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Plasma amyloid-β oligomers level is a biomarker for Alzheimer's disease diagnosis

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ABSTRACT

Amyloid beta (Aβ), especially Aβ oligomers, is important in Alzheimer's disease (AD) pathogenesis. We studied plasma $A\beta_{40}$, $A\beta_{42}$, and Aβ oligomers levels in 44 AD patients and 22 non-demented controls. Cognitive functions were assessed by Chinese version of mini-mental state examination (MMSE), Abbreviated Metal Test (AMT), Alzheimer's Disease Assessment Scale Cognitive Subscale (ADAS-cog). Plasma Aβ monomers and oligomers levels were measured by ELISA. We found that the median plasma $A\beta_{40}$ and $A\beta_{42}$ levels were similar between AD and controls, and without significant correlation with cognition. Plasma Aβ oligomers level was higher in AD than controls (642.54 ng/ml [range 103.33–2676.93] versus 444.18 ng/ml [range 150.19–1311.18], $p = 0.047$), and negatively correlated with cognition. In multivariate logistic regression analysis, the highest tertile of Aβ oligomers levels showed an increased risk of AD than the combined group of middle and lowest tertiles (OR = 8.85, $p = 0.013$), after adjustment of gender, age and APOE4 genotype. Increased plasma Aβ oligomers level was associated with decreased MMSE and AMT scores ($p = 0.037$, $p = 0.043$, respectively) and increased ADAS-cog score ($p = 0.036$), suggesting negative correlation with cognitive function. We concluded that plasma Aβ oligomers level is an useful biomarker for AD diagnosis.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia [1]. It is pathologically characterized by the abnormal accumulation of extracellular senile plaques containing amyloid beta (Aβ) and intracellular neurofibrillary tangles containing hyperphosphorylated tau [2]. Aβ is generated from proteolytic cleavage of amyloid precursor protein (APP) [2,3]. $A\beta_{40}$ and $A\beta_{42}$ are the most common monomeric Aβ isoforms in vivo [4,5]. There is an equilibrium of Aβ between the brain and peripheral blood [6,7]. Recent evidences suggest that soluble Aβ oligomers are the major neurotoxic species especially in early AD [8–10]. Immunization with $A\beta_{42}$ significantly reduced amyloid plaque density and improved cognitive function in AD animal models and AD patients [11–13]. Unfortunately, severe aseptic meningoencephalitis developed in 6% AD patients after active immunization. Aβ antibodies may be effective to remove cerebral Aβ and/or reduce Aβ neurotoxicity [14,15]. Human plasma-derived antibodies, intravenous immunoglobulin, contain Aβ autoantibodies and is a potentially effective treatment for AD [16,17].

Studies on plasma $A\beta_{42}$ and $A\beta_{40}$ levels in AD patients as biomarkers of AD yield inconsistent results. There are only a few studies on plasma Aβ oligomers level as a biomarker of AD. We studied the plasma levels of $A\beta_{42}$, $A\beta_{40}$ peptides and Aβ oligomers of Chinese AD patients and non-demented elderly controls, and their relationship with cognitive function.

2. Subjects

The study was approved by the our regional IRB. This cross-sectional study involved 66 subjects (44 Chinese AD patients and 22 Chinese non-demented elderly controls). All subjects were recruited from the Memory Clinic of Queen Mary Hospital. Written informed consents were obtained from all study subjects and their relatives. The inclusion criteria for controls included no complaints of memory or other cognitive difficulties during the history taking or system review components of examination, and no examination findings suggestive of cognitive compromise. For all participants, exclusion criteria were age below 58 years or over 90 years, active infection, end-stage illness (e.g. renal failure, heart failure), cancer within 5 years, depression by DSM-IV criteria (American

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Psychiatric Association 2000), deafness and other communication barriers. AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [18]. Cognitive status of studied subjects were assessed by the Mini-Mental State Examination (MMSE), Abbreviated Mental Test (AMT) and Alzheimer's disease Assessment Scale-Cognitive Subscale (ADAS-cog).

3. Materials and methods

3.1. Plasma and serum collection

Five milliliters venous blood was collected in EDTA-containing collection tubes for plasma and 5 ml venous blood was collected in clotted blood tubes for serum. After centrifuged at 3000 rpm for 15 min, plasma and serum was stored at −80 °C until used.

3.2. ELISA for plasma Aβ monomers levels

C-terminal capturing antibodies and N-terminal or midregion detecting antibodies are used in standard for ELISA measuring Aβ levels. We employed sandwich ELISA kits (Invitrogen) for measuring plasma $A\beta_{42}$ and $A\beta_{40}$ levels.

3.3. Preparation of Aβ oligomers and western blot to detect Aβ oligomers

Aβ oligomers were prepared as described previously [19]. Solid $A\beta_{42}$ peptide (American peptide company) stored at −80 °C was placed on ice when ready to prepare stock peptide films. 221.7 μl cold HFIP was added to 1 mg $A\beta_{42}$ (final peptide concentration was 1 mM). Peptides were incubated at room temperature (RT) for 1 h, and then placed on ice for 5–10 min. Solution was then aliquotted into non-siliconized microcentrifuge tubes (100 μl solution containing 0.45 mg $A\beta_{42}$) and evaporated overnight at RT. A thin clear film remained at the bottom of the tubes. Twenty micro liters dimethyl sulfoxide (DMSO) was added to 0.45 mg $A\beta_{42}$ (final peptide concentration was 5 mM). After the solution was mixed thoroughly, 980 μl F12 medium without phenol red was added to the solution (final peptide concentration was 100 μM). Solution was incubated at 5 °C overnight and then centrifuged at 14,000*g* for 10 min at 4 °C. The supernatant contained Aβ oligomers. Standard western blot using 12% SDS–PAGE gel, Aβ oligomers antibody (7A1a, New England Agent), and antibody against Aβ residues 1–16 (2C8, Santa Cruz) was performed to confirm successful synthesis of synthetic Aβ oligomers.

3.4. ELISA for plasma Aβ oligomers levels

Rabbit antibody against Aβ N-terminal, Aβ residues1–14 (Abcam) was coated in PBS (50 μl/well) at 5 μg/ml into 96-well opaque microplates at 4 °C. The plates were then washed 4X with PBS-T and blocked with PBS containing 2% bovine serum albumin (BSA) and 0.05% Tween-20 (300 μl/well) for at least 1hr at RT, and stored dessicated at 4 °C until use. An 8-point standard curve (1–1000 ng/ml) was prepared in duplicate using oligomerised $A\beta_{42}$. Serine protease inhibitor, 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) was added to diluted samples (diluted 1:2 with PBS containing 1% BSA) and the standards at a final concentration of 1 mM in order to prevent proteolysis of Aβ peptides. The samples and standards (100 μl/well) were added to the plates and incubated at RT for 2 h. The plates were washed 4× with PBS-T. 7A1a antibody diluted at 1:2500 with PBS containing 1% BSA was added to wells (100 μl/well). The plates were then incubated for 1.5 h at RT with shaking. Rabbit anti-mouse HRP conjugated antibody diluted at 1:5000 in PBS containing 1% BSA was added (100 μl/well) and plates were then incubated for 30 min at RT with shaking. The enhanced chemiluminescent substrate (SuperSignal ELISA Femto Maximum Sensitivity Substrate) was added (100 μl/well) and solution was mixed in wells for 1 min using a microplate mixer. Relative light units (at ~425 nm) were measured by a luminometer 1–5 min after addition the substrate.

3.5. Apolipoprotein E genotyping

The assay of Apolipoprotein E genotyping has been reported previously [31].

3.6. Statistical analysis

In this study, the distributions of Aβ levels were non-normal, so the levels were transformed (log10) before analysis. Correlational analysis was performed using Pearson's simple correlation. The AD group and control group were compared using the Mann–Whitney *U* test. Plasma Aβ oligomers levels were also categorized by tertiles, and the proportions of the highest, middle and lowest tertiles between the AD and control groups were analyzed by Chi-square statistics. Multivariate analyses were then performed to adjust for potential confounders, including gender, age and APOE4 genotype. We used logistic regression analyses to analyze the association between the highest tertile of plasma Aβ oligomer levels and Alzheimer's disease, with gender, age and APOE4 genotype included as covariates in the final model. A p-value of less than 0.05 is considered as statistically significant. All analyses were performed with SPSS 18.0 software.

4. Results

4.1. Clinical features of studied subjects

Demographic characteristics of the AD patients and control subjects recruited in the study are shown in Table 1. AD patients were older than control subjects. As expected, AD patients were more frequently women and had shorter duration of education than controls. The cognitive scores of AD patients and controls are shown in Table 2.

4.2. No differences in plasma $A\beta_{42}$ and $A\beta_{40}$ levels between AD patients and control subjects

There was no difference in the median plasma levels of $A\beta_{42}$, $A\beta_{40}$ and the $A\beta_{42}/A\beta_{40}$ ratio between AD patients and controls ($A\beta_{42}$, 9.94 pg/ml versus 8.42 pg/ml for AD and control respec-

Table 1
Demographic characteristics of AD patients and normal controls.

	AD subjects	Non-demented Subjects
Number of Subjects	44	22
Women (%)	35 (79.5%)	10 (45.5%)†
Median Age (range) in year	81.50 (58–89)	73 (58–86)‡
Median duration of symptoms (range) in year	4 (1–12)	
Median Education (range) in year	0.625 (0–16)	11 (0–16)‡
Smoking history (%)	3 (6.8%)	2 (9.1%)‡
Hypertension (%)	31 (70.5%)	13 (59.1%)
Diabetes mellitus (%)	11 (25%)	5 (22.7%)
APOE4 (1 or 2 allele)	13 (29.5%)	(18.2%)

† $P = 0.005$.
‡ $P < 0.001$.

Table 2
Cognitive scores of AD patients and control subjects.

Group	Median MMSE (range)	Median AMT (range)	Median ADAS-cog (range)
AD patients (44)	17.50 (3–28)	6.00 (0–10)	26.84 (11.67–69)
Controls (22)	29.00 (23–30)†	10.00 (6–10)†	5.00(1–13)†

† $P < 0.001$.

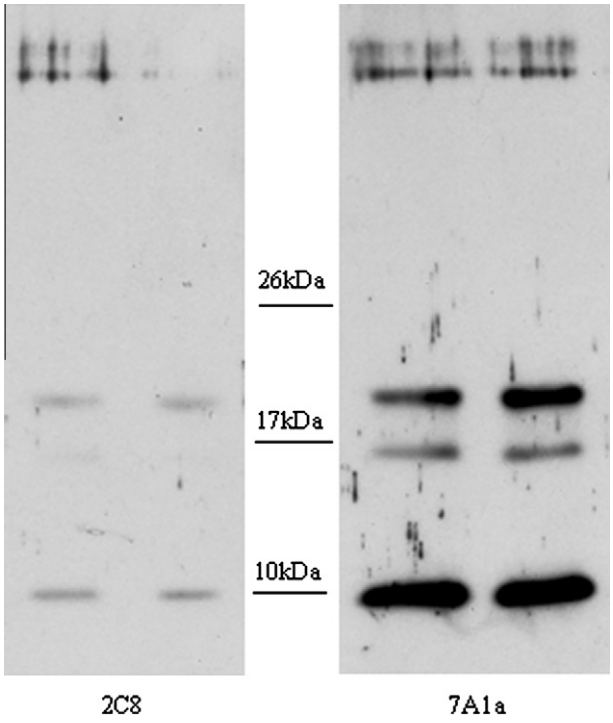

Fig. 1. Western blot of synthetic Aβ oligomers. 2C8 is an Aβ N-terminal antibody and can recognize both Aβ monomers and oligomers. 7A1a is Aβ oligomeric antibody and only recognize Aβ oligomers. Western blot using 7A1a showed three bands at ~10, ~16 and ~20 kDa which represented Aβ dimers, tetramers and pentamers respectively, illustrating that our synthetic Aβ oligomers predominantly contained Aβ dimers and pentamers. Western blot using 2C8 also revealed similar bands but weaker.

tively, $p = 0.187$; $A\beta_{40}$, 145.93 pg/ml versus 130.34 pg/ml for AD and control respectively, $p = 0.196$; $A\beta_{42}/A\beta_{40}$ ratio, 0.071 versus 0.062 for AD and control respectively, $p = 0.550$).

4.3. Correlation of plasma $A\beta_{42}$ and $A\beta_{40}$ levels and cognitive function

There was no significant correlation found between plasma levels of $A\beta_{42}$ and $A\beta_{40}$ with cognitive function in both control subjects and AD patients (not shown).

4.4. Plasma Aβ oligomers level was higher in AD patients and showed an independent association with AD

Western blot analysis confirmed the presence of synthetic Aβ oligomers (Fig. 1). The calibration curve of ELISA for Aβ oligomers is shown in Fig. 2. Plasma Aβ oligomers levels of AD patients and control subjects were estimated according to this curve. The properties of synthetic Aβ oligomers are not identical to that of natural plasma Aβ oligomers. Hence, the Aβ oligomers concentrations measured in our ELISA are the relative concentrations to that of synthetic Aβ oligomers. The median plasma Aβ oligomers level was higher in AD patients than control subjects (642.5 ng/ml versus 441.8 ng/ml, $p = 0.047$) (Fig. 3). Analysis of plasma Aβ oligomers level by tertiles showed a significantly higher proportion of the highest tertile of plasma Aβ oligomer level in AD patients than in controls (43.2% vs 13.6%, $p = 0.041$) (Table 3). There were no correlations detected between gender or age with plasma Aβ oligomers level in either AD patients or controls. Logistic regression analysis, with adjustment of confounders including gender, age and APOE4 genotype, showed that the highest tertile of plasma Aβ oligomer levels was independently associated with an increased risk of AD (O.R. 8.85, 95% CI 1.58 to 49.55) (Table 4).

4.5. Correlation between plasma Aβ oligomers level and cognitive status

There was no significant correlation between sex or age with the median plasma Aβ oligomers level in either AD patients or control subjects (not shown). Increased plasma Aβ oligomers level was associated with decreased MMSE and AMT scores ($p = 0.037$, $p = 0.043$, respectively) and increased ADAS-cog scores ($p = 0.036$) (Fig. 4). These suggested that plasma Aβ oligomers level was negatively correlated with cognitive function.

5. Discussion

We observed that the median plasma $A\beta_{42}$ and $A\beta_{40}$ levels were indifferent between AD and controls. This is consistent with some cross-sectional studies [20,21]. Other studies support that high plasma $A\beta_{42}$ level is a risk factor of AD and plasma $A\beta_{42}$ level declines gradually after development of AD [22,23]. Mehta et al. re-

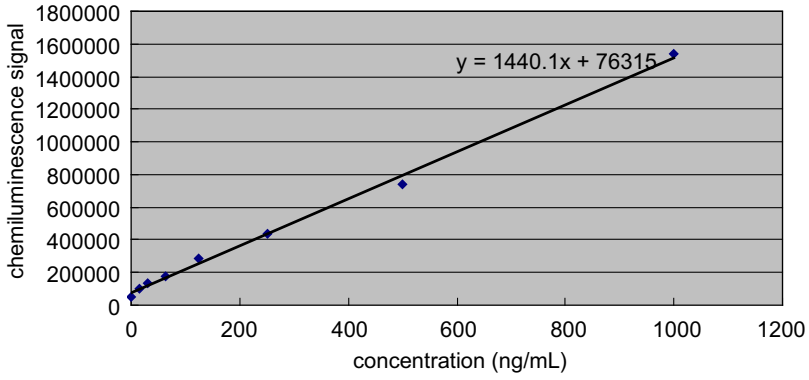

Fig. 2. Calibration curve of ELISA for Aβ oligomers. The 8-point calibration curve of chemiluminescence signal was made at various synthetic Aβ oligomers concentrations (0–1000 ng/ml). Plasma levels of Aβ oligomers of AD patients and control subjects were estimated according to this curve.

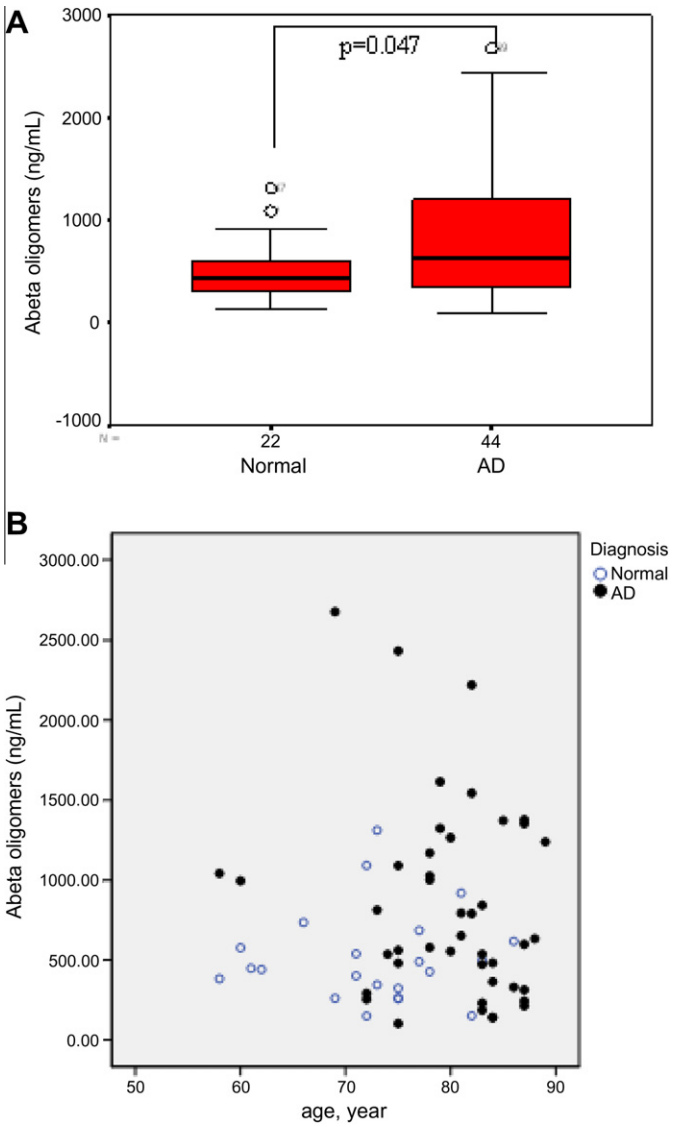

Fig. 3. Plasma Aβ oligomers levels of AD patients and control subjects. (A) The middle line indicates the median Aβ oligomers level whereas the upper and lower lines indicate the upper-quartile and lower-quartile respectively. AD patients have a higher median Aβ oligomers level than control subjects (642.5 ng/ml versus 441.8 ng/ml, $p = 0.047$). (B) Scatter plot of plasma Aβ oligomers level of studied subjects; solid dots indicate AD patients and empty dots indicate normal controls.

Table 3
Plasma Aβ monomer and oligomers levels of AD patients and control subjects.

	AD	Controls
Median $A\beta_{42}$ (range), pg/ml	9.94 (2.42–21.47)	8.42 (3.94–17.64)
Median $A\beta_{40}$ (range), pg/ml	145.93 (57.09–218.13)	130.34 (84.97–231.54)
Median Aβ oligomers (range)	642.54 (103.33–2676.93)*	444.18 (150.19–1311.18)
Plasma Aβ oligomers tertiles		
Lowest tertile (103.3–437.5)	12 (27.3%)	10 (45.5%)
Midddle tertile (431.6–806.6)	13 (29.5%)	9 (40.9%)
Highest tertile (806.7–2676.9)	19 (43.2%)**	3 (13.6%)

* $p = 0.047$.
** $p = 0.041$.

Table 4
Logistic regression analysis of plasma Aβ oligomers (in tertile) and risk of AD.

	Odds Ration (OR)	95% Confidence interval of OR		p
Gender (Women vs Men)	5.07	1.29	19.97	0.02
Age, years	1.19	1.07	1.32	0.01
APOE genotype (4+ vs 4−)	2.82	0.53	15.10	0.23
Plasma Aβ oligomers (Highest tertile vs middle and lowest tertile combined)	8.85	1.58	49.55	0.013

Note: Model adjusted for gender, age and APOE genotypes.

ported that the mean plasma $A\beta_{40}$ level was higher in AD than controls but with substantial overlap while plasma $A\beta_{42}$ levels were similar [24]. The indifference in median plasma $A\beta_{42}$ levels between AD and controls may be explained as despite increased $A\beta_{42}$ production in AD patients, deposition of plasma $A\beta_{42}$ into brain is also increased [23,25]. $A\beta_{42}$ deposits into the brain earlier than $A\beta_{40}$ and constitutes the main form of cerebral Aβ deposits [25]. A cross-sectional study reported that in women with AD, the lowest plasma $A\beta_{42}$ level was found in moderate to severe patients as compared with mild patients ($p < 0.05$) and healthy subjects ($p < 0.01$) [26].

Accumulating evidence supports that soluble nonfibrillar Aβ oligomers are the important neurotoxic species in AD especially in early disease [32]. The amount of soluble Aβ oligomers in postmortem AD brain is correlated with disease symptoms [33,34] whereas amyloid plaque load is not correlated with severity of dementia in AD [35]. In-vitro and in vivo studies suggest that the major pathogenic mechanisms of Aβ oligomers in AD is impairment of synaptic transmission [36–40]. Shankar et al. demonstrated that Aβ oligomers isolated directly from cortex of AD brain potently inhibited long-term potentiation, enhanced long-term depression and reduced dendritic spine density when injected into hippocampus of mice. In addition, Aβ oligomers also impaired memory of a learned behavior in normal rats and these synaptotoxic effects were specifically attributed to Aβ dimers.[41] Other soluble low molecular weight Aβ oligomers such as trimers are also synaptotoxic [36,44]. However, other studies revealed that Aβ oligomers in synapses were between 50 and 100 kDa in molecular weight, [37,39] consistent with the finding of a prominent Aβ oligomer in AD brain extract [45] and that Aβ oligomers of ~56 kDa (Aβ*56) led to cognitive impairment in vivo [38].

CSF Aβ oligomers level was reported to be increased in AD patients [27], and two studies reported that plasma Aβ oligomers level was increased in AD patients [28,29]. Using a combination of immunoprecipitation with immobilization of immunocomplexes to magnetic beads and flow cytometry, Santos et al. studied plasma levels of $A\beta_{40}$, $A\beta_{42}$ and Aβ oligomers from AD patients and normal controls. They reported that plasma Aβ oligomers level allowed differentiation of AD and control with sensitivity of 71% and specificity of 81% [28]. Using sandwich ELISA in which the same Aβ N-terminal antibody was used to capture and detect antigen, Xia et al. found that more than half AD patients had detectable plasma Aβ oligomers levels whereas most control subjects had plasma Aβ oligomers below detection limit, and Aβ oligomers level was higher in brain tissue and plasma of AD patients [29]. In addition, some AD patients had decline in plasma $A\beta_{42}$ and Aβ oligomers levels over a 1–2-year period; and neither the plasma levels of $A\beta_{42}$ nor $A\beta_{40}$ was different between AD patients and controls [29]. We employed sandwich ELISA using a Aβ oligomers conformation-specific antibody, 7A1a, as the detection antibody to measure plasma Aβ oligomers level. Similar ELISA has been employed to

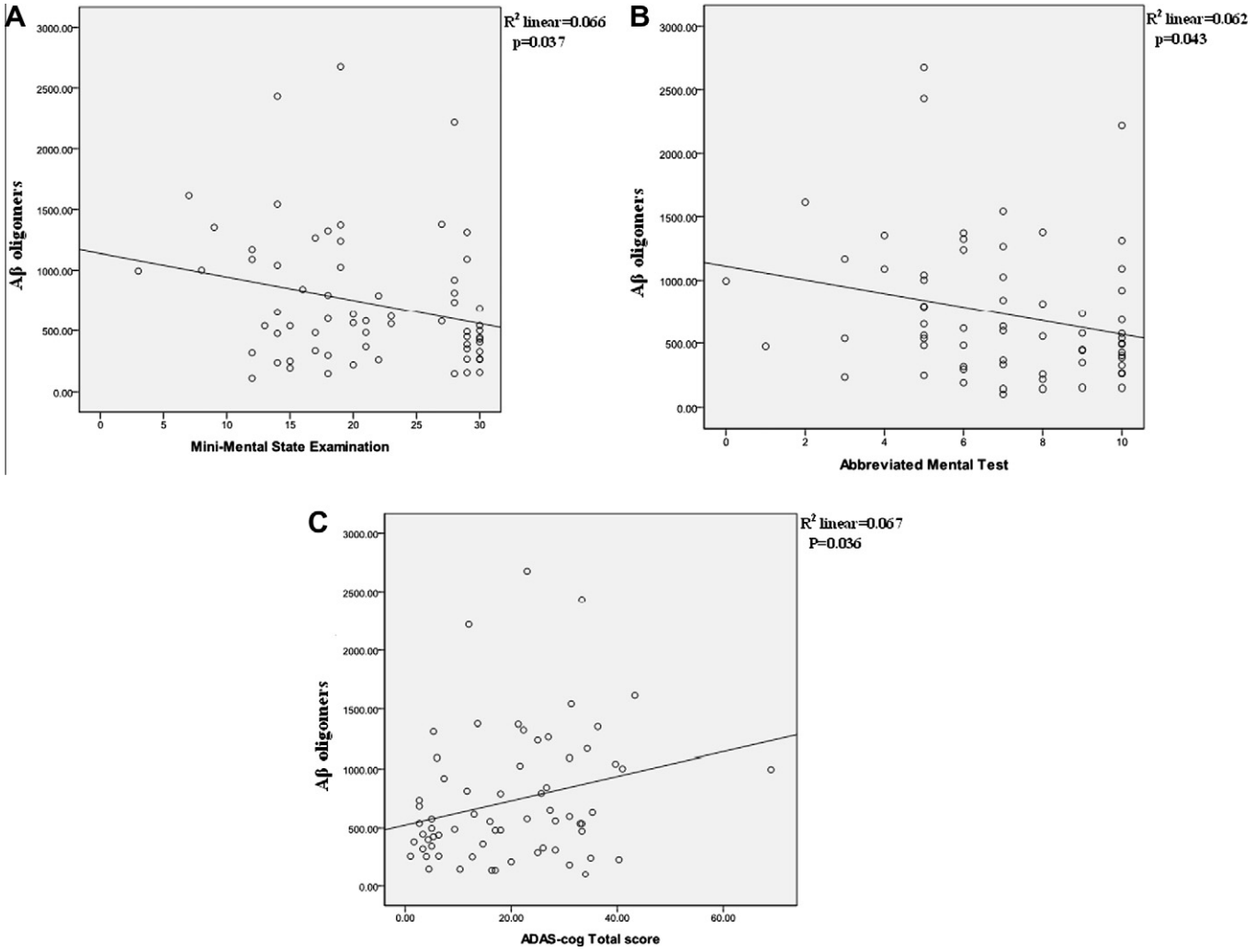

Fig. 4. Correlation between plasma Aβ oligomers level and cognitive function. Plasma level of Aβ oligomers was negatively correlated with cognitive functions as increased plasma Aβ oligomers level was associated with decreased MMSE score ($p = 0.037$) (A) and AMT score ($p = 0.043$) (B), and increased ADAS-cog score ($p = 0.036$) (C).

measure cerebral cortex homogenates Aβ oligomers level by van Helmond et al. who reported that brain homogenates Aβ oligomers level was significantly higher in AD than controls [43]. 7A1a is shown to bind to synthetic Aβ oligomers with different sizes including low molecular weight species such as dimers, trimers and tetramers (~10 to ~18 kDa) and high molecular weight species ranging from 50 kDa to greater than 150 kDa [43]. To adjust for the confounding effects of age, gender and APOE4 genotype, we examined the relationship of the tertiles of plasma Aβ oligomers level with AD by multivariate logictic regression analyses. We found a strong independent association of the highest tertile of plasma Aβ oligomers level with AD versus the combined group of middle and lowest tertiles. Our result is consistent with that of Xia et al. and Santos et al. [28,29]. Our ELISA is a simple convenient assay which measures plasma level of soluble Aβ oligomers of different sizes (low and high molecular weight species) known to be synaptotoxic, which may be used clinically to facilitate AD diagnosis.

Tomic et al. reported that soluble fibrillar Aβ oligomers level was elevated in multiple brain regions of AD patients, and negatively correlated with cognitive function [30]. We found that plasma Aβ oligomers level is negatively correlated with cognitive function in AD, hence it may reflect dementia severity in AD. Our results support a direct pathogenic role of Aβ oligomers in AD, which is clinically relevant as Aβ oligomers-specific immunotherapy such as Aβ oligomers-specific monoclonal antibody is potentially efficacious for prevention and treatment of AD [42].

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References

[1] R. Brookmeyer, E. Johnson, K. Ziegler-Graham, H.M. Arrighi, Forecasting the global burden of Alzheimer's disease, Alzheimers Dement 3 (2007) 186–191.
[2] H.W. Querfurth, F.M. LaFerla, Mechanism of disease: Alzheimer's Disease, N. Engl. J. Med. 362 (2010) 329–344.
[3] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[4] S.A. Gravina, L. Ho, C.B. Eckman, K.E. Long, L. Otvos Jr., L.H. Younkin, N. Suzuki, S.G. Younkin, Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43), J. Biol. Chem. 270 (1995) 7013–7016.
[5] N. Schupf, B. Patel, W. Silverman, W.B. Zigman, N. Zhong, B. Tycko, P.D. Mehta, R. Mayeux, Elevated plasma amyloid beta-peptide 1–42 and onset of dementia in adults with Down syndrome, Neurosci. Lett. 301 (2001) 199–203.
[6] B.V. Zlokovic, Clearing amyloid through the blood-brain barrier, J. Neurochem. 89 (2004) 807–811.

[7] R.E. Tanzi, R.D. Moir, S.L. Wagner, Clearance of Alzheimer's Abeta peptide: the many roads to perdition, Neuron 43 (2004) 605–608.
[8] D.J. Selkoe, Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior, Behav. Brain Res. 192 (2008) 106–113.
[9] C.G. Glabe, Structural classification of toxic amyloid oligomers, J. Biol. Chem. 283 (2008) 29639–29643.
[10] D.M. Walsh, D.J. Selkoe, Aβ oligomers: a decade of discovery, J. Neurochem. 101 (2007) 1172–1184.
[11] D. Schenk, R. Barbour, W. Dunn, G. Gordon, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, Z. Liao, I. Lieberburg, R. Motter, L. Mutter, F. Soriano, G. Shopp, N. Vasquez, C. Vandevert, S. Walker, M. Wogulis, T. Yednock, D. Games, P. Seubert, Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse, Nature 400 (1999) 173–177.
[12] C. Janus, J. Pearson, J. McLaurin, P.M. Mathews, Y. Jiang, S.D. Schmidt, M.A. Chishti, P. Horne, D. Heslin, J. French, H.T. Mount, R.A. Nixon, M. Mercken, C. Bergeron, P.E. Fraser, St George-Hyslop, P., and Westaway, D. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease, Nature 408 (2000) 979–982.
[13] D. Morgan, D.M. Diamond, P.E. Gottschall, K.E. Ugen, C. Dickey, J. Hardy, K. Duff, P. Jantzen, G. DiCarlo, D. Wilcock, K. Connor, J. Hatcher, C. Hope, M. Gordon, G.W. Arendash, A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease, Nature 408 (2000) 982–985.
[14] F. Bard, C. Cannon, R. Barbour, R.L. Burke, D. Games, H. Grajeda, T. Guido, K. Hu, J. Huang, K. Johnson-Wood, K. Khan, D. Kholodenko, M. Lee, I. Lieberburg, R. Motter, M. Nguyen, F. Soriano, N. Vasquez, K. Weiss, B. Welch, P. Seubert, D. Schenk, T. Yednock, Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease, Nat. Med. 6 (2000) 916–919.
[15] J. McLaurin, R. Cecal, M.E. Kierstead, X. Tian, A.L. Phinney, M. Manea, J.E. French, M.H. Lambermon, A.A. Darabie, M.E. Brown, C. Janus, M.A. Chishti, P. Horne, D. Westaway, P.E. Fraser, H.T. Mount, M. Przybylski, And St George-Hyslop, P, Therapeutically effective antibodies against amyloid-beta peptide target amyloid-beta residues 4–10 and inhibit cytotoxicity and fibrillogenesis, Nat. Med. 8 (2002) 1263–1269.
[16] R.C. Dodel, Y. Du, C. Depboylu, H. Hampel, L. Frolich, A. Haag, U. Hemmeter, S. Paulsen, S.J. Teipel, S. Brettschneider, A. Spottke, C. Nolker, H.J. Moller, X. Wei, M. Farlow, N. Sommer, W.H. Oertel, Intravenous immunoglobulins containing antibodies against beta-amyloid for the treatment of Alzheimer's disease, J. Neurol. Neurosurg. Psychiatry 75 (2004) 1472–1474.
[17] N.R. Relkin, P. Szabo, B. Adamiak, T. Burgut, C. Monthe, R.W. Lent, S. Younkin, L. Younkin, R. Schiff, M.E. Weksler, 18-Month study of intravenous immunoglobulin for treatment of mild Alzheimer disease, Neurobiol. Aging 30 (2009) 1728–1736.
[18] G. McKhann, D. Drachman, M. Folstein, R. Katzman, D. Price, E.M. Stadlan, Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's Disease, Neurology 34 (1984) 939–944.
[19] W.L. Klein, Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets, Neurochem. Int. 41 (2002) 345–352.
[20] A. Tamaoka, T. Fukushima, N. Sawamura, K. Ishikawa, E. Oguni, Y. Komatsuzaki, S. Shoji, Amyloid beta protein in plasma from patients with sporadic Alzheimer's disease, J. Neurol. Sci. 141 (1996) 65–68.
[21] H. Fukumoto, M. Tennis, J.J. Locascio, B.T. Hyman, J.H. Growdon, M.C. Irizarry, Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels, Arch. Neurol. 60 (2003) 958–964.
[22] R. Mayeux, M.X. Tang, D.M. Jacobs, J. Manly, K. Bell, C. Merchant, S.A. Small, Y. Stern, H.M. Wisniewski, P.D. Mehta, Plasma amyloid beta-peptide 1–42 and incipient Alzheimer's disease, Ann. Neurol. 46 (1999) 412–416.
[23] R. Mayeux, L.S. Honig, M.X. Tang, J. Manly, Y. Stern, N. Schupf, P.D. Mehta, Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk, Neurology 61 (2003) 1185–1190.
[24] P.D. Mehta, T. Pirttila, S.P. Mehta, E.A. Sersen, P.S. Aisen, H.M. Wisniewski, Plasma and cerebrospinal fluid levels of amyloid beta proteins 1–40 and 1–42 in Alzheimer disease, Arch. Neurol. 57 (2000) 100–105.
[25] N. Graff-Radford, Analysis of plasma Aβ42 as a premorbid marker for late onset Alzheimer's disease, Neurobiol. Aging 25 (2004) 354.
[26] A. Baranowska-Bik, W. Bik, E. Wolinska-Witort, L. Martynska, M. Chmielowska, M. Barcikowska, B. Baranowska, Plasma beta amyloid and cytokine profile in women with Alzheimer's disease, Neuro. Endocrinol. Lett. 29 (2008) 75–79.
[27] M. Pitschke, R. Prior, M. Haupt, D. Riesner, Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy, Nat. Med. 4 (1998) 832–834.
[28] A.N. Santos, A. Simm, V. Holthoff, G. Boehm, A method for the detection of amyloid-beta1-40, amyloid-beta1-42 and amyloid-beta oligomers in blood using magnetic beads in combination with flow cytometry and its application in the diagnostics of Alzheimer's disease, J. Alzheimers Dis. 14 (2008) 127–131.
[29] W. Xia, T. Yang, G. Shankar, I.M. Smith, Y. Shen, D.M. Walsh, D.J. Selkoe, A specific enzyme-linked immunosorbent assay for measuring beta-amyloid protein oligomers in human plasma and brain tissue of patients with Alzheimer disease, Arch. Neurol. 66 (2009) 190–199.
[30] J.L. Tomic, A. Pensalfini, E. Head, C.G. Glabe, Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction, Neurobiol. Disease 35 (2009) 352–358.
[31] L.H. Chen, P.Y. Kao, Y.H. Fan, D.T. Ho, C.S. Chan, P.Y. Yik, J.C. Ha, L.W. Chu, Y.Q. Song, Polymorphisms of CR1, CLU and PICALM confer susceptibility of Alzheimer's disease in a southern Chinese population, Neurobiology of Aging 33 (2012) 210e1–210e7.
[32] M.P. Lambert, A.K. Barlow, B.A. Chromy, C. Edwards, R. Freed, M. Liosatos, T.E. Morgan, I. Rozovsky, B. Trommer, K.L. Viola, P. Wals, C. Zhang, C.E. Finch, G.A. Krafft, W.L. Klein, Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins, Proc. Natl. Acad. Sci. USA 95 (1998) 6448–6453.
[33] L.F. Lue, Y.M. Kuo, A.E. Roher, L. Brachova, Y. Shen, L. Sue, T. Beach, J.H. Kurth, R.E. Rydel, Rogers J Soluble amyloid β peptide concentration as a predictor of synaptic change in Alzheimer disease, Am. J. Pathol. 155 (1999) 853–862.
[34] C.A. McLean, R.A. Cherny, F.W. Fraser, S.J. Fuller, M.J. Smith, K. Beyreuther, A.I. Bush, C.L. Masters, Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in Alzheimer disease, Ann. Neurol. 46 (1999) 860–866.
[35] R. Katzman, R. Terry, R. DeTeresa, T. Brown, P. Davies, P. Fuld, X. Renbing, A. Peck, Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques, Ann. Neurol. 23 (1988) 138–144.
[36] D.M. Walsh, I. Klyubin, J.V. Fadeeva, W.K. Cullen, R. Anwyl, M.S. Wolfe, M.J. Rowan, D.J. Selkoe, Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo, Nature 416 (2002) 535–539.
[37] P.N. Lacor, M.C. Buniel, L. Chang, S.J. Fernandez, Y. Gong, K.L. Viola, M.P. Lambert, P.T. Velasco, E.H. Bigio, C.E. Finch, G.A. Krafft, W.L. Klein, Synaptic targeting by Alzheimer's-related amyloid β oligomers, J. Neurosci. 24 (2004) 10191–10200.
[38] S. Lesné, M.T. Koh, L. Kotilinek, R. Kayed, C.G. Glabe, A. Yang, M. Gallagher, K.H. Ashe, A specific amyloid-β protein assembly in the brain impairs memory, Nature 440 (2006) 352–357.
[39] P.N. Lacor, M.C. Buniel, P.W. Furlow, A.S. Clemente, P.T. Velasco, M. Wood, K.L. Viola, W.L. Klein, Aβ oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease, J. Neurosci. 27 (2007) 796–807.
[40] G.M. Shankar, B.L. Blood good, M. Townsend, D.M. Walsh, D.J. Selkoe, B.L. Sabatini, Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor dependent signaling pathway, J. Neurosci. 27 (2007) 2866–2875.
[41] G.M. Shankar, S. Li, T.H. Mehta, A. Garcia-Munoz, N.E. Shepardson, I. Smith, F.M. Brett, M.A. Farrell, M.J. Rowan, C.A. Lemere, C.M. Regan, D.M. Walsh, B.L. Sabatini, D.J. Selkoe, Amyloid-β protein dimers isolated directly from Alzheimer's brain impair synaptic plasticity and memory, Nat. Med. 14 (2008) 837–842.
[42] H. Hillen, S. Barghorn, A. Striebinger, B. Labkovsky, R. Müller, V. Nimmrich, M.W. Nolte, C. Perez-Cruz, I. van der Auwera, F. van Leuven, M. van Gaalen, A.Y. Bespalov, H. Schoemaker, J.P. Sullivan, U. Ebert, Generation and therapeutic efficacy of highly oligomer-specific beta-amyloid antibodies, J. Neurosci. 30 (2010) 10369–10379.
[43] Z. van Helmond, K. Heesom, S. Love, Characterisation of two antibodies to oligomeric A beta and their use in ELISAs on human brain tissue homogenates, J. Neurosci. Meth. 176 (2009) 206–212.
[44] M. Townsend, G.M. Shankar, T. Mehta, D.M. Walsh, D.J. Selkoe, Effects of secreted oligomers of amyloid beta-protein on hippocampal synaptic plasticity: a potent role for trimers, J. Physiol. 572 (2006) 477–492.
[45] Y. Gong, L. Chang, K.L. Viola, P.N. Lacor, M.P. Lambert, C.E. Finch, G.A. Krafft, W.L. Klein, Alzheimer's disease-affected brain: presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss, Proc. Natl. Acad. Sci. USA 100 (2003) 10417–10422.