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Neurochemical Ameliorating of the Hippocampus in Dyslipidemic Alzheimer Patients Following Silymarin; a Double-Blind Placebo-Controlled Randomized Clinical Trial

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Abstract

Background: Amyloid-beta (Aβ) production is a normal physiological process, and an imbalance in Aβ production/excretion rate is the basis of the plaque load increase in AD. LRP1 is involved in both central clearance of Aβ from the CNS and transport of Aβ toward peripheral organs. In this study, the effect of silymarin combination compared to rosuvastatin and placebo on neuro-metabolites and serum levels of LRP1 and Aβ1-42 proteins and oxidative stress enzymes and lipid and cognitive tests of Iranian AD patients.

Methods: In this double-blind placebo-controlled study, thirty-six mild AD patients were divided into groups (n=12) of silymarin 140mg, placebo, and rosuvastatin 10mg. Medications were administered 3 times a day for 6 months. Clinical tests, lipid profile (TG, HDL, TC, and LDL), Aβ1-42, and LRP1 markers were measured at the beginning and end of the intervention. Magnetic resonance spectroscopy (MRS) was used to measure metabolites. Using SPSS software a one-way ANOVA test was used to compare the means of the quantitative variables and Pearson and Spearman's correlations to measure the correlation. GraphPad Prism software was used for drawing graphs. $P < 0.05$ was considered a significant.

Results: The levels of LRP1 and Aβ1-42 in the silymarin group were significantly increased compared to the other groups ($P < 0.05$). NAA/mI in the silymarin group had a significant increase compared to both placebo and rosuvastatin groups ($P < 0.05$). Right and left hippocampal mI/Cr directly correlated with TG (r = 0.603, $P = 0.003$ and r = 0.595, $P = 0.004$, respectively). NAA/Cr of the right and left hippocampus was inversely related to TG (r = -0.511, $P = 0.0033$, and r = -0.532, $P = 0.0021$, respectively). NAA/Cr and NAA/mI of bilateral hippocampi directly correlated with HDL ($P < 0.05$). An inverse correlation was observed between the Aβ1-42 and mI/Cr of the right and left hippocampus (r = -0.661, $P = 0.000$ and r = -0.638, $P = 0.000$, respectively).

Conclusion: Donepezil and silymarin improved lipid profile associated with increased NAA/Cr, and decreased mI/Cr, in AD patients. Biomarker NAA/mI can be clinically significant in examining AD pathology. Measurement of the lipid factors and neurometabolites can be a suitable method for monitoring this disease.

Keywords: Neuroimaging, Magnetic Resonance Spectroscopy, Alzheimer's Disease, Amyloid-Beta

Conflicts of Interest: *None declared*
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Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly and a progressive neurodegenera-

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↑What is "already known" in this topic:
The production/excretion imbalance of amyloid-beta (Aβ) is important signaling in Alzheimer's pathology and depends on the low-density lipoprotein receptor-related protein-1 (LRP1) carrier, which causes plaque deposition and neuronal dysfunction. Dyslipidemia leads to disruption of the amyloid processing pathway and neuronal damage. Enhancing the peripheral "sink effect" mechanism leads to the reduction of brain Aβ burden.

→What this article adds:
Silymarin causes a significant decrease in triglyceride (TG) levels and an increase in LRP1, Aβ1-42, and concentration of neurometabolite N-acetyl-aspartate/myoinositol (NAA/mI). Silymarin is a potent antioxidant for improving lipid profile, modulating neurometabolites, and enhancing the Aβ clearance system in dyslipidemic Alzheimer's patients.

tive disease that eventually leads to huge neuron loss, especially in the cortex corresponding to the limbic system, known as the circuit of Papez (1). As the disease progresses, body functions decline, and patients often withdraw from the family and community, leading to death (2). Nearly 50 million people worldwide live with dementia, and it is predicted that by 2050, it will reach about 152 million, which puts additional socio-economic-cultural pressure on patients, families and communities (3, 4). The level of steady-state amyloid-beta (Aβ) in the brain is determined by the balance of Aβ production/excretion rate, as the imbalance in this process is the basis for (Aβ) deposition in the brain (5). There is a relationship between the increased risk of AD and high plasma cholesterol (6). Nakamura et al. (2018) reported that the plasma $A\beta_{1\text{-}42}$ has a significant and robust correlation with regions of high Aβ deposition in the brain. Since the measurement of the $A\beta_{1\text{-}42}$ plasma biomarker has an accuracy of 80.4%, it can reduce the cost of unnecessary positron emission tomography (PET) scans and is significantly valuable for clinical decision-making (7). Magnetic resonance spectroscopy (MRS) is able to detect neurodegenerative metabolic changes by quantifying metabolites such as lactate (Lac), choline (Cho), Myo-inositol (mI), N-acetyl aspartate (NAA), creatine (Cr) and also demonstrating tissue damage in the early stages, before the signs of atrophy appear on routine imaging (8). Marjańska et al. (2014) showed that the MRS technique can depict the therapeutic effects of the monoclonal antibody ponezumab, including the level of mI/Cr, which was reduced in AD mice treated with ponezumab. It seems that MRS is a valid in vivo tool for evaluating the effectiveness of treatment in preclinical studies and can significantly accelerate the process of new drug discovery (9). Bittner et al. (2013) showed that hippocampal NAA/Cr has the highest sensitivity (94.1%) and specificity (92.3%) for differentiating AD patients from cognitively normal subjects, and there had been a direct correlation between the right hippocampal NAA/Cr and $A\beta_{1\text{-}42}$ (10). Huang et al. (2017) reported that the administration of simvastatin 80mg daily for 18 months improved the MMSE score and slow cognitive decline of AD patients (11). Rosuvastatin reduces microglial load and ,inhibits inflammatory factors, and has a high affinity for binding to proteins such as acetylcholinesterase (AChE) and Aβ (12). Molecular pharmacokinetics analysis studies show that rosuvastatin has a high affinity for binding to proteins such as AChE and amyloid beta (Aβ), and is involved in the clearance of Aβ (12, 13). Hu et al. (2020) demonstrated that Rosuvastatin 10mg once daily for 6 months significantly alleviated the cognitive impairment progression and the risks of dementia in older hypertensive patients (14). Duan et al. (2015) showed that administration of 200 mg/kg silybin once a day for 28 days reduced Aβ and AChE and converted them into stable complexes in vitro, as well as the Morris-Water-Maze test score of rats was higher and their spatial learning ability was improved (15). Murata et al. (2010) showed that AD mice fed a diet containing 0.1% silymarin for 6 months had reduced brain Aβ plaques and improved behavioral disorders (16). Bai et al. (2017) showed that 200 mg/kg of silibinin daily for 8 weeks caused decreased memory impairment, oxidative stress, apoptosis, and increased synapse protection (17). In this study, the effect of silymarin (compared to rosuvastatin and placebo) on the brain metabolites (as imaging biomarkers), and the serum level of LRP1 and $A\beta_{1\text{-}42}$ peptides (as blood biomarkers) in Alzheimer patients with secondary dyslipidemia, was investigated.

Methods

Patient selection

In this double-blind, placebo-controlled study,AD patients with the age range of 60 to 80 years were confirmed by a neurologist. The inclusion criteria for entering the study are the following:

a) Confirmation of mild sporadic Alzheimer's disease based on neuropsychological clinical questionnaire;

b) Confirmation of secondary lipid disorder (dyslipidemia) based on biochemical tests and demographic questionnaire;

c) Confirmation of routine MRI imaging based on medial temporal atrophy (MTA), global cortical atrophy (GCA), and expansion of cerebral ventricles (Figure 1).

d) Not having a history of viral hepatitis and active rheumatic disorders,

e) Not having diabetes, advanced heart failure, and uncontrolled hypertension,

f) Not having a history of brain surgery, chronic kidney failure, thyroid disorder, alcohol abuse, smoking, and narcotic drugs.

AD patients with mild dementia were recruited from five medical centers, including Ziaeian (n=197), Roozbeh (n=122), Rasol Akram (n=59), Jam Neurology Clinic (n=217), and Noor neurology clinic (n=65), with a total number of 660 cases in Tehran (Iran), between October 2021 and November 2022. After screening for eligible participants, 36 patients with definite secondary dyslipidemia remained and were randomly allocated into three groups with a 6-block method. All patients took a blood test to evaluate their lipid profiles including triglycerides (TG), low-density lipoprotein (LDL), total cholesterol (TC), and high-density lipoprotein (HDL). After confirmation of the presence of dyslipidemia according to blood tests, patients were divided into 3 groups of 12 patients by random allocation rule and double-blind method

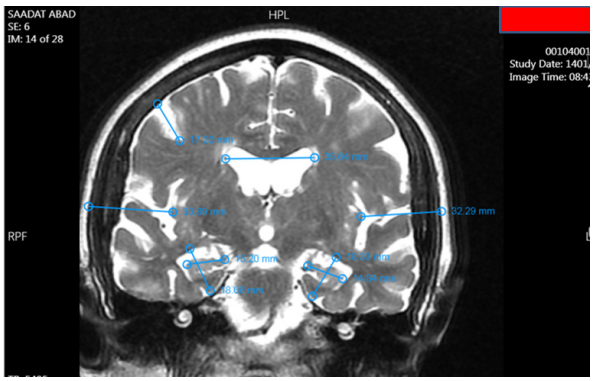

Figure 1. Atrophy of the cerebral and hippocampal cortices in T_2W-MRI image for AD screening

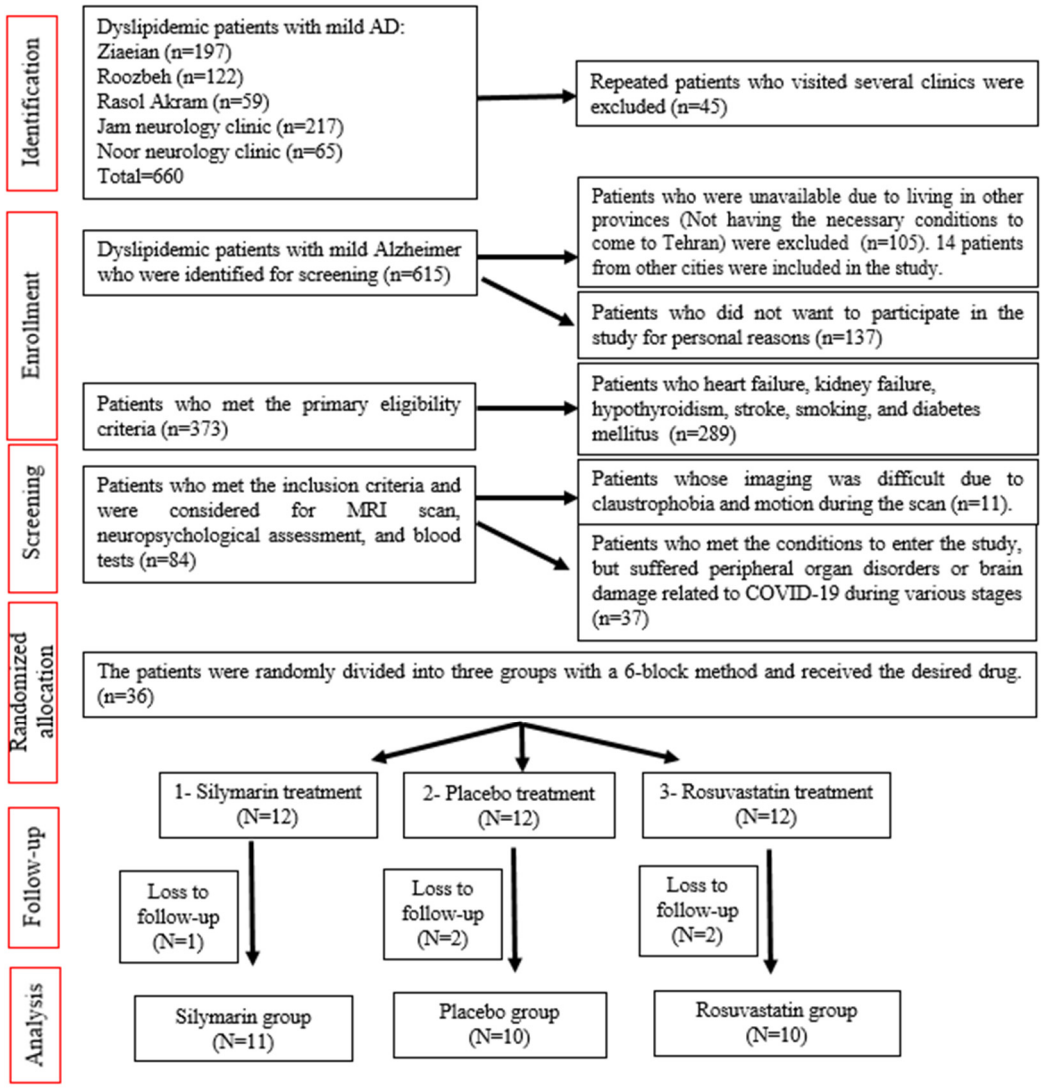

Figure 2. Study flow diagram according to international CONSORT guidelines

(Figure 2). The randomization tool of this study was the website https://www.sealedenvelope.com, which randomly determined the block identifier, block size, sequence within the block, treatment type, and intervention code. Enrollment of participants in interventions was conducted by the study`s neurologists, but the assignment of participants was random to three groups as per the mentioned website-generated schedule (provided by https://www.sealedenvelope.com/). An experienced faculty member with expertise in clinical trials from the Department of Medical Ethics of Iran University of Medical Sciences, who was not among the collaborators of the project, was the Clinical Trial Auditor of the study. After registering the information of the participants, she assigned an allocation code from the codes created by the mentioned system to each patient and recorded it in his confidential file. One of the important capabilities of the sealed envelope website is to create favorable conditions for Allocation concealment. In our study trial participants, investigators, care providers, outcome assessors, and data analysts were blinded. All participants received the medications in sterile pill boxes provided with a unique code.

Study groups including 1- silymarin group: routine medications and 140mg oral silymarin tablet (Livergol 140mg manufactured by GolDaro Pharmaceutical Company; Isfahan, Iran); 2-placebo group (placebo tablet manufactured by GolDaro Pharmaceutical Company; Isfahan, Iran): 140mg placebo along with routine medications; and 3- rosuvastatin group: 10mg oral rosuvastatin (Ropixon, Abidi Pharmaceutical Company; Tehran, Iran) along with routine medications. In all three groups, medications were taken three times a day for 6 months. Exclusion criteria include claustrophobia for magnetic resonance imaging (MRI) scanning, COVID-19 infection, failure to meet medication instructions in the protocol, use of additional therapeutic interventions during the study, and traumatic injury during the study.

Neuropsychological tests

All patients took the Mini-Mental State Examination (MMSE) test, which is 30 points and evaluates brain functions such as 1) Short term memory, 2) Visuospatial abilities, 3) Executive functions, 4) Attention and working memory, 5) Word and Language, and 6) Orientation (18). The Persian language MMSE reliably differentiates cognitively normal individuals from mild cognitive impairment (sensitivity of 83.3% and specificity of 87.5%) and Alzheimer patients (sensitivity and specificity of 100%). According to the results of Ansari et al. (2010) (19) and Barekatain et al. (2010) (20), the Persian version of the MMSE questionnaire is a valid, reliable, and clinically useful tool for the assessment of cognitive disorders or neuropsychiatric illnesses in the Persian-speaking community. The Clinical Dementia Rating (CDR) is a test that assesses cognitive and executive performance (21).

Blood sampling and parameters

Six milliliters of venous blood samples were collected from each fasting participant after neuropsychological tests. Blood samples were collected and all biochemical tests including lipid profile (TC, HDL, LDL, and TG) were performed. Also, the serum levels of LRP1 and Ab_{1-42} were measured with the related ELISA kit (Human $A\beta_{1-42}$ peptide and LRP1 ELISA Kits- ZellBio GmbH, Germany, Veltinerweg-29).

MRS and metabolite processing

Before and after the intervention, patients were scanned at the SaadatAbad Medical Imaging Center with an MRI scanner (1.5 Tesla, Optima MR360 model, GE company, USA), which was capable of spectrometry and multivoxel ^{1}H-MRS, using a standard head coil. MRI sequences included: axial T1-weighted fast spin-echo (T1-FSE) with repetition time/echo time/number of excitations (TR/TE/NEX) 2/14/500 and axial T2-weighted fast spin-echo (T2 -FSE) with TR/TE/NEX was 2/126/4000, respectively. 1H-MRS was performed using multi-voxel two-dimensional point-resolved spatially localized spectroscopy (PRESS) (TE= 144 ms), to evaluate the concentration of metabolites on the T2W images. For post-processing of MRS data was used water suppression, lipid filter, zero filling, frequency shift correction, eddy current correction, and phase correction. The voxel grid (for Voxel Placement) was adjusted so that it matches the bilateral hippocampi. According to the Foy method (2011) (22), the anterior border of the voxel was where the amygdala nucleus disappeared, and its posterior border was 10mm behind this point. In all patients, spectroscopic parameters included TR=1500, TE=144, the field of view (FOV)= 24 cm, and data points=1024. The acquisition time for each sequence was 6 minutes and 12 seconds (Figure 3). Signal reference data was extracted as a file from the MRI console and processed with TARQUIN software. For each MRS spectrum to the international unit (mM), the MRSCloud tool (23) was used to simulate the base set as an external reference. NAA/Cr, Cho/Cr, mI/Cr, and NAA/mI ratios were calculated both before and after intervention, bilaterally for the hippocampus (24).

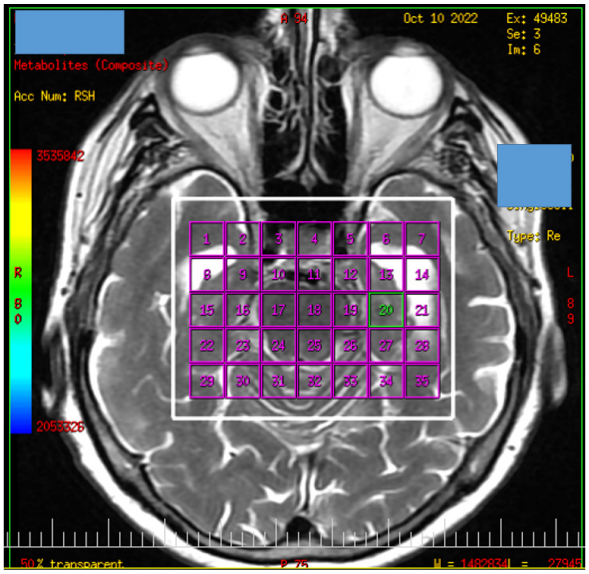

Figure 3. Anatomical localization in multi-voxel MRS on the patient's hippocampi, and capturing metabolite spectra.

Outcomes

According to previous studies, the change in $A\beta_{1-42}$ level (7, 18) and NAA/Cr concentration (25, 26) with a pooled sensitivity of PS=0.81 and PS=0.85, respectively, were considered the primary outcomes. MMSE was considered as a secondary outcome (18). To determine the clinical effectiveness of drugs, the level of clinical significance equal to 1.1 points of increase in MMSE score between groups was considered (27), because statistical significance is different from clinical significance. CDR cognitive test was another secondary outcome. A change of -1.63 points in the CDR- sum of boxes (CDR-SB) score equal to -0.27 in the CDR-global (CDR-G) score (27, 28) was the cutoff point for clinical effectiveness. The change in MMSE and CDR scores was equivalent to a daily dose of 10 mg of Donepezil over a 26-week period (27).

Data analysis

After data collection, it was entered into SPSS software version 20 (SPSS Statistics for Windows, IBM Corp., Armonk, NY, United States). Delta (Δ) of each variable was calculated for patients, and their mean ± standard deviation was used to report the quantitative values. Qualitative data were reported as frequency percentages. A one-way analysis of variance (one-way ANOVA) test was used to compare the means of the quantitative variables that had a normal distribution, and Tukey's post hoc test was used to compare groups. Kruskal–Wallis test and Bonferroni post hoc test were used for the data that did not have a normal distribution. $P < 0.05$ was considered a significant. To measure the correlation between imaging biomarkers, MMSE-CDR scores, and $A\beta_{1-42}$ and LRP1 levels, Pearson and Spearman's correlations were used. GraphPad Prism software (GraphPad Software, version 9) was used for drawing graphs and graphic analysis.

Results

Demographic status and serum markers

All sociodemographic data and baseline levels of variables are mentioned in Table 1. In this study, although statistically there was no significant difference between the groups in the ΔMMSE score ($P > 0.05$), an increase of 1.73 points was seen in the MMSE score of the silymarin group, compared to the placebo group, which is considered higher than the cutoff point of clinical significance (27). Also, in the rosuvastatin group, the clinical score improved by 1.68 points compared to the placebo group. There is no statistically significant difference between the groups in the ΔCDR score ($P > 0.05$). Also, a 0.2-point decrease in the CDR score of patients receiving silymarin was seen, compared to the placebo group, which was below the cut-off point defined for CDR and has no clinical significance. The serum level of ΔLRP1 in the silymarin group (+0.61±0.47) had a statistically significant increase compared to both placebo (+0.04±0.41) and rosuvastatin (-0.19±0.22) groups ($P = 0.0067$ and $P = 0.0002$, respectively). There was a significant increase in the serum level of $\Delta A\beta_{1\text{-}42}$ in the silymarin group (+6.01±3.02), compared to the placebo (-6.66±6.82) and rosuvastatin groups (-1.28±1.71, respectively) ($P = 0.0004$ and $P = 0.0098$) (Figure 4).

Neurometabolites and imaging biomarkers

The ΔmI/Cr of the right hippocampus in the silymarin group (0.85±1.19) had a significant decrease ($P = 0.001$ and $P = 0.015$), compared to both placebo (0.27±0.48) and rosuvastatin (-0.18±1.41) groups. The ΔmI/Cr of the left hippocampus had a significant decrease in both silymarin (-0.66±0.57) and rosuvastatin groups (-0.43±0.97), compared to the placebo group (0.39±0.58) (p=0.008 and $P = 0.034$, respectively) (Figure 5). The ΔNAA/Cr of the right hippocampus was significantly increased in the silymarin group (1.9±1.62) and rosuvastatin (-0.66±2.34) ($P = 0.009$), but in the left hippocampus, there was a significant increase in the silymarin group (1.93±1.74), compared to the placebo group (-1.13±2.55) ($P = 0.003$) (Figure 6). The ΔNAA/mI of the right hippocampus in the silymarin group (13.93±9.62) had a significant increase compared to both placebo (-1.22±1.88) and rosuvastatin (1.73±21.09) groups ($P = 0.002$ and $P = 0.005$, respectively), but in the left hippocampus, there was only a significant increase in the silymarin group (13.95±8.23) rather than the placebo group (-4.71±5.29) ($P = 0.0002$) (Figure 7).

The ΔmI/Cr of the right and left hippocampus directly correlated with ΔTG level (r = 0.603, $P = 0.003$ and r=0.595, P=0.004, respectively). ΔNAA/Cr of the right and left hippocampi was inversely correlated to serum ΔTG level (r = -0.511, $P = 0.003$ and r = -0.532, $P = 0.002$, respectively). The ΔNAA/mI of the right and left hippocampi was inversely correlated to the ΔTG factor (r= -0.610, $P = 0.000$ and r = -0.521, $P = 0.002$, respectively). The ΔmI/Cr and ΔCho/Cr ratios of bilateral hippocampi were inversely correlated to ΔHDL serum level. On the other hand, ΔNAA/Cr and ΔNAA/mI ratios of bilateral

Table 1. Comparison of baseline findings and sociodemographic characteristics in study groups

Variable		Silymarin (n=12)	Placebo (n=12)	Rosuvastatin (n=12)
age (years) Mean (±SD)		71 (6.46)	72.5 (6.02)	72.58 (4.85)
gender (percentage)	Female	66.7	66.7	75
	Male	33.3	33.3	25
Marital status (percentage)	with a partner	58.3	66.7	75
	alone	41.7	33.3	25
Duration of AD (years) Mean(±SD)		2.94 (2.48)	3.16 (1.17)	3 (1.45)
Duration of medication (years) Mean(±SD)		1.29 (0.76)	1.3 (1.04)	1.68 (1.26)
Education level (percentage)	Illiterate	83.3	75	75
	Literate	16.7	25	25
Alzheimer's drug type	Donepezil	66.7	41.7	50
	Rivastigmine	33.3	58.3	50
COVID-19 vaccine	AstraZeneca	16.7 n=2	33.3 n=4	50 n=6
	Sinopharm	45.5 n=10	31.8 n=7	22.7 n=5
	COVIran Barekat	0	50 n=1	50 n=1
MMSE score Mean(±SD)		20.5 (1.73)	20.67 (1.43)	21.17 (1.19)
CDR score Mean(±SD)		0.96 (0.54)	0.88 (0.22)	0.96 (0.39)
SBP (mmHg) Mean(±SD)		127.08 (10.10)	124.58 (7.21)	127.50 (7.53)
DBP (mmHg) Mean(±SD)		80.83 (7.93)	80.83 (6.68)	80 (7.38)
BMI (kg/m^2) Mean(±SD)		27.16 (2.99)	26.70 (2.70)	26.05 (2.62)
TC (mg/dL) Mean(±SD)		229.58 (54.91)	197.92 (43.44)	210.08 (34.79)
TG (mg/dL) Mean(±SD)		210.17 (63.57)	188.75 (96.65)	175.75 (111.42)
HDL (mg/dL) Mean(±SD)		45.59 (7.68)	54.23 (15.45)	55.24 (13.52)
LDL (mg/dL) Mean(±SD)		127.35 (46.61)	106.95 (46.03)	119.7 (46.52)
$A\beta_{1\text{-}42}$ (ng/L) Mean(±SD)		17.04 (1.83)	22.46 (6.10)	19.02 (3.17)
LRP1 (mg/L) Mean(±SD)		2.01 (0.32)	2.15 (0.40)	2.26 (0.42)
mI/Cr	Right hippocampus	1.12(1.17)	0.71(0.46)	0.71(1.06)
Mean(±SD)	Left hippocampus	0.89(0.62)	0.74(0.56)	0.87(0.93)
NAA/Cr	Right hippocampus	3.32(2.42)	5.58(3.65)	6.02(2.59)
Mean(±SD)	Left hippocampus	3.21(2.50)	6.60(3.54)	4.98(2.24)
Cho/Cr	Right hippocampus	1.40(0.98)	0.93(0.60)	1.68(1.14)
Mean(±SD)	Left hippocampus	1.19(0.48)	1.15(0.50)	1.93(2.38)
NAA/mI	Right hippocampus	6.97(11)	25.27(6.93)	16.29(11.29)
Mean(±SD)	Left hippocampus	6.87(9.54)	11.77(6.55)	11.47(10.91)

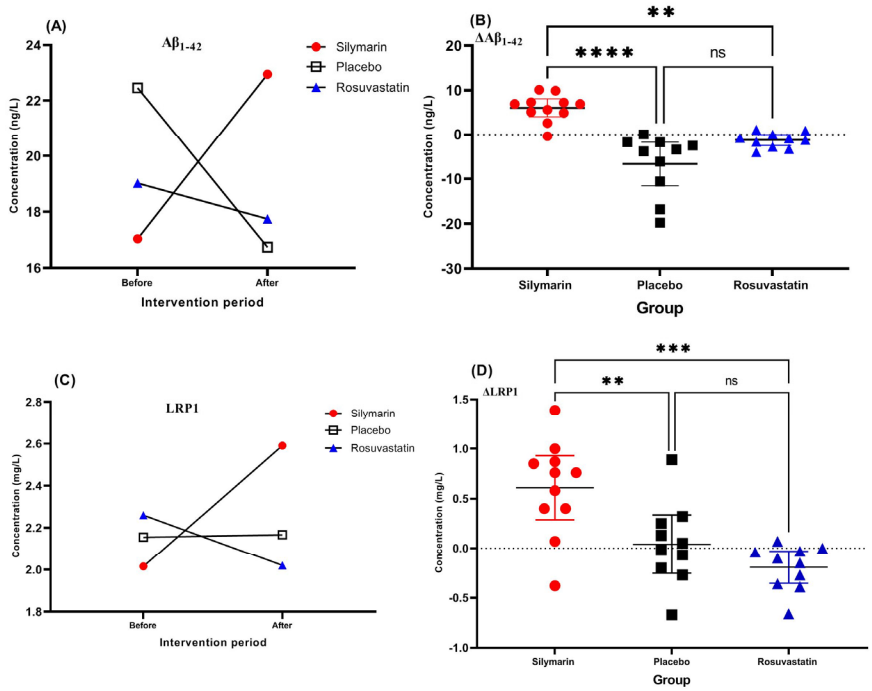

Figure 4. A and C show mean $A\beta_{1\text{-}42}$ and LRP1 levels at the beginning and end of the intervention within the groups, respectively. B and D show the delta (difference) after 6 months of intervention, between the groups, based on the mean with a 95% confidence coefficient (CI 95%). ** $P < 0.001$ and *** $P < 0.0001$.

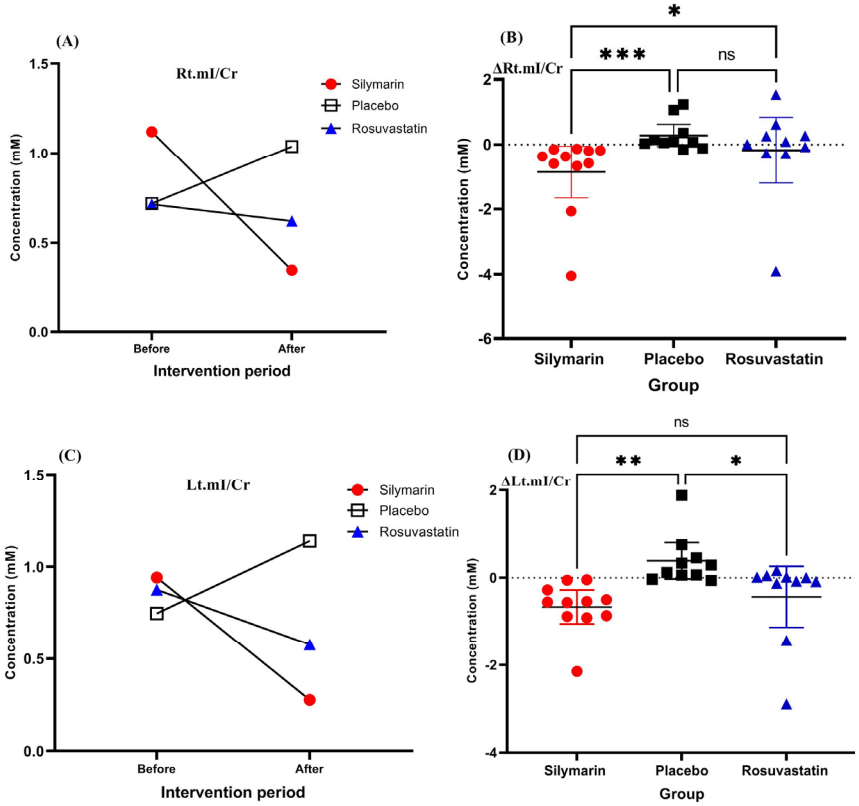

Figure 5. A and C show the mean concentration of mI/Cr in the right and left hippocampus, respectively, at the beginning and end of the intervention within the groups. Images B and D show the delta (difference) after 6 months of intervention between the groups based on the average with a 95% confidence coefficient (CI 95%). * $P < 0.05$, ** $P < 0.001$ and *** $P < 0.0001$.

hippocampi had a direct relationship with ΔHDL serum level (Table 2). An inverse correlation was observed between the $\Delta A\beta_{1\text{-}42}$ marker and ΔmI/Cr of the right and left hippocampi (r = -0.661, $P = 0.000$, and r = -0.638, $P = 0.000$, respectively). The ΔNAA/Cr of the left hippocampus had a direct relationship with $\Delta A\beta_{1\text{-}42}$ (r = 0.681, $P = 0.000$). There was also a direct relationship between $\Delta A\beta_{1\text{-}42}$ and ΔNAA/mI ratio of the bilateral hippocampus.

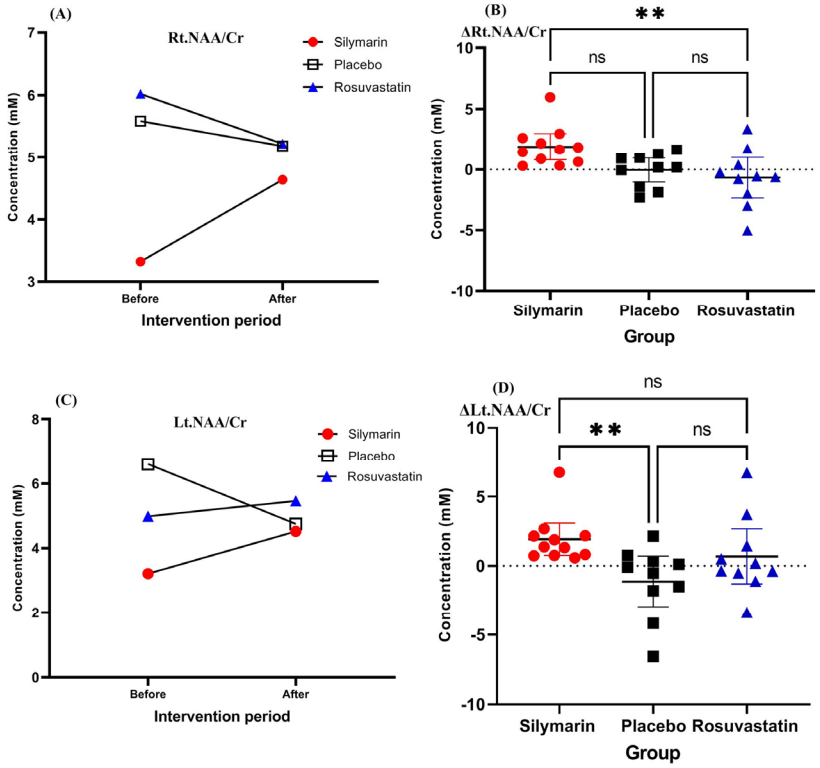

Figure 6. A and C show the mean concentration of NAA/Cr in the right and left hippocampus, respectively, at the beginning and end of the intervention within the groups. Images B and D show the delta (difference) after 6 months of intervention between the groups, based on the average with a 95% confidence coefficient (CI 95%). ** $P < 0.001$.

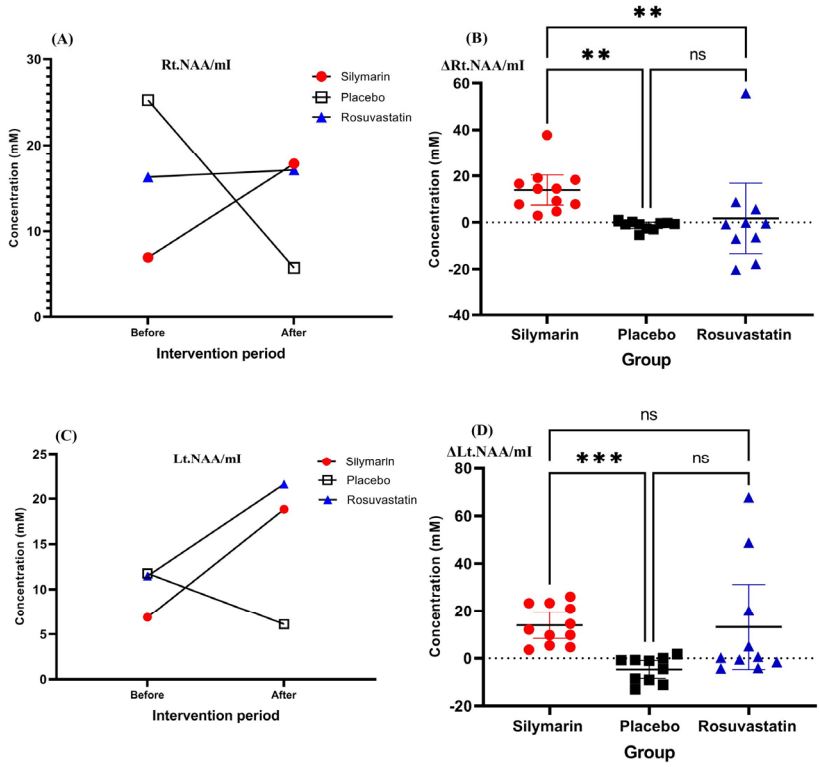

Figure 7. A and C show the mean concentration of NAA/mI, respectively, in the right and left hippocampus at the beginning and end of the intervention within the groups. Images B and D show the delta (difference) after 6 months of intervention between the groups, based on the average with a 95% confidence coefficient (CI 95%). ** $P < 0.001$ and *** $P < 0.0001$.

Inverse, direct, and direct correlations were observed between ΔLRP1 and mI/Cr, ΔLRP1 and ΔNAA/Cr, ΔLRP1 and ΔNAA/mI ratios of the right hippocampus, respectively (Table 3).

Side effects report in the study groups

The adverse events (AE) in the silymarin group were very few; one patient complained of headache and dizziness, and another patient complained of sleep disturbance.

Table 2. Correlations of quadruple neurometabolite ratios with quadruple lipid profile

Variable	Hippocampus	ΔLDL		ΔHDL		ΔTG		ΔTC	
		r [95% CI]	*P*	r [95% CI]	*P*	r [95% CI]	*P*	r [95% CI]	*P*
ΔmI/Cr	Right	0.089 [-0.2840-0.4388]	0.634	0.389 [-0.6590--0.02840]	0.031	0.603 [0.3066-0.7930]	<0.000	0.136 [-0.2395-0.4765]	0.464
	Left	0.183 [-0.1933-0.5131]	0.323	-0.534 [-0.7514--0.2102]	0.002	0.595 [0.2946-0.7881]	<0.000	0.205 [-0.1712-0.5297]	0.267
ΔNAA/Cr	Right	0.176 [-0.2325-0.4822]	0.341	0.378 [0.08951-0.6923]	0.036	-0.511 [-0.7377--0.1808]	0.003	0.232 [-0.1909-0.5149]	0.216
	Left	0.013 [-0.3530-0.3747]	0.946	0.394 [0.02768-0.6586]	0.028	-0.532 [-0.7507--0.2088]	0.002	-0.081 [-0.4319-0.2918]	0.665
ΔCho/Cr	Right	0.071 [-0.3010-0.4236]	0.706	-0.360 [-0.6398-0.004898]	0.047	0.345 [-0.02174-0.6297]	0.057	0.239 [-0.1373-0.5543]	0.196
	Left	0.051 [-0.3186-0.4075]	0.784	-0.366 [-0.6450--0.003933]	0.043	0.225 [-0.1512-0.5444]	0.223	-0.054 [-0.4095-0.3164]	0.774
ΔNAA/mI	Right	0.015 [-0.3512-0.3764]	0.938	0.382 [0.01729-0.6527]	0.034	-0.610 [-0.7975--0.3176]	<0.000	-0.030 [-0.3900-0.3372]	0.871
	Left	-0.127 [-0.4689-0.2487]	0.497	0.487 [0.1425-0.7193]	0.006	-0.521 [-0.7442--0.1946]	0.002	-0.219 [-0.5397-0.1577]	0.237

r=correlation strength, p=significance, CI=Confidence interval.

Table 3. Correlations of quadruple neurometabolite ratios with amyloid-beta and LRP1

Variable	Hippocampus	ΔLRP1		$\Delta A\beta_{1-42}$	
		r [95% CI]	*P*	r [95% CI]	*P*
ΔmI/Cr	Right	-0.364 [-0.6431--0.0006]	0.044	-0.661 [-0.8268--0.3929]	<0.000
	Left	-0.304 [-0.6012-0.0676]	0.097	-0.638 [-0.8117--0.3533]	<0.000
ΔNAA/Cr	Right	0.384 [0.04415-0.6679]	0.033	0.282 [0.06173-0.6775]	0.123
	Left	0.269 [-0.1053-0.5764]	0.144	0.681 [0.4235-0.8381]	<0.000
ΔCho/Cr	Right	-0.014 [-0.3762-0.3514]	0.939	-0.220 [-0.5411-0.1558]	0.234
	Left	0.092 [-0.2813-0.4411]	0.623	-0.219 [-0.5391-0.1584]	0.237
ΔNAA/mI	Right	0.373 [0.01048-0.6488]	0.039	0.688 [0.4316-0.8410]	<0.000
	Left	0.293 [-0.0788-0.5939]	0.109	0.747 [0.5278-0.8740]	<0.000

r=correlation strength, p=significance, CI=Confidence interval.

Table 4. The adverse events (AE) of drugs in three study groups

Type of complication	Silymarin (n=11)	Placebo (n=10)	Rosuvastatin (n=10)
Nausea	0%	0%	10% (n=1)
Urinary disorder	0%	40% (n=4)	20% (n=2)
Defecation disorder	0%	0%	0%
Dizziness	9.1% (n=1)	20% (n=2)	30% (n=3)
Muscular pain	0%	10% (n=1)	10% (n=1)
Headache	9.1% (n=1)	30% (n=3)	20% (n=2)
Tinnitus	0%	10% (n=1)	0%
Anxiety/Restlessness	0%	0%	0%
Sleep disturbance	9.1% (n=1)	20% (n=2)	20% (n=2)
Appetite status	0%	50% (n=5)	30% (n=3)

The most important complication in the rosuvastatin group was dizziness and loss of appetite (30%). Then the frequency of urination, headache, and irregular sleep were other complaints of patients in this group (20%). Placebo caused loss of appetite in half of the patients in this group. Other complications in the placebo were urinary disorder (40%) in the form of a burning sensation and frequent urination and diffuse headache (30%) (Table 4). There were no serious adverse events (SAEs) including death and heart failure in the groups. One patient died during the study due to hospitalization due to a fracture of the femur, followed by COVID-19.

Discussion

It is reported that silybin could act as a dual inhibitor of Aβ and AChE aggregation and a potential therapeutic strategy for the treatment of AD (15). In AD patients and in the elderly, brain LRP1 levels are significantly reduced and inversely related to the age of AD onset, suggesting that decreased LRP1 function causes cognitive decline (29). As a result of these events, Aβ enters the brain parenchyma from the blood, and causes the deposition of Aβ plaques (30). Dyslipidemia causes impairment of Aβ clearance by reducing the expression of LRP1 in the endothelial cells of cerebral vessels, which results in decreased peripheral clearance of Aβ, leading to the accumulation of Aβ in the form of senile plaques (31). Silymarin potentially inhibits Aβ oligomerization (16) and can cross the blood-brain barrier (BBB), so the improvement of MMSE score in the silymarin group is probably due to this property and its neuroprotective effects on brain tissue. MRS can provide a direct and accurate assessment of the damage caused by AD in brain tissue (32), especially the NAA/Cr metabolite reflects the volume of nerve tissue,

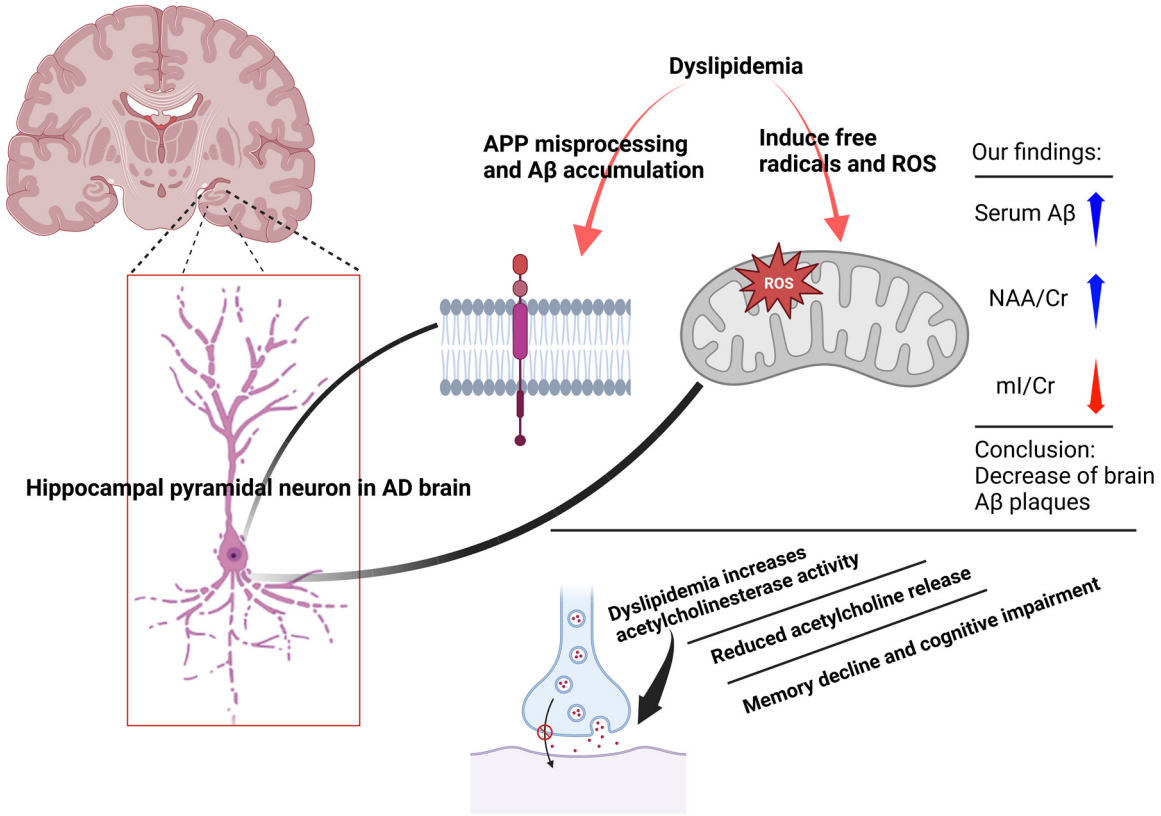

Figure 8. There are inverse and direct correlations of ΔNAA/Cr (as a neuron integrity marker) and ΔmI/Cr (as a glial/neuroinflammation marker) metabolites with dyslipidemia. Silymarin decreased ΔmI/Cr and increased ΔNAA/Cr, and probably through this increased acetylcholine activity improved cognitive status. ΔNAA/Cr and ΔmI/Cr concentrations bi-directionally depend on the peripheral clearance rate of Aβ and predict the progression of AD pathology.

and is of great importance for evaluating the effectiveness of drug trials in AD patients (33). Voevodskaya et al. (2019) showed that mI/Cr and NAA/mI are potential markers for investigating Aβ-related pathology (34). According to the report of Schott et al. (2010), the concentration of NAA/mI in AD patients, decreases by 3.7%, compared to cognitively normal subjects (35). Kantarci et al. (2008) reported that there is a relationship between increased Aβ deposition in the brain, and high levels of mI/Cr (25). So in our study, it can be concluded that silymarin probably reduces brain Aβ deposition by reducing mI/Cr concentration. Lee et al. (2007) showed that there is a significant relationship between NAA/Cr and MMSE in AD patients, and the gradual decrease of NAA/Cr in these patients was accompanied by a decrease of MMSE score (36). One other feature of the present study was the direct correlation of ΔmI/Cr, and the inverse correlation of ΔNAA/Cr and ΔNAA/mI metabolites of the bilateral hippocampi with the ΔTG factor (Figure 8). Banks et al. (2018) showed that TG crosses the BBB and reduces cognitive capacity through leptin resistance (37). Nedelska et al. (2017) showed that mI levels increase with age and Aβ accumulation occurs in brain tissue. Also, high mI in cognitively normal subjects, reflects the increase of Aβ accumulation, over time (38). Targosz et al. (2013) showed that mI/Cr can predict cognitive decline in AD patients, with 70% sensitivity and 85% specificity (39). Voevodskaya et al. (2016) showed that the reduction of $A\beta_{1\text{-}42}$ in CSF, has a statistically significant relationship with the increase of mI/Cr, and also the decrease of NAA/Cr. They reported that mI/Cr in the precuneus lobe was increased in patients with normal $A\beta_{1\text{-}42}$ levels in CSF, so the evaluation of this metabolite could detect AD pathological changes before pathological amyloid deposition (40). Andersson et al. (2023) showed that the $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio in CSF and serum of AD mice significantly decreases with age and is associated with an increase in Aβ plaque burden in the brain (41). In our study, there was an inverse and direct correlation between the serum level of $\Delta A\beta_{1\text{-}42}$ with ΔmI/Cr and $\Delta A\beta_{1\text{-}42}$ with ΔNAA/mI of the bilateral hippocampus, respectively, which is in line with the results of Voevodskaya et al. (2016), Nakamura et al. (2018), and Andersson et al. (2023).

Conclusion

This study showed the relationship between plasma lipids, especially TG and HDL, with Aβ clearance in dyslipidemic AD patients and suggests the measurement of these lipids as easily accessible biomarkers to monitor the response to medications. Donepezil combined with silymarin is effective in modulating lipid profile, increasing LRP1-depended Aβ clearance, and improving antioxidant enzymes in mild dyslipidemic AD patients after 6 months. Also, NAA/Cr, mI/Cr, and NAA/mI ratios have a

significant relationship with the level of $A\beta_{1-42}$, TG, and HDL factors, which can promote monitoring of the prognosis and clinical status of the patients. Moreover, high mI/Cr concentration and low NAA/Cr (and overall low NAA/mI ratio) in the silymarin group are indicators of neurodegenerative processes in AD patients after a 6-month intervention.

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Ethics Approval and Consent to Participate

This BIBA project (Blood and Imaging Biomarkers of Alzheimer's Disease) was performed based on Good Clinical Practice (GCP) guidelines. This study was conducted under the supervision of the Iran University of Medical Sciences (IUMS) Ethics Committee IR.IUMS.FMD.REC.1400.409 and Iran Clinical Trial Registration Center (approved IRCT code: IRCT20210901052360N1). The written consent obtained from the participants or their legal representatives was approved by the IUMS ethics committee.

Conflict of Interests

The authors declare that they have no competing interests.

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