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Association of Circulating Neurodegenerative Biomarkers with Cognitive Impairment in Type 2 Diabetes Mellitus

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Abstract

Background

Type 2 diabetes mellitus (T2DM) has been increasingly associated with neurocognitive and neurodegenerative changes, but the underlying mechanisms and the potential of circulating biomarkers for early detection remain unclear. We measured serum levels of neurofilament light chain (NfL), carbonic anhydrase II (CA-II), and amyloid-beta 42 (Aβ42) and examined their associations with diabetes status and screening-defined cognitive status.

Methods

This cross-sectional study included 90 individuals with T2DM and 90 non-diabetic controls. Cognitive function was evaluated using the Montreal Cognitive Assessment (MoCA), and blood samples were collected to measure NfL, Aβ42, and CA-II concentrations. We compared cognitive scores, biomarker concentrations, and clinical characteristics between the groups. Participants were also classified according to their cognitive status. Age-adjusted analysis of covariance (ANCOVA) was then used to examine the independent and combined associations of diabetes and cognitive status with biomarker levels.

Results

The study included 180 participants (90 with diabetes and 90 without diabetes). Compared with non-diabetic participants, those with diabetes had significantly higher serum neurofilament light chain (NfL) levels (27.85 ± 12.88 vs. 22.53 ± 8.34 pg/mL, $P = 0.001$) and lower amyloid-beta 42 (Aβ42) levels (26.86 ± 13.29 vs. 34.21 ± 17.11 pg/mL, $P = 0.002$), whereas carbonic anhydrase II (CA-II) levels did not differ significantly. Participants with cognitive impairment (MoCA < 26) had significantly lower Aβ42 and higher CA-II levels than those with normal cognition (both $P < 0.001$), while NfL levels were comparable between cognitive groups ($P = 0.462$). In age-adjusted analyses, diabetes was significantly associated with higher NfL levels ($P = 0.001$), whereas cognitive status and the diabetes–cognitive status interaction were not significant. Both diabetes and cognitive status were significantly associated with Aβ42 levels (both $P < 0.001$), with a significant interaction between them ($P < 0.001$). For CA-II, cognitive status ($P = 0.015$) and its interaction with diabetes ($P = 0.001$) were significant, whereas the main effect of diabetes was not ($P = 0.724$). The highest adjusted CA-II levels were observed in participants with both diabetes and cognitive impairment.

Conclusion

Circulating NfL, Aβ42, and CA-II showed different patterns of association with diabetes and cognitive status. NfL was associated with diabetes irrespective of cognitive status, which may reflect diabetes-related neuroaxonal injury. Aβ42 was associated with both diabetes and cognitive status, with evidence of an interaction between the two. CA-II was not associated with diabetes alone but was associated with cognitive impairment, particularly among participants with T2DM. These findings suggest that a multi-marker approach may help characterize neurocognitive changes in T2DM. Longitudinal studies are needed to determine whether these biomarkers predict cognitive decline and to define their clinical utility.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder associated with vascular and neurological complications, including cognitive dysfunction. Type 2 diabetes mellitus (T2DM), in particular, is associated with an increased risk of cognitive decline and dementia, although cross-sectional studies do not consistently show differences in cognitive performance(1, 2). This variability may reflect the heterogeneous effects of diabetes on the brain, which are influenced by disease duration, metabolic control, and aging. Moreover, diabetes-related neurobiological changes may precede clinically apparent cognitive impairment, underscoring the need for biomarkers that capture early or distinct biological changes associated with cognitive vulnerability(3–5).

A major challenge in understanding diabetes-related cognitive dysfunction is its biological and clinical heterogeneity. Neuroimaging, cerebrospinal fluid (CSF) analysis, and positron emission tomography (PET) provide valuable information on structural, functional, and pathological brain changes, but their invasiveness, cost, and limited availability restrict routine clinical use. Blood-based biomarkers offer a more accessible, minimally invasive means of investigating neurobiological changes associated with cognitive dysfunction. Circulating proteins may provide complementary information on neuroaxonal injury, amyloid-related processes, and metabolic or cellular disturbances, allowing different biological pathways potentially involved in cognitive impairment to be examined within the same population(6, 7).

Among candidate biomarkers, neurofilament light chain (NfL), amyloid-beta 42 (Aβ42), and carbonic anhydrase II (CA-II) may represent different aspects of neurobiological dysfunction(8–10). NfL is a neuronal cytoskeletal protein and a sensitive circulating marker of axonal injury across a range of neurological disorders(9, 11). Aβ42 is a proteolytic fragment of amyloid precursor protein that is closely related to amyloid pathology; however, circulating Aβ42 concentrations should not be regarded as a direct measure of cerebral amyloid deposition(10, 12). CA-II is a zinc-dependent metalloenzyme involved in acid–base regulation and cellular homeostasis and has also been implicated in neuronal and neurovascular processes. This provides a rationale for examining CA-II as a potential marker of metabolic or neurovascular alterations relevant to cognition in T2DM. Nevertheless, the relationship between circulating CA-II and cognitive impairment in T2DM has not been well characterized(8, 13, 14).

Despite growing evidence linking T2DM with cognitive dysfunction and with changes in individual circulating biomarkers, it remains unclear whether these biomarkers show distinct patterns of association with diabetes and with cognitive status, and whether diabetes modifies the relationship between biomarker levels and cognitive impairment. Addressing these questions may help distinguish biomarker patterns related primarily to diabetes from those more closely associated with cognitive dysfunction.

We therefore measured serum NfL, Aβ42, and CA-II in adults with and without T2DM and examined their associations with diabetes status and MoCA-defined cognitive status. We first compared biomarker levels according to each factor and then tested whether the association between each biomarker and cognitive status differed by diabetes status. Given the distinct biological characteristics of these markers, we examined whether their associations with diabetes and cognitive status followed different patterns, with particular attention to CA-II as a candidate biomarker of cognitive vulnerability in T2DM.

Methods

Study participants and design

This cross-sectional study was conducted at the Diabetes Clinic of Royan Institute, Tehran, Iran. It enrolled 180 adults aged 45–70 years: 90 with T2DM and 90 non-diabetic controls. Participants in the T2DM group had a documented diagnosis of T2DM for at least 5 years before enrollment, according to American Diabetes Association (ADA) criteria. Controls had no self-reported history of diabetes and did not meet ADA criteria for diabetes based on fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) measurements. We collected demographic and clinical data, including age, sex, education level, body mass index (BMI), duration of T2DM, smoking status, and alcohol use, together with biochemical parameters: FBG, HbA1c, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), thyroid hormones (TSH, T3, T4), liver enzymes (ALT, AST), and platelet count (PLT). The study was approved by the Ethical and Research Committee of Royan Institute (No. IR.ACECR.ROYAN.REC.1401.093). Written informed consent was obtained from all participants after a full explanation of the study procedures, including consent for the collection and use of blood samples and relevant personal information for research purposes.

Inclusion and exclusion criteria

Participants were eligible if they were aged 45–70 years and, for the T2DM group, had been diagnosed with T2DM at least 5 years before enrollment. Exclusion criteria were: (1) other types of diabetes; (2) fewer than 6 years of education; (3) inability to complete neuropsychological screening (e.g., speech impairment, hearing loss, or unwillingness to cooperate); (4) acute cerebral illness within the previous 3 months or significant sequelae of cerebrovascular disease; (5) psychosis, Parkinson’s disease, brain tumor, encephalitis, epilepsy, delirium, or head trauma that might affect cognitive function; (6) overt

anxiety or depression; (7) thyroid dysfunction (including hypothyroidism), carbon monoxide poisoning, syphilis, or other systemic diseases that could cause cognitive impairment; (8) severe lung or kidney disease, a history of heart failure, or malignancy; (9) acute metabolic events within the previous 3 months (severe hypoglycemia, diabetic ketoacidosis, or hyperosmolar hyperglycemic state), acute diabetic complications, or a history of severe infection; and (10) alcohol or drug dependence or abuse.

Biochemical measurements

HbA1c was measured with an automated glycohemoglobin analyzer. FBG, TC, TG, LDL-C, and HDL-C were measured with an automated biochemical analyzer (Hitachi 7060C). BMI was calculated as weight in kilograms divided by height in meters squared. Serum T3, T4, and TSH were measured by chemiluminescence immunoassay (CLIA). Liver enzymes (ALT and AST) were measured in all participants.

Cognitive assessment

Cognitive function was assessed with the validated Persian version of the Montreal Cognitive Assessment (MoCA), a brief 30-point screening instrument covering visuospatial and executive functions, naming, memory, attention, language, abstraction, calculation, and orientation. Participants were classified as cognitively normal (MoCA ≥ 26) or cognitively impaired (MoCA < 26). Because the MoCA is a screening instrument, the latter category denotes screening-defined cognitive impairment rather than a clinical diagnosis of mild cognitive impairment or dementia. Diabetes status and cognitive status were then combined to form four analytical subgroups: (1) non-diabetic participants with normal cognition, (2) non-diabetic participants with cognitive impairment, (3) participants with T2DM and normal cognition, and (4) participants with T2DM and cognitive impairment.

Blood collection and serum biomarker measurements

After a 12–14-hour overnight fast, venous blood was collected into plain tubes (without ethylenediaminetetraacetic acid [EDTA] or heparin). Samples were kept on ice for 1 hour and then centrifuged at 3000 × g for 10 minutes. Serum was separated and stored at − 80°C until analysis.

Serum carbonic anhydrase II (CA-II), amyloid-beta 42 (Aβ42), and neurofilament light chain (NfL) concentrations were measured with commercially available human ELISA kits (ZellBio GmbH, Germany) according to the manufacturer's instructions, and are reported in pg/mL. The CA-II assay was based on a biotin double-antibody sandwich method, and concentrations were calculated from the standard curve by point-to-point interpolation. The Aβ42 assay used a double-biotin antibody sandwich method; absorbance was measured at 450 nm, and concentrations were calculated from the standard curve by point-to-point interpolation. NfL concentrations were calculated from the corresponding standard curve.

Statistical analysis

Statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 9. Normality was assessed with the Kolmogorov–Smirnov test. Continuous variables are presented as mean ± standard deviation (SD) if normally distributed and as median (interquartile range, IQR) otherwise; categorical variables are presented as frequencies and percentages. Comparisons by diabetes status and by cognitive status used independent-samples t-tests or Mann–Whitney tests, as appropriate. For the four-group comparisons, one-way ANOVA or Kruskal–Wallis tests were used descriptively. Categorical variables were compared using chi-square or Fisher's exact tests. Because age differed significantly across the four groups, age was included as a covariate in ANCOVA models that tested the effects of diabetes status, cognitive status, and their interaction on each biomarker. A two-sided $P < 0.05$ was considered statistically significant.

Results

Comparison by diabetes status

Age, BMI, sex distribution, education, alcohol consumption, and smoking did not differ significantly between participants with and without diabetes (all $P > 0.05$; Table 1).

Table 1
Baseline characteristics of participants grouped by diabetes status

Variables		Diabetic N = 90	Non-Diabetic N = 90	P value
Age		62.41 ± 8.43	60.73 ± 8.22	0.178[1]
BMI		27.44 ± 3.94	27.66 ± 4.25	0.714[1]
Sex	Male	39(43.3)	31(34.4)	0.248[2]
	Female	51(56.7)	59(65.6)	
Education	Below diploma	15(16.7)	12(13.3)	0.775[2]
	Diploma	41(45.6)	40(44.4)	
	University degree	34(37.8)	38(42.2)	
Alcohol use	Yes	8(8.9)	5(5.6)	0.566[2]
Smoking	Yes	6(6.7)	5(5.6)	1.000[2]

Data are mean ± SD or n (%). [1]Independent-samples t-test and [2]Chi-square test. SD: standard deviation.

As shown in Table 2, participants with diabetes had significantly lower total cholesterol, LDL-C, and HDL-C levels than non-diabetic participants (all P < 0.01). Triglycerides, thyroid function markers, liver enzymes, platelet count, and MoCA scores did not differ significantly between the groups.

Table 2
Clinical and biochemical characteristics of participants grouped by diabetes status

Variables	**Diabetic** **N = 90**	**Non-Diabetic** **N = 90**	**P value**
Total cholesterol (mg/dl)	149.87 ± 35.33	185.98 ± 45.48	< 0.001[1]
Triglycerides (mg/dl)	148.27 ± 101.01	147.48 ± 93.16	0.961[1]
LDL cholesterol (mg/dl)	80.52 ± 25.09	107.10 ± 37.39	< 0.001[1]
HDL cholesterol (mg/dl)	46.23 ± 10.84	52.39 ± 13.59	0.003[1]
TSH (mIU/mL)	1.90(3.28)	1.88(1.66)	0.427[2]
T3 (ng/mL)	1.60(5.50)	1.20(0.41)	0.423[2]
T4 (µg/dL)	8.40(2.67)	8.20(4.10)	0.479[2]
ALT (IU/L)	29.35 ± 19.43	24.32 ± 12.00	0.073[1]
AST(IU/L)	24.72 ± 12.80	22.98 ± 6.92	0.345[1]
PLT (1000/ µl)	224.19 ± 52.43	220.17 ± 51.43	0.688[1]
MoCA	24.60 ± 3.35	24.38 ± 3.78	0.677[1]

Data are mean ± SD or median (IQR). [1]Independent-samples t-test and [2]Mann–Whitney test.

As shown in Fig. 1, participants with diabetes had significantly higher NfL levels than non-diabetic participants (27.85 ± 12.88 vs. 22.53 ± 8.34 pg/mL, P = 0.001) and significantly lower Aβ42 levels (26.86 ± 13.29 vs. 34.21 ± 17.11 pg/mL, P = 0.002). CA-II levels did not differ significantly between the groups (153.70 ± 48.32 vs. 155.80 ± 54.15 pg/mL, P = 0.775).

Comparison by MoCA-defined cognitive status

Participants with MoCA < 26 were significantly older and had lower educational attainment than those with MoCA ≥ 26 (both P < 0.001), and the sex distribution also differed between cognitive groups (P = 0.022). BMI, alcohol consumption, and smoking did not differ significantly (Table 3).

Table 3
Demographic characteristics of participants by cognitive status

Variables		MoCA ≥ 26 N = 87	MoCA < 26 N = 93	P value
Age		58.43 ± 7.41	64.50 ± 8.14	< 0.001[1]
BMI		27.48 ± 4.38	27.61 ± 2.48	0.839[1]
Sex	Male	26(37.1)	44(62.9)	0.022[2]
	Female	61(55.5)	49(44.5)	
Education	Below diploma	3(11.1)	24(88.9)	< 0.001[3]
	Diploma	42(51.9)	39(48.1)	
	University degree	42(58.3)	30(41.7)	
Alcohol use	Yes	7(53.8)	6(46.2)	0.777[2]
Smoking	Yes	7(63.6)	4(36.4)	0.360[3]

Data are mean ± SD or n (%). [1]Independent-samples t-test, [2]Chi square test and [3]Fisher exact test.

Among clinical variables, only total cholesterol differed significantly between cognitive groups, with higher values in participants with MoCA < 26 (P = 0.018); the remaining clinical and metabolic variables did not differ significantly (Table 4).

Table 4
Biochemical characteristics of participants by cognitive status

Variables	MoCA ≥ 26 N = 87	MoCA < 26 N = 93	P value
FBG (mg/dL)	123.32 ± 45.00	127.77 ± 41.09	0.499[1]
HbA1c (%)	6.57 ± 1.69	6.53 ± 1.26	0.859[1]
Total cholesterol (mg/dl)	147(32)	169(63.50)	0.018[2]
Triglycerides (mg/dl)	131(73.5)	123(76)	0.850[2]
LDL cholesterol (mg/dl)	95.61 ± 32.24	91.03 ± 35.65	0.415[1]
HDL cholesterol (mg/dl)	48(19)	49(12)	0.504[2]
TSH (mIU/mL)	1.30(2.31)	2.00(3.48)	0.516[2]
T3 (ng/mL)	2.80(8.90)	1.20(0.67)	0.050[2]
T4 (µg/dL)	8.80(3.80)	8.10(3.40)	0.372[2]
ALT (IU/L)	29(18)	19(8.50)	0.058[2]
AST(IU/L)	24(7)	22(10.50)	0.660[2]
PLT (1000/ µl)	218(33)	209(61)	0.572[2]

Data presented as: mean ± SD or Median (IQR), SD: Standard Deviation, IQR: Interquartile range, [1]Independent-samples t-test, [2] Mann whitney test.

Participants with MoCA < 26 had significantly lower Aβ42 levels than those with MoCA ≥ 26 (26.50 [14.02] vs. 34.85 [16.34] pg/mL, P < 0.001) and significantly higher CA-II levels (167 [42.2] vs. 142.2 [50.2] pg/mL, P < 0.001). NfL levels did not differ significantly between the groups (P = 0.462; Fig. 2).

Combined analysis by diabetes status and cognitive status

Age differed significantly among the four groups (P < 0.001), with participants with MoCA < 26 being older than cognitively normal participants. Education also differed significantly (P = 0.001), with lower educational attainment more common among participants with diabetes and MoCA < 26. BMI, sex, alcohol consumption, and smoking did not differ significantly among the groups (Table 5).

Table 5
Demographic characteristics of participants in the four groups

Variables		Non-DM, MoCA < 26	Non-DM, MoCA ≥ 26	DM, MoCA < 26	DM, MoCA ≥ 26	P value
Age		63.45 ± 8.11	57.61 ± 7.25	65.62 ± 8.08	59.20 ± 7.56	< 0.001
BMI		27.78 ± 3.89	27.52 ± 4.65	27.42 ± 3.75	27.45 ± 4.16	0.973
Sex	Male	21(30)	10(14.3)	23(32.9)	16(22.9)	0.058
	Female	27(24.5)	32(29.1)	22(20)	29(26.4)	
Education	Below diploma	10(37)	2(7.4)	14(51.9)	1(3.7)	0.001
	Diploma	19(23.5)	21(25.9)	20(24.7)	21(25.9)	
	University degree	19(26.4)	19(26.4)	11(15.3)	23(31.9)	
Alcohol use	Yes	3(23.1)	2(15.4)	3(23.1)	5(38.5)	0.710
	No	45(26.9)	40(24)	42(25.1)	40(24)	
Smoking	Yes	2(18.2)	3(27.3)	2(18.2)	4(36.4)	0.775
	No	46(27.2)	39(23.1)	43(25.4)	41(24.3)	

Data presented as: mean ± SD or N(%), SD: Standard Deviation, [1] one-way ANOVA, [2] Chi square test, [3]Fisher exact test.

Total cholesterol, LDL-C, and HDL-C differed significantly among the four groups, with the lowest values in participants with diabetes and MoCA < 26. Triglycerides, thyroid function markers, liver enzymes, and platelet counts did not differ significantly (Table 6).

Table 6
Biochemical characteristics of the four subgroups

Variables	Non-DM, MoCA < 26	Non-DM, MoCA ≥ 26	DM, MoCA < 26	DM,MoCA ≥ 26	P value
Total cholesterol (mg/dl)	182.87 ± 51.22	190.00 ± 37.24	139.43 ± 30.51	161.73 ± 37.06	< 0.001[1]
Triglycerides (mg/dl)	140.05 ± 63.64	156.84 ± 121.12	139.50 ± 62.06	157.94 ± 131.66	0.747[1]
LDL cholesterol (mg/dl)	107.31 ± 39.38	106.84 ± 35.29	75.52 ± 23.04	86.20 ± 26.39	< 0.001[1]
HDL cholesterol (mg/dl)	51.34 ± 12.50	53.71 ± 14.96	45.81 ± 11.43	46.72 ± 10.24	0.021[1]
TSH (mIU/mL)	1.90(2.01)	1.60(2.22)	4.60(8.21)	1.60(2.22)	0.365[2]
T3 (ng/mL)	1.15(0.44)	2.80(9.00)	1.06(1.18)	2.80(9.00)	0.239[2]
T4 (μg/dL)	8.20(3.02)	8.80(2.80)	8.10(6.31)	8.80(2.80)	0.712[2]
ALT (IU/L)	20.50(6.00)	24.00(19.00)	18.00(19.00)	24(19)	0.248[2]
AST(IU/L)	22.00(8.50)	22.00(7.00)	22.00(12.50)	22.00(7.00)	0.897[2]
PLT (1000/ μl)	220.84 ± 51.09	218.94 ± 53.51	231.69 ± 57.51	215.85 ± 45.96	0.669[1]

Data are mean ± SD or median (IQR). [1] one-way ANOVA and [2] Kruskal–Wallis test.

Age-adjusted ANCOVA was used to assess the associations of diabetes status, MoCA-defined cognitive status, and their interaction with NfL levels (Table 7). After adjustment for age, diabetes status was significantly associated with NfL levels (F = 10.91, P = 0.001), with a higher adjusted mean in participants with diabetes than in those without (27.95 ± 1.15 vs. 22.58 ± 1.14). Cognitive status was not significantly associated with NfL (F = 1.54, P = 0.216), and the diabetes × cognitive-status interaction was not significant (F = 1.92, P = 0.168), indicating no evidence that the association between diabetes and NfL differed by cognitive status. Age was not significantly associated with NfL in the adjusted model (F = 0.31, P = 0.581).

Table 7
ANCOVA of NfL (age-adjusted)

Variables			Adjusted mean ± SE	df	SS	F	P
Intercept			-	1	2305.97	19.77	< 0.001
Age			-	1	35.75	0.31	0.581
DM	NO		22.58 ± 1.14	1	1272.80	10.91	0.001
	YES		27.95 ± 1.15				
MoCA	≥ 26		26.34 ± 1.20	1	180.02	1.54	0.216
	< 26		24.19 ± 1.16				
Diabetes × MoCA	No	≥ 26	22.54 ± 1.63	1	223.55	1.92	0.168
		< 26	22.62 ± 1.66				
	Yes	≥ 26	30.14 ± 1.72				
		< 26	25.75 ± 1.60				
Error		-		175	20415.40	-	-
Total		-		180	136455.83	-	-

Age-adjusted ANCOVA was likewise used to examine the associations of diabetes status, cognitive status, and their interaction with Aβ42 levels (Table 8). After adjustment for age, diabetes status was significantly associated with Aβ42 (F = 17.42, P < 0.001), with lower mean levels in participants with diabetes than in those without (26.86 ± 13.29 vs. 34.21 ± 17.11). Cognitive status was also significantly associated with Aβ42 (F = 18.77, P < 0.001), with lower mean levels in participants with MoCA < 26 than in those with MoCA ≥ 26 (26.50 ± 14.02 vs. 34.85 ± 16.34). The diabetes × cognitive-status interaction was also significant (F = 47.46, P < 0.001), indicating that the combined association of diabetes and cognitive impairment with Aβ42 was not simply additive; cell means are shown in Table 8. Age was not significantly associated with Aβ42 in the adjusted model (F = 0.41, P = 0.521).

Table 8
ANCOVA of Aβ42 (age-adjusted)

Variables			Mean ± SD	df	SS	F	P
Intercept			-	1	1857.81	10.78	0.001
Age			-	1	71.15	0.41	0.521
Diabetic	Yes		26.86 ± 13.29	1	3002.98	17.42	< 0.001
	No		34.21 ± 17.11				
MoCA	≥ 26		34.85 ± 16.34	1	3236.02	18.77	< 0.001
	< 26		26.50 ± 14.02				
Diabetic*MoCA	Yes	≥ 26	29.30 ± 16.83	1	8179.17	47.46	< 0.001
		< 26	46.03 ± 15.69				
	No	≥ 26	23.87 ± 10.26				
		< 26	24.41 ± 7.87				
Error		-		175	3060.65	-	-
Total		-		180	212020.70	-	-

In the age-adjusted ANCOVA for CA-II (Table 9), diabetes status was not significantly associated with CA-II (P = 0.724), whereas cognitive status was (P = 0.015). The diabetes × cognitive-status interaction was also significant (P = 0.001): the highest CA-II levels were observed in participants with diabetes and cognitive impairment, and the lowest in participants with diabetes and normal cognition.

Table 9
ANCOVA of CA-II (age-adjusted)

Variables			Mean ± SD	df	SS	F	P
Intercept			-	1	59535.58	24.71	< 0.001
Age			-	1	195.69	0.081	0.776
Diabetic	Yes		153.66 ± 48.32	1	302.07	0.125	0.724
	No		155.85 ± 54.15				
MoCA	≥ 26		144.27 ± 49.49	1	14501.62	6.02	0.015
	< 26		164.55 ± 51.06				
Diabetic*MoCA	Yes	≥ 26	130.96 ± 39.50	1	28440.92	11.81	0.001
		< 26	176.35 ± 45.92				
	No	≥ 26	158.53 ± 55.31				
		< 26	153.50 ± 53.57				
Error		-		175	421626.62	-	-
Total		-		180	4779578.80	-	-

Discussion

Our findings reveal distinct patterns of association among T2DM, cognitive status, and three circulating biomarkers. Global cognitive performance, assessed by the MoCA, did not differ between participants with and without diabetes. Participants with T2DM had higher NfL and lower Aβ42 levels. When participants were classified by cognitive status, those with MoCA < 26 had lower Aβ42 and higher CA-II levels, whereas NfL did not differ. In the four-group analysis, the diabetes × cognitive-status interaction was significant for Aβ42 and CA-II but not for NfL. These findings suggest that diabetes-related changes in circulating biomarkers are not uniform and that the association between some biomarkers and cognitive status may vary by diabetes status.

Diabetes status and circulating biomarkers

MoCA scores did not differ significantly between participants with and without diabetes. Although meta-analyses of prospective studies have reported an increased risk of cognitive impairment and dementia in individuals with T2DM (2), we found no significant difference in global cognitive performance between the groups. This may be partly explained by the comparability of the groups in age, sex, BMI, and education (Table 1) and by the relatively narrow age range (45–70 years). The discrepancy with earlier findings may also reflect differences in study populations, diabetes duration, metabolic control, or

cognitive assessment methods. Importantly, the absence of a difference in MoCA scores does not exclude diabetes-related neurobiological changes that a brief global screening measure may not detect.

NfL was associated with diabetes status irrespective of cognitive status. Participants with T2DM had higher circulating NfL levels than non-diabetic participants, and the association remained significant after age adjustment (P = 0.001). Cognitive status was not significantly associated with NfL (P = 0.216), nor was the diabetes × cognitive-status interaction (P = 0.168). These findings are consistent with a population-based analysis of 2,070 US adults, in which diabetes was associated with higher serum NfL after adjustment for age, sex, race/ethnicity, alcohol use, and kidney function. Higher serum NfL has also been linked to diabetic sensorimotor polyneuropathy and peripheral nerve dysfunction in people with recently diagnosed diabetes(11, 15, 16). and, in type 1 diabetes, to higher HbA1c, accelerated brain aging on MRI, and a greater likelihood of impaired cognition (4). Together, these reports suggest that elevated NfL in diabetes may reflect neuroaxonal injury of central and/or peripheral origin. Chronic hyperglycemia, oxidative stress, mitochondrial dysfunction, inflammation, and microvascular abnormalities may contribute to such injury; however, these mechanisms were not evaluated in our study and should be regarded as possible explanations rather than established mechanisms(16, 17).

The absence of an association between NfL and cognitive status suggests that the higher NfL levels in T2DM were not accompanied by measurable differences in MoCA-defined cognition; elevated circulating NfL may therefore occur in T2DM without clinically detectable cognitive impairment. A similar pattern was observed in the Look AHEAD cohort of individuals with T2DM and overweight or obesity, in which baseline blood biomarker levels were not associated with cognition, whereas increases in NfL and glial fibrillary acidic protein over time marked incident cognitive impairment. Because our data are cross-sectional, we cannot determine whether elevated NfL is an early marker of neurological injury that precedes cognitive decline; longitudinal studies are needed to test whether individuals with higher NfL are at increased risk of subsequent decline(18, 19).

Aβ42 showed a different pattern: circulating Aβ42 levels were significantly lower in participants with T2DM than in non-diabetic participants, and this difference remained significant after age adjustment. Published findings on blood amyloid in diabetes are not uniform. In the HABS-HD cohort, T2D diagnosis, glucose, and HbA1c were related to plasma NfL but not to the plasma Aβ42/40 ratio or total tau, and plasma Aβ40 and Aβ42 have also been examined in relation to T2DM in Chinese adults (20, 21). Differences in assay platform, analyte (Aβ42 alone versus the Aβ42/40 ratio), and population may contribute to these discrepancies. A meta-analysis of prospective studies found that plasma Aβ42 or Aβ40 alone was not significantly associated with later dementia or Alzheimer's disease, whereas the Aβ42/Aβ40 ratio was more informative but heterogeneous across studies (22, 23). More recent work emphasizes the plasma Aβ42/Aβ40 ratio measured on automated platforms. Because we measured Aβ42 alone by ELISA and did not measure Aβ40, our results are not directly comparable with ratio-based studies. Alterations in circulating Aβ42 may reflect changes in amyloid production, clearance, or peripheral–central Aβ dynamics. Serum Aβ42 should not be considered a direct measure of cerebral amyloid deposition; in the absence of CSF or amyloid PET data, our findings indicate an association

between T2DM and altered circulating amyloid-related processes rather than direct evidence of cerebral amyloid pathology(20, 24, 25).

CA-II levels did not differ significantly between participants with and without diabetes, which suggests that CA-II was not simply elevated as a consequence of diabetes. Its association became apparent only when diabetes status and cognitive status were considered together.

Cognitive status and circulating biomarkers

Comparison by cognitive status showed a different biomarker pattern: participants with MoCA < 26 had significantly lower Aβ42 and higher CA-II levels than those with MoCA ≥ 26, whereas NfL levels did not differ between the groups.

The association between CA-II and cognitive status is of particular interest. CA-II plays an important role in acid–base regulation and cellular ion homeostasis, and carbonic anhydrase-related pathways have been implicated in neuronal and neurovascular processes.The higher CA-II levels in participants with lower MoCA scores may indicate an association between altered cellular or metabolic regulation and cognitive vulnerability. However, the biological significance of circulating CA-II in cognitive impairment remains uncertain, and the cross-sectional design does not allow us to determine whether higher CA-II contributes to cognitive impairment, results from it, or reflects another process associated with both.

The lower Aβ42 levels in participants with MoCA < 26 support an association between circulating amyloid-related biology and cognitive performance. However, circulating Aβ42 does not directly reflect cerebral amyloid deposition, and the association may reflect changes in amyloid metabolism or clearance rather than established Alzheimer-type pathology. NfL, in contrast, did not differ between cognitive groups. Taken together, these findings suggest that the three biomarkers may reflect partly distinct biological processes: NfL was more closely associated with diabetes status, whereas Aβ42 and CA-II showed stronger associations with cognitive status. These observations should nevertheless be interpreted cautiously, as they do not establish distinct biological roles for the biomarkers.

Diabetes × cognitive-status interaction

We examined the interaction between diabetes and cognitive status in relation to serum biomarkers using ANCOVA. Age differed significantly among the four groups and was therefore included as a covariate in all models. The three biomarkers showed different patterns of association with diabetes and cognitive status.

For NfL, diabetes was independently associated with higher levels after age adjustment, whereas neither cognitive status nor the diabetes × cognitive-status interaction was significant. Neurofilament proteins have emerged as body-fluid biomarkers of neuroaxonal injury across a range of neurological disorders; although NfL is not disease-specific, it may provide information on neuroaxonal damage, prognosis, and treatment response. The higher serum NfL in individuals with diabetes may therefore reflect neuroaxonal

injury that could occur before clinically detectable cognitive impairment, although our cross-sectional design cannot establish this(16, 26).

For Aβ42, both diabetes and cognitive impairment were associated with lower circulating levels, and the significant interaction indicates that the relationship between cognitive status and Aβ42 differed according to diabetes status; that is, the associations of diabetes and cognitive impairment with Aβ42 were not simply additive. The mechanisms underlying this finding are unclear. Diabetes may affect amyloid-β production, clearance, transport, or peripheral metabolism, which could influence circulating Aβ42 and its relationship with cognitive performance, but our cross-sectional data cannot distinguish among these possibilities. Moreover, lower circulating Aβ42 should not be interpreted as evidence of cerebral amyloid deposition, because circulating and brain amyloid measures may reflect different processes. Studies combining blood-based biomarkers with established measures of cerebral amyloid pathology are needed to clarify the relationship between diabetes, Aβ42, and cognitive performance(20, 22, 27).

For CA-II, the association with cognitive status was significant after age adjustment, as was the interaction with diabetes. The difference between cognitively impaired and cognitively normal participants was concentrated among those with T2DM, whereas non-diabetic participants showed little difference, suggesting that the relationship between CA-II and cognitive status differs by diabetes status. Plasma CA-II has been reported to be higher in patients with Alzheimer's disease than in cognitively normal controls and to increase with age, and carbonic anhydrases have been proposed as therapeutic targets in Alzheimer's disease and neurovascular dysfunction. In a mouse model of Alzheimer's disease with cerebral amyloid angiopathy, FDA-approved carbonic anhydrase inhibitors reduced amyloid-β pathology and improved cognition, although the isoform most prominently implicated was the mitochondrial CA-VB rather than CA-II. These data support the biological plausibility of carbonic anhydrase involvement in cognitive impairment but do not show that circulating CA-II marks it. To our knowledge, direct clinical evidence linking circulating CA-II to cognitive status in T2DM is very limited; our finding should therefore be regarded as a novel observation requiring replication(8, 13, 28).

These findings are consistent with accumulating evidence that diabetes, particularly through chronic hyperglycemia and insulin resistance, may contribute to cognitive dysfunction through multiple interconnected mechanisms, including oxidative stress, neuroinflammation, mitochondrial dysfunction, cerebrovascular injury, and impaired cerebral insulin signaling. These mechanisms can ultimately disrupt neuronal metabolism, synaptic plasticity, and neurotransmission. In this context, chronic hyperglycemia and oxidative stress might create conditions in which altered carbonic anhydrase-dependent pH and bicarbonate homeostasis further influence neuronal function. This link is hypothetical and should not be interpreted as evidence that carbonic anhydrase dysfunction directly causes diabetes-associated cognitive impairment.

Strengths and limitations

Strengths of this study include simultaneously measuring three biomarkers reflecting different biological processes, including a non-diabetic comparison group, and explicitly testing diabetes × cognitive-status interactions. Several limitations should also be acknowledged. First, the cross-sectional design precludes inference about temporal or causal relationships. Second, cognition was assessed with a single screening test (MoCA); the “cognitive impairment” group was defined by a cut-off rather than a clinical diagnosis, and the groups differed in education, which was not included in the ANCOVA models. Third, the models were adjusted for age only. Sex differed between cognitive groups, and CA-II has been reported to differ by sex (18); renal function, BMI, and medication use, which can influence NfL and amyloid levels, were not modeled, and neuropathy was not assessed, so a peripheral contribution to NfL cannot be excluded. Fourth, Aβ42 was measured alone by ELISA, without Aβ40 or the Aβ42/Aβ40 ratio, and ELISA is less sensitive than the ultrasensitive platforms commonly used for blood biomarkers such as NfL and Aβ. In addition, hemolysis was not assessed; because CA-II is abundant in erythrocytes, hemolysis could influence serum CA-II measurements. Fifth, the sample size was modest, no correction was made for multiple comparisons, and no CSF, PET, or MRI measures were available. Larger longitudinal studies with ratio-based amyloid measures, comprehensive neuropsychological testing, and neuroimaging are needed.

Conclusion

T2DM was associated with higher NfL and lower Aβ42 levels, although MoCA scores did not differ significantly between participants with and without diabetes. Cognitive impairment was associated with lower Aβ42 and higher CA-II levels. Significant diabetes × cognitive-status interactions were observed for Aβ42 and CA-II, indicating that the relationship between these biomarkers and cognitive status differed according to diabetes status.

NfL was more closely associated with diabetes status, whereas CA-II showed a more pronounced association with cognitive impairment among participants with T2DM, and Aβ42 showed a more complex, diabetes-dependent relationship with cognitive status. These findings support further investigation of CA-II as a potential blood-based marker of diabetes-related cognitive vulnerability. Longitudinal studies with larger samples, comprehensive cognitive assessment, and measures of cerebral pathology are needed before its predictive value or clinical utility can be determined.

Declarations

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(analysis and interpretation of data), Hossein Fallahi, Negin Omidzahir, and Pegah Rahimizadeh(questionnaire and data collection); Roya Hosseini and Zahra Vahabi(consultant).

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Competing interests: The authors declare no competing interests.

References

1. Yu F, Pituch KA, Maxfield M, Baena E, Geda YE, Pruzin JJ, et al. The associations between type 2 diabetes and plasma biomarkers of Alzheimer's disease in the Health and Aging Brain Study: Health Disparities (HABS-HD). PLoS ONE. 2024;19(4):e0295749.
2. Xue M, Xu W, Ou Y-N, Cao X-P, Tan M-S, Tan L, et al. Diabetes mellitus and risks of cognitive impairment and dementia: A systematic review and meta-analysis of 144 prospective studies. Ageing Res Rev. 2019;55:100944.
3. Talbot K. Brain insulin resistance in Alzheimer's disease and its potential treatment with GLP-1 analogs. Neurodegenerative disease Manage. 2014;4(1):31–40.
4. Sadanand S, Balachandar R. Bharath SJDmr, reviews. Memory and executive functions in persons with type 2 diabetes: a meta-analysis. 2016;32(2):132–42.
5. Amin AM, Mostafa H, Khojah HMJ. Insulin resistance in Alzheimer’s disease: The genetics and metabolomics links. Clin Chim Acta. 2023;539:215–36.
6. Dash SKJRPE, Metabolic, Discovery ID. Cogn Impairment Diabetes. 2008;2(3):218–23.
7. Dhivya KSS, Manigandan G. Cognitive decline in type II diabetes mellitus among post-menopausal women: A cross-sectional study in South India %J. J Appl Pharm Science: ssue: 12; 2024. 139 – 44 p.
8. Lionetto MG. Carbonic Anhydrase and Biomarker Research. New Insights. 2023;24(11):9687.
9. Maalmi H, Strom A, Petrera A, Hauck SM, Strassburger K, Kuss O, et al. Serum neurofilament light chain: a novel biomarker for early diabetic sensorimotor polyneuropathy. Diabetologia. 2023;66(3):579–89.
10. Peng X, Xu Z, Mo X, Guo Q, Yin J, Xu M, et al. Association of plasma β-amyloid 40 and 42 concentration with type 2 diabetes among Chinese adults. Diabetologia. 2020;63(5):954–63.

11. Määttä LL, Andersen ST, Parkner T, Hviid CVB, Witte DR, John J, et al. Serum Neurofilament Light Chain and Structural and Functional Nerve Fiber Loss in Painful and Painless Diabetic Polyneuropathy. Diabetes Res Clin Pract. 2025;223:112098.
12. Huimin C, Xiaofeng F, Shuiyue Q, Ziye R, Changbiao C, Longfei J. Amyloid-β-targeted therapies for Alzheimer's disease: currently and in the future. 2023;3(3):13.
13. Lemon N, Canepa E, Ilies MA, Fossati S. Carbonic Anhydrases as Potential Targets Against Neurovascular Unit Dysfunction in Alzheimer's Disease and Stroke. 2021;Volume 13–2021.
14. Poggetti V, Salerno S, Baglini E, Barresi E, Da Settimo F, Taliani S. Carbonic Anhydrase Activators for Neurodegeneration: An Overview. Molecules. 2022;27(8).
15. Fridman V, Sillau S, Ritchie A, Bockhorst J, Coughlan C, Araya P, et al. Plasma neurofilament light chain concentrations are elevated in youth-onset type 2 diabetes and associated with neuropathy. J peripheral Nerv system: JPNS. 2023;28(3):460–70.
16. Chen Y, Huang S, Cai W, Song J, Sui C. Combined serum neurofilament light chain and Tau as biomarkers of diabetic peripheral neuropathy. 2026;Volume 17–2026.
17. Škorvanová M, Verešpejová AI, Hanes J, Plášil R, Huňarová N, Šofranko J, et al. Plasma neurofilament light chain in type 2 diabetes: Associations with diabetic peripheral neuropathy, age, and renal function in a pilot cross-sectional study. PLoS ONE. 2026;21(9):e0357207.
18. Li L, Li C, Zhu L, Zhu J. Association between serum NfL and cognitive functioning in older adults with diagnosed diabetes: A cross-sectional study from NHANES. Medicine. 2025;104(44):e45602.
19. Vijiaratnam N, Lawton M, Real R, Heslegrave AJ, Guo T, Athauda D, et al. Diabetes and Neuroaxonal Damage in Parkinson's Disease. Mov disorders: official J Mov Disorder Soc. 2022;37(7):1568–9.
20. Wu Y, Wang Z, Yin J, Yang B, Fan J, Cheng Z. Association Plasma Aβ42 Levels with Alzheimer's Disease and Its Influencing Factors in Chinese Elderly Population. Neuropsychiatr Dis Treat. 2022;18:1831–41.
21. Peters K, Davis W, Taddei K, Martins R, Masters C, Davis T, et al. Plasma Amyloid-β Peptides in Type 2 Diabetes: A Matched Case-Control Study. J Alzheimer's Disease. 2017;56:1127–33.
22. Martínez-Dubarbie F, Guerra-Ruiz A, López-García S, Irure-Ventura J, Lage C, Fernández-Matarrubia M et al. Influence of Physiological Variables and Comorbidities on Plasma Aβ40, Aβ42, and p-tau181 Levels in Cognitively Unimpaired Individuals. 2024;25(3):1481.
23. Zhang Y, Chen H, Li R, Sterling K, Song W. Amyloid β-based therapy for Alzheimer's disease: challenges, successes and future. Signal Transduct Target therapy. 2023;8(1):248.
24. Zhang W, Li M, Zhou X, Huang C, Wan K, Li C, et al. Altered serum amyloid beta and cerebral perfusion and their associations with cognitive function in patients with subcortical ischemic vascular disease. Front NeuroSci. 2022;16:993767.
25. Li H, Wang Z. Blood biomarkers for clinical applications in Alzheimer's disease: A narrative review. NeuroMarkers. 2025;2(2):100078.

26. Ciardullo S, Muraca E, Bianconi E, Cannistraci R, Perra S, Zerbini F, et al. Diabetes Mellitus is Associated With Higher Serum Neurofilament Light Chain Levels in the General US Population. J Clin Endocrinol Metab. 2023;108(2):361–7.
27. Odetti P, Piccini A, Giliberto L, Borghi R, Natale A, Monacelli F, et al. Plasma levels of insulin and amyloid beta 42 are correlated in patients with amnestic Mild Cognitive Impairment. J Alzheimer's disease: JAD. 2005;8(3):243–5.
28. Canepa E, Parodi-Rullan R, Vazquez-Torres R, Gamallo-Lana B, Guzman-Hernandez R, Lemon NL, et al. FDA-approved carbonic anhydrase inhibitors reduce amyloid β pathology and improve cognition, by ameliorating cerebrovascular health and glial fitness. Alzheimer's Dement J Alzheimer's Assoc. 2023;19(11):5048–73.

Figures

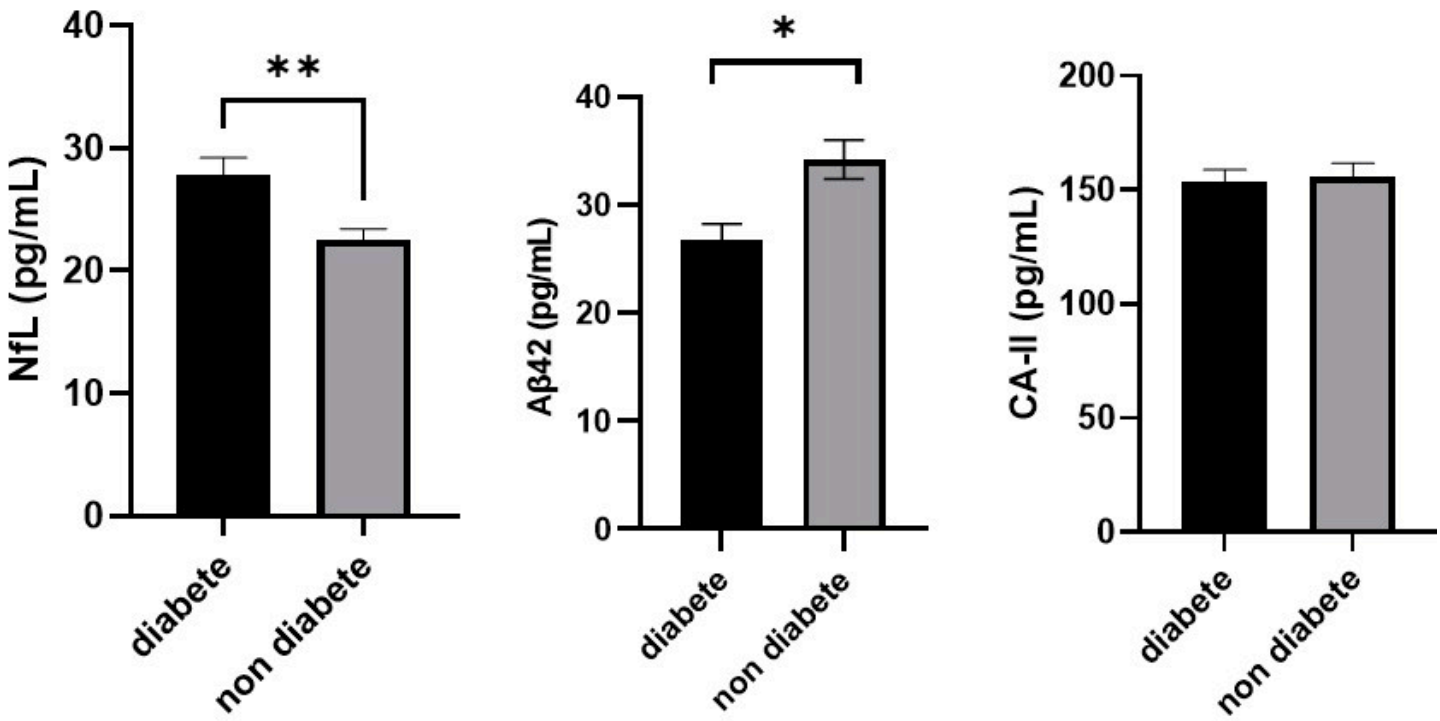


Figure 1

Serum concentrations of NfL, Aβ42, and CA-II in participants with and without diabetes. * P < 0.05, ** P < 0.01, *** P < 0.001.

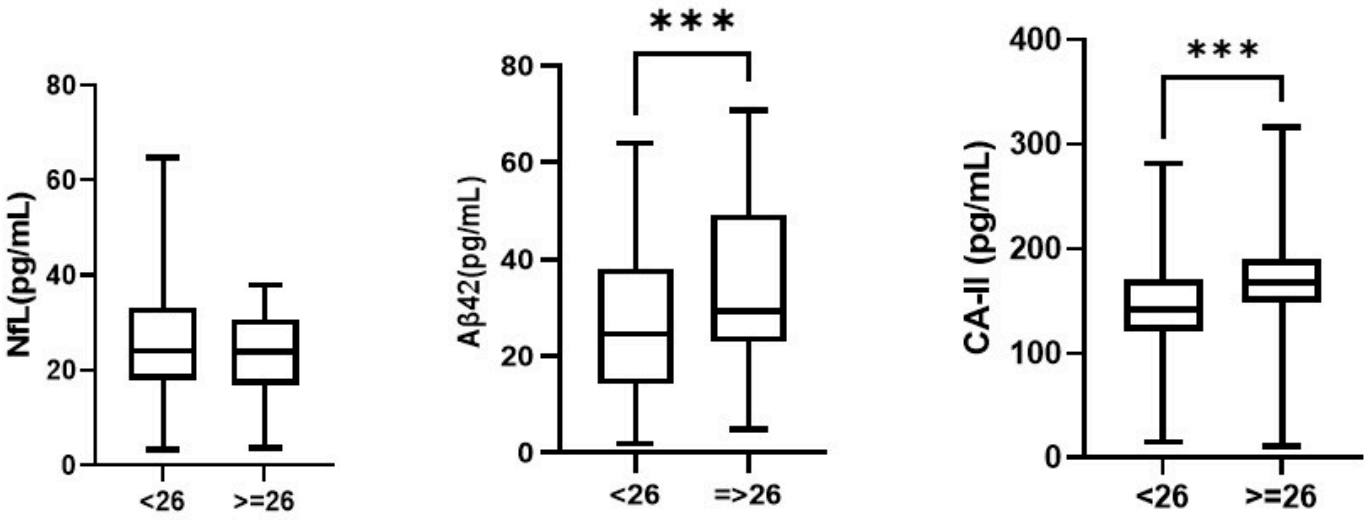


Figure 2

Serum concentrations of NfL, Aβ42, and CA-II by MoCA-defined cognitive status (box plots). * P < 0.05, ** P < 0.01, *** P < 0.001.