation. According to the amyloid cascade hypothesis, the disease's significant pathohistological features include the extracellular accumulation of β-amyloid (Aβ), primarily consisting of Aβ1-42 peptide in brain tissue. Additionally, there is the formation of neurofibrillary tangles (NFT) composed of hyperphosphorylated and misfolded tau proteins (p-Tau) in nerve cell axons. These processes lead to neurodegeneration, brain atrophy, and subsequent cognitive impairment [4].

The diagnosis of AD involves the use of neuroradiological and biochemical biomarkers. Magnetic resonance imaging (MRI) plays a crucial role in assessing morphological changes, as well as distinctive patterns of brain perfusion [5]. In line with the National Institute on Aging-Alzheimer's Association diagnostic guidelines (NIA-AA, 2011) [6], the concentrations of biochemical biomarkers in cerebrospinal fluid (CSF) are analyzed, including Aβ42, Aβ40, followed by Aβ42/40 ratio calculation, total Tau (t-Tau), and phosphorylated Tau (p-Tau,181P). The positivity of specific CSF AD biomarkers is determined using the A/T/N classification (2018). CSF Aβ42 and/or Aβ42/Aβ40 ratio assess Aβ pathology (biomarker "A"), CSF p-Tau evaluates tau pathology (biomarker "T"), and CSF t-Tau evaluates neurodegeneration (biomarker "N") [7].

Correspondence to: Jelena Bašić
Department of Biochemistry, Faculty of Medicine, University of Niš, Dr. Zoran Đinđić Blvd 81, 18000 Niš, Serbia
E-mail: jelena.basic@medfak.ni.ac.rs
Received October 12th, 2023 / Accepted November 26th, 2023

It has been shown that the inflammatory response plays a crucial role in AD, regulated by microglia and astrocytes. When these cells sense signals of damage or injury, they undergo gradual activation, releasing pro-inflammatory mediators that lead to chronic inflammation, further neuronal damage, and disease progression. Studies have shown that Aβ protofibrils activate microglia, triggering the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukins (IL-1β, IL-6, IL-12), interferon-gamma (INF-γ), and chemokines like monocyte chemoattractant protein-1 (MCP-1) and eotaxin-1. This activation also results in the generation of reactive oxygen species (ROS) and nitric oxide (NO). In response, astrocytes express various receptors for inflammatory cytokines (e.g., IL-1β and TNFα), chemokines, and damage signals, including Toll-like Receptor (TLR) ligands. Moreover, other receptors and mediators of inflammation may be induced after appropriate activation signals from other brain cells [8].

APOE is a glycoprotein produced by the APOE gene, and plays a crucial role in Aβ clearance. Two key Single Nucleotide Polymorphisms (SNPs), rs429358 and rs7412, are essential in determining whether an individual possesses the *APOE*ε2, ε3, or ε4 allele, and the corresponding APOE2, E3, or E4 protein isoform. Carrying the *APOE*ε4 allele has been linked to a higher risk of accumulating Aβ and developing sporadic AD [3, 9]. Nevertheless, the connection between this allele and the levels of inflammatory cytokines in patients with AD remains unclear.

This study aimed to investigate the influence of the *APOE*ε4 allele on pro-inflammatory cytokine levels in the cerebrospinal fluid of patients with AD dementia.

Materials and Methods

The study involved 36 patients diagnosed with probable dementia due to AD at the Neurology Clinic, University Clinical Center Niš, based on the NIA-AA guidelines [6]. Patients underwent neurological examination and cognitive function assessments, including Mini-Mental State Examination (MMSE), Addenbrooke's Cognitive Examination Revised (ACE-R), Clinical Dementia Rating (CDR), and Instrumental activities of daily living (IADL) using the recently validated Serbian version of the Amsterdam IADL questionnaire (A-IADL-Q) [10, 11]. Brain MRI was performed according to dementia protocol. CSF samples were obtained in all patients, taken in a polypropylene tube, centrifuged at 2000 g for 10 min at +4°C, and the supernatant was separated, aliquoted, and stored at -80 °C until analysis. Biomarkers in the CSF were analyzed using the Enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EUROIMMUN, Germany), including Aβ42/40 ratio, t-Tau, p-Tau, as described previously [12). Patients with ACE-R scores less than 88, MMSE scores ranging from 10-26, CDR scores greater than 0.5, A-IADL-Q scores less than or equal to 51.4, as well as positive MRI and biochemical biomarkers were included in the study.

DNA isolation was performed using a commercial DNA isolation kit (Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit, Thermo Fisher Scientific). *APOE* rs429358 and rs7412 were determined by Real-Time PCR using allele-specific TaqMan assays (ThermoFisher Scientific, California, USA). The analysis of both *APOE* SNPs was followed by the determination of *APOE*ε4 allele carrier status, as we have previously described (12). Patients carrying at least one *APOE*ε4 allele were assigned as *APOE*ε4 positive (*APOE*ε4+), while patients carrying *APOE*ε2 or ε3 alleles were considered *APOE*ε4 negative (*APOE*ε4-).

Levels of TNF-α and IL-1β were determined in the patient's CSF by ELISA according to the manufacturer's instructions (Abexxa, Cambridge, UK; R&D Systems, Abingdon, UK, respectively) and expressed in pg/mL. Minimum detectable dose for TNF-α was 0.52 pg/mL, and for IL-1β was 1 pg/mL.

All participants were fully informed and gave their consent to participate in the study. The Ethical Committee of the University Clinical Center Niš and the Faculty of Medicine University of Niš approved the research. The study was conducted in the Laboratory for Medical Genetics, at the Faculty of Medicine, University of Niš, Serbia.

Statistical Analysis

Variables are expressed as mean (M) ± standard deviation (SD). The statistically significant differences between the groups were determined by the t-test for two independent samples. Pearson's correlation analysis was performed to examine associations between variables of interest. Statistical significance was set at a p-value of <0.05. SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Demographic, clinical, and laboratory parameters of the subjects are presented in Table 1.

All patients were A+/T+/N+ according to cut-off values for core CSF biomarkers (Aβ42/40, t-Tau, p-Tau). Seventeen out of 36 patients (47.22%) were assigned as *APOE*ε4+, while 19 (52.78%) patients were *APOE*ε4-.

Levels of TNF-α, t-Tau, and p-Tau in the CSF were significantly higher in *APOE*ε4+ AD dementia patients in comparison to *APOE*ε4– patients ($p<0.001$; $p=0.007$; $p=0.003$, respectively), while the Aβ42/40 ratio was significantly lower in *APOE*ε4 carriers compared to non-carriers ($p=0.006$). There were no significant differences in IL-1β levels between these two groups ($p=0.261$; Table 2).

Correlation analysis showed that TNF-α negatively correlated with the Aβ42/40 ratio ($r=-0.356$, $p=0.033$), while positive correlation with t-Tau and p-Tau was observed ($r=0.539$, $p=0.001$, $r=0.401$, $p=0.015$, respectively). Levels of IL-1β did not show a significant

correlation with the Aβ42/40 ratio (p=0.745), t-Tau (p=0.235), and p-Tau levels (p=0.771; Table 3).

Table 1 Demographic, clinical, and laboratory parameters of the subjects

	AD dementia patients N=36	
Gender N (%)		
Male	15	(41.67)
Female	21	(58.33)
Age M (SD)	71.05	(7.78)
MMSE M (SD)	16.97	(5.88)
CSF biomarkers (M (SD))		
Aβ42/40 ratio	0.07	(0.01)
t-Tau (pg/mL)	653.00	(215.46)
p-Tau (pg/mL)	175.64	(90.43)
TNF-α (pg/mL)	4.66	(0.81)
IL-1β (pg/mL)	22.84	(11.04)
APOE ε4 positivity N (%)		
APOE ε4+	17	(47.22)
APOE ε4–	19	(52.78)

AD – Alzheimer's disease, APOE – Apolipoprotein E, CSF – cerebrospinal fluid, M – mean, SD – standard deviation, MMSE – Mini-mental State Examination, N – number of subjects, TNF – Tumor necrosis factor, IL – Interleukin

Table 2 CSF biomarkers in *APOE* ε4+ and *APOE* ε4– AD dementia patients

CSF biomarkers	*APOE* ε4– M (SD)		*APOE* ε4+ M (SD)		p-value
Aβ42/40 ratio	0.08	(0.01)	0.06	(0.01)	0.006
t-Tau (pg/mL)	564.53	(138.06)	751.87	(245.81)	0.007
p-Tau (pg/mL)	134.90	(76.8)	221.16	(84.16)	0.003
TNF-α (pg/mL)	4.21	(0.47)	5.16	(0.82)	0.000
IL-1β (pg/mL)	20.86	(10.70)	25.06	(11.31)	0.261

AD – Alzheimer's disease, APOE – Apolipoprotein E, CSF – cerebrospinal fluid, M – mean, SD – standard deviation, TNF – Tumor necrosis factor, IL – Interleukin

Table 3 Correlation analysis of CSF biomarkers in AD dementia patients

		Aβ42/40 ratio	t-Tau	p-Tau
TNF-α	Pearson's r	-0.356	0.539	0.401
	p-value	0.033	0.001	0.015
IL-1β	Pearson's r	-0.056	0.203	0.050
	p-value	0.745	0.235	0.771

AD – Alzheimer's disease, IL – Interleukin, TNF – Tumor necrosis factor

Discussion

While rs429358 and rs7412 SNPs are frequently studied variations in the APOE gene, the relationship between the *APOE*ε4 allele and pro-inflammatory cytokine levels in CSF of patients with AD dementia is not fully comprehended. The findings of this study revealed a significant increase in CSF TNF-α levels among patients with AD dementia who carried the APOEε4 allele, compared to non-carriers. Additionally, TNF-α exhibited a positive correlation with t-Tau and p-Tau, while a negative correlation with the Aβ42/40 ratio was observed.

Prior research has demonstrated elevated levels of specific inflammation-related biomarkers in the blood serum or CSF of AD patients. These biomarkers include TNFα, IL-1β, IL-4, IL-5, IL-9, IL-13, YKL-40 (also known as chitinase-3-like protein-1 (CHI3L1)), and glial fibrillary acidic protein (GFAP). Additionally, two chemokines, MCP-1, and eotaxin-1, have previously been associated with more significant memory impairment in individuals with Mild Cognitive Impairment (MCI) and AD [8, 13, 14].

The *APOE*ε4 allele is widely acknowledged as the primary risk factor for sporadic AD and is linked to an earlier onset of the disease, contingent on an individual's carrier status. People who have the *APOE*ε4 allele typically develop the disease approximately 12 years earlier than those who do not carry this allele [15]. Additionally, studies have indicated that patients carrying the *APOE*ε4 allele display specific patterns of brain region involvement in the pathological process, differing from *APOE*ε4-negative patients. This variance leads to distinct clinical presentations. Specifically, *APOE*ε4-negative patients undergo a cognitive decline in other domains before experiencing memory decline, which is the initial symptom in *APOE*ε4+ patients [3]. Furthermore, our previously published results revealed that individuals with the *APOE*ε4 allele had a 3-fold higher risk of developing dementia due to AD compared to those with the reference allele *APOE*ε3 in the Serbian population [12].

APOE plays a pivotal role in clearing Aβ, which is eliminated from the brain through the blood-brain barrier (BBB) or cellular and enzymatic degradation pathways. It is synthesized in microglia, astrocytes, and neurons under stressful conditions [9]. After becoming lipidated, APOE binds with soluble Aβ and aids in its clearance, involving uptake through the LDL receptor (LDLR) and LDL Receptor-Related Protein 1 (LRP1) as well as transport across the BBB. The APOE E4 isoform has a reduced affinity for Aβ, binding to it less efficiently than APOE E3. This weakened binding hampers both cellular and perivascular clearance, resulting in the accumulation of Aβ in the brain and further progression of the disease in *APOE*ε4+ individuals [3, 16].

Research studies have shown that microglia expressing the APOE4 significantly increase the production of pro-inflammatory mediators such as TNFα, IL-6, and NO. Additionally, these microglia demonstrate impaired migration and reduced phagocytic function. Moreover, APOE's involvement in neuroinflammation is further substantiated by the discovery that APOE signaling through the triggering receptor expressed on myeloid cells 2 (TREM2) prompts a shift in microglia toward a neurodegenerative phenotype [9, 17]. Additionally, it has been observed that all APOE isoforms can induce increased expression of IL-1β, TNFα, and IL-6 in human astrocytes, with APOE4 leading to the highest level of cytokine expression. Nevertheless, the specific function

of APOE4 in astrocytes and its detailed link to pathological neuroinflammation is not yet fully understood. It has been shown that Transgelin 3 (TAGLN3) may play an important role in the negative regulation of nuclear factor κB (NF-kB) activity in human astrocytes. Moreover, TAGLN3 downregulation has been observed in AD. A recent research investigation has highlighted the involvement of microglial NF-κB signaling in facilitating the spread and harmful effects of tau proteins in a mouse model of tauopathy [18]. Furthermore, in APOE4 iPSC-derived astrocytes, it was found that TAGLN3 downregulation exacerbates inflammation through NF-kB activation, identifying TAGLN3 as an interacting partner of IkBa [19]. NF-kB plays a crucial role in TNF-α signaling and can be activated through the TNF-α signaling cascade. Interestingly, NF-kB not only responds to TNF-α signaling but can also trigger the expression of TNF-α, leading to the increased production of this cytokine and prolonged inflammation. We suggest that the increased levels of TNF-α in CSF observed in *APOE4*ε4+ patients with dementia due to AD in our study occur as a response to microglial activation. This activation is induced by *APOE4*ε4-mediated increased accumulation of Aβ. Additionally, this elevation in TNF-α might be attributed to the downregulation of TAGLN3 in astrocytes, resulting in NF-kB activation and intensified neuroinflammation.

On the other hand, the presence of the *APOE*ε4 allele is linked to the accumulation of tau, α-synuclein, TDP43, neurofilament-light (NfL), and other proteins associated with neurodegeneration, regardless of Aβ presence [3, 9, 17, 20]. Moreover, the presence of certain anti-inflammatory cytokines, including IL-10, IL-13, and IL-4, showed a negative correlation with tau pathology, specifically in the aging cohort of *APOE*ε4- individuals. This implies a protective effect against tau pathology in those lacking *APOE*ε4 [21]. In line with these results, Abe et al. [22] found that *APOE*ε4+ MCI-AD patients exhibited a significantly faster decline in hippocampal volume in comparison to *APOE*ε4- patients during a 15-year follow-up period. Additionally, it has been shown that *APOE*ε4 carriers with elevated levels of TNF-α had shorter times to conversion from MCI-AD to AD dementia [23]. In line with previous studies, a positive correlation of TNF-α with t-Tau and p-Tau in our study indicates the role of this cytokine in neurodegeneration. Prolonged neuroinflammation is a key factor contributing to the hyperphosphorylation of tau protein, the accumulation of NFTs, neurodegeneration, and brain atrophy, ultimately resulting in cognitive decline. Importantly, the presence of the *APOE*ε4 allele could be implicated in mediating and accelerating these processes. However, more evidence is needed to fully understand the exact roles of APOEε4 and TNF-α as biomarkers in predicting neurodegeneration and disease pregression.

Conclusion

The results of this study demonstrate that *APOE*ε4+ patients with dementia due to AD exhibit significantly higher levels of TNF-α in the CSF compared to the group of *APOE*ε4- patients, as well as a negative correlation of this cytokine with Aβ42/40 ratio, and positive correlation with t-Tau and p-Tau. These results emphasize the involvement of TNF-α in both neuroinflammation and neurodegeneration in this disease, particularly in individuals with *APOE*ε4+ status. Future research involving a large sample size and correlation with neuroimaging will be necessary to confirm the specific mechanisms underlying the interplay between the *APOE*ε4 allele and TNF-α in disease progression.

ACKNOWLEDGMENTS: *This work was financially supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200113), and by the Faculty of Medicine University of Niš, Serbia (internal scientific project No. 44).*

References

1. Nichols E, Szoeke CE, Vollset SE, et al. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2019; 18(1):88–106.
2. Nichols E, Steinmetz JD, Vollset SE, et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Health 2022; 7(2):e105–125.
3. Frisoni GB, Altomare D, Thal DR, et al. The probabilistic model of Alzheimer disease: the amyloid hypothesis revised. Nat Rev Neurosci 2022; 23(1):53–66.
4. Ando K, Houben S, Homa M, et al. Alzheimer's disease: tau pathology and dysfunction of endocytosis. Front Mol Neurosci 2021; 13:583755.
5. Patel KP, Wymer DT, Bhatia VK, Duara R, Rajadhyaksha CD. Multimodality imaging of dementia: clinical importance and role of integrated anatomic and molecular imaging. Radiographics 2020; 40(1):200–22.
6. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7(3):263–9.
7. Jack Jr CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018; 14(4):535–62.
8. Prins S, de Kam ML, Teunissen CE, Groeneveld GJ. Inflammatory plasma biomarkers in subjects with preclinical Alzheimer's disease. Alzheimers Res Ther 2022; 14(1):1–9.
9. Troutwine BR, Hamid L, Lysaker CR, Strope TA, Wilkins HM. Apolipoprotein E and Alzheimer's disease. Acta Pharm Sin B 2022; 12(2):496–510.
10. Milošević V, Malobabić M, Stojanović I, Bašić J. Importance of a functional measure in the evaluation of patients in a memory clinic: Validation of the Serbian version of the Amsterdam instrumental activities of daily living questionnaire. Clin Neurol Neurosurg 2022; 214:107165.

11. Milošević V, Malobabić M, Antić E, Trenkić AA, Stojanov D, Bašić J. Assessment of the instrumental activities of daily living in mild cognitive impairment and dementia due to alzheimer's disease: diagnostic accuracy of the serbian version of the amsterdam iadl questionnaire. Facta Univ Ser Med Biol 2022; 24(2):35–9.
12. Bašić J, Milošević V, Đorđević B, et al. Association of the apolipoprotein E ε4 allele in a Serbian population with Alzheimer's dementia. Arch Biol Sci 2023; 75(3):319–25.
13. Angiulli F, Conti E, Zoia CP, et al. Blood-based biomarkers of neuroinflammation in Alzheimer's disease: A central role for periphery? Diagnostics 2021; 11(9):1525.
14. de la Monte SM, Tong M, Hapel AJ. Concordant and Discordant Cerebrospinal Fluid and Plasma Cytokine and Chemokine Responses in Mild Cognitive Impairment and Early-Stage Alzheimer's Disease. Biomedicines 2023; 11(9):2394.
15. Belloy ME, Napolioni V, Greicius MD. A quarter century of APOE and Alzheimer's disease: progress to date and the path forward. Neuron 2019; 101(5):820–38.
16. Bachmeier C, Shackleton B, Ojo J, Paris D, Mullan M, Crawford F. Apolipoprotein E isoform-specific effects on lipoprotein receptor processing. Neuromolecular Med 2014; 16:686–96.
17. Iannucci J, Sen A, Grammas P. Isoform-specific effects of apolipoprotein E on markers of inflammation and toxicity in brain glia and neuronal cells in vitro. Curr Issues Mol Biol 2021; 43(1):215–25.
18. Wang C, Fan L, Khawaja RR, et al. Microglial NF-κB drives tau spreading and toxicity in a mouse model of tauopathy. Nat Commun 2022; 13(1):1969.
19. Arnaud L, Benech P, Greetham L, et al. APOE4 drives inflammation in human astrocytes via TAGLN3 repression and NF-κB activation. Cell Rep 2022; 40(7):111200.
20. Wang C, Najm R, Xu Q, et al. Gain of toxic apolipoprotein E4 effects in human iPSC-derived neurons is ameliorated by a small-molecule structure corrector. Nat Med 2018; 24(5):647–57.
21. Friedberg JS, Aytan N, Cherry JD, et al. Associations between brain inflammatory profiles and human neuropathology are altered based on apolipoprotein E ε4 genotype. Sci Rep 2020; 10(1):2924.
22. Abe K, Chiba Y, Ide K, et al. Plasma MMP-9 Levels as the Future Risk of Conversion to Dementia in ApoE4-Positive MCI Patients: Investigation Based on the Alzheimer's Disease Neuroimaging Initiative Database. J Prev Alzheimers Dis. 2022; 9(2):331–7.
23. Contreras JA, Aslanyan V, Albrecht DS, Mack WJ, Alzheimer's Disease Neuroimaging Initiative (ADNI), Pa J. Higher baseline levels of CSF inflammation increase risk of incident mild cognitive impairment and Alzheimer's disease dementia. Alzheimers Dement Diagn Assess Dis Monit. 2022; 14(1):e12346.