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Research Report

Assessment of High Risk for Alzheimer's Disease Using Plasma Biomarkers in Subjects with Normal Cognition in Taiwan: A Preliminary Study

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Abstract.
Background: In Alzheimer's disease (AD), cognitive impairment begins 10–15 years later than neurodegeneration in the brain. Plasma biomarkers are promising candidates for assessing neurodegeneration in people with normal cognition. It has been reported that subjects with the concentration of plasma amyloid-β 1-42 × total tau protein higher than 455 pg^2/ml^2 are assessed as having a high risk of amnesic mild impairment or AD, denoted as high risk of AD (HRAD).
Objective: The prevalence of high-risk for dementia in cognitively normal controls is explored by assaying plasma biomarkers.
Methods: 422 subjects with normal cognition were enrolled around Taiwan. Plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau levels were assayed using immunomagnetic reduction to assess the risk of dementia.
Results: The results showed that 4.6% of young adults (age: 20–44 years), 8.5% of middle-aged adults (age: 45–64 years), and 7.3% of elderly adults (age: 65–90 years) had HRAD. The percentage of individuals with HRAD dramatically increased in middle-aged and elderly adults compared to young adults.
Conclusion: The percentage of HRAD in cognitively normal subjects are approximately 10%, which reveals that the potentially public-health problem of AD in normal population. Although the subject having abnormal levels of Aβ or tau is not definitely going on to develop cognitive declines or AD, the risk of suffering cognitive impairment in future is relatively high. Suitable managements are suggested for these high-risk cognitively normal population. Worth noting, attention should be paid to preventing cognitive impairment due to AD, not only in elderly adults but also middle-aged adults.

Keywords: Alzheimer's disease, immunomagnetic reduction, normal cognition, plasma biomarkers

INTRODUCTION

The prevalence of dementia in elderly people (≥65 years) in Taiwan increased from 2% in 1995 to 8% in 2014, and most dementia patients had Alzheimer's disease (AD) [1–4]. The main method of diagnosis for dementia in the clinic in Taiwan is neuropsychological tests and structural neuroimaging examinations [5]. As reported, the onset of cognitive impairment occurs 10–15 years later than the initiation of amyloid accumulation or neurofibrillation in the brain [6]. This implies that clinically cognitively normal subjects might suffer the risk of amyloid accumulation or neurofibrillation. Cognitively normal subjects with amyloid accumulation or neurofibrillation could be assessed as having a high risk for AD (HRAD). It is important to identify HRAD patients because interventions or management could be applied to delay or prevent cognitive impairment in HRAD patients. Thus, the prevalence of dementia could be effectively lowered.

Amyloid positron emission tomography (PET) is a powerful tool to observe pathological evidence of HRAD [7, 8]. Due to its high cost and low availability, amyloid PET is not practically applied to screen HRAD. The changes in the levels of biomarkers such as amyloid-β and tau protein in cerebrospinal fluid (CSF) could reflect the amyloid accumulation or neurofibrillation [9–11]. The detection of biomarkers in CSF is suggested to be an alternative way to identify HRAD [9–11]. However, lumbar puncture is an invasive process, and its potential side effects limit the clinical practice of CSF biomarker assays [12, 13].

In recent years, with the successful development of ultrahigh-sensitivity assay technologies, the precise quantitative detection of AD-related biomarkers such as amyloid-β 1-40 ($A\beta_{1\text{-}40}$), $A\beta_{1\text{-}42}$, and total tau protein (T-Tau) in blood has become feasible [14–19]. Lots of clinical evidence for validating the uses of plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau for assessing the cognitive impairment were reported [20, 21]. The associations between plasma-biomarker assays and current diagnosis of AD were demonstrated [22–24]. For example, the correlations between plasma $A\beta_{1\text{-}42}$ or T-Tau and CSF $A\beta_{1\text{-}42}$ or T-Tau were revealed [25–27]. This implies that peripheral circulating $A\beta_{1\text{-}42}$ and T-Tau reflect the levels of $A\beta_{1\text{-}42}$ and T-Tau in central neurological system. Furthermore, the concentration ratio of $A\beta_{1\text{-}42}$ to $A\beta_{1\text{-}40}$ in plasma was found to be related to standardized uptake value ratios of amyloid PET [28–30]. Thus, plasma $A\beta_{1\text{-}42}$-to-$A\beta_{1\text{-}40}$ ratio significantly represents the

deposition of amyloid in brain. Clinical validations using plasma biomarkers for the assessments of AD were performed in Taiwan from 2011 to 2016 [31, 32]. According to reports [31, 32], normal controls have the lowest levels of plasma $A\beta_{1\text{-}42}$ or T-Tau, which becomes higher in amnestic mild cognitive impairment (aMCI) or AD. The results show that the cut-off value in terms of plasma $A\beta_{1\text{-}42}$ x T-Tau for assessing aMCI or AD is 455 pg^2/ml^2 [24]. The agreement between plasma biomarker diagnosis and clinical diagnosis is higher than 80%. Such cut-off value is helpful to identify HRAD in normal subjects. Besides, it was found that the concentration of plasma T-Tau negatively correlated to Mini-Mental State Examination (MMSE) scores in aMCI or AD [33]. Concentrations of plasma $A\beta_{1\text{-}42\text{-}}$ is promisingly an index for predicting the cognitive decline in aMCI [34, 35]. All the reported results demonstrate the feasibility of assessing the risk of aMCI or AD, monitoring the cognitive impairment in aMCI or AD, and predicting the cognitive decline in aMCI using plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$ and T-Tau.

In [31], the subjects were divided into three groups: normal controls (NC), aMCI, and AD according to NIA-AA guidelines. Meanwhile, the plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau were assayed blindly using IMR. After unblinding the results, the values of $A\beta_{1\text{-}42}$ x T-Tau were found to be relatively low than those in aMCI and AD. Through RCO curve analysis, the cut-off value to discriminate aMCI/AD from NC was found to be 455 pg^2/ml^2, having clinical sensitivity of 0.96 and specificity of 0.97. The results demonstrate the high accuracy to differentiate aMCI/AD from normal controls by utilizing plasma $A\beta_{1\text{-}42}$ x T-Tau as an assessment parameter for (cut-off value = 455 pg^2/ml^2) aMCI/AD. Although there are other published papers proposing cut-off values of 382 or 400 pg^2/ml^2, the clinical sensitivity and specificity are not as high as those in [31]. Therefore, in this study, the reported cut-off value 455 pg^2/ml^2 is cited for assessing the risk for AD. The subjects showing plasma $A\beta_{1\text{-}42}$ x T-Tau higher than 455 pg^2/ml^2 were referred to as having a high risk for aMCI or AD, denoted as HRAD, while the subjects showing plasma $A\beta_{1\text{-}42}$ x T-Tau between 455 and 382 pg^2/ml^2 were referred to as being subhealthy subjects.

The assay kits for plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau utilizing immunomagnetic reduction (IMR) have been approved by the Taiwan Food and Drug Administration. With the approved kits, it is convenient to explore the prevalence of HRAD in cognitively normal subjects via blood testing. In this work, kits were used to assay plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau levels in clinically cognitively normal subjects aged 20 to 89 years, in order to explore the prevalence of HRAD.

MATERIALS AND METHODS

Recruitment of subjects

Four hundred and twenty-two subjects were enrolled at eleven hospitals in major cities around Taiwan, such as Taipei, New Taipei, and Taoyuan in northern Taiwan, Taichung and Changhua in central Taiwan, and Tainan and Kaohsiung in southern Taiwan. Each subject was evaluated for past medical history, physical and neurological examinations, and laboratory tests. All subjects were examined with Mini-Mental State Examination (MMSE) and Clinical Dementia Rating (CDR). The score of CSD referred as the global score of CDR in this study. For subjects with suspicious aMCI, tests of Activities of Daily Living (ADL), Instrumental ADL (IADL), or Wechsler Memory Scale III (WMS-III) were performed. Participants with cardiac failure, end-stage renal disease, HbA1c > 8.5, or Geriatric Depression Scale (GDS) > 8 were excluded. All enrolled subjects were diagnosed as cognitively normal according to NIA-AA 2011 guidelines [5]. All participants provided written informed consent prior to study enrollment. The data of this study were collected from eleven hospitals that were approved by their institutional review boards.

Plasma preparation

For IMR measurements, the well-defined protocol of plasma preparation is proposed. A 6-ml EDTA blood collection tube was used for blood draw. The blood was centrifuged at a speed ranging from 1500–2500 g for 15 min at room temperature with the aid of a swing-bucket rotor. Notably, the centrifugation was finished no later than 4.5 h after the blood draw [36]. In addition, the blood tube was at room temperature during the period between blood draw and centrifugation. The plasma in the EDTA tube was transferred and aliquoted into 0.5-ml microcentrifuge tubes and stored at –80°C until biomarker assays. Plasma was frozen no later than 4.5 h after blood draw. Collected plasma samples were delivered to MagQu Co., Ltd. in New Taipei City, Taiwan, with a dry-ice package for blind assays of plasma biomarkers.

Assays of plasma biomarkers

Plasma biomarkers were assayed with IMR. Different IMR kits (MF-AB0-0060, MF-AB2-0060, MF-TAU-0060, MagQu) and an IMR analyzer (XacPro-S, MagQu) were used for assaying $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau. The pre-clinical performances of IMR $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau were published [27, 37]. Duplicated measurements were conducted for each biomarker per sample. The mean value of the duplicated measurements was reported for every individual biomarker of a subject. IMR measurements for $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau were performed by following the manufactures' instructions described in package inserts.

Statistical methods

Continuous variables for each measurement are presented as the mean ± standard deviation. Continuous variables were compared using *t*-tests, and the *p* values were determined. The analysis of Pearson correlation, r, was performed with GraphPad Prism.

Distribution of biomarker concentrations

For a given biomarker, the concentration distributions was analyzed by fitting the measured results to the Gaussian distribution as expressed

$$\text{Portion}\ (\%) = A_o \exp\left(-0.5x\left(\frac{\phi - \overline{\phi}}{\sigma}\right)^2\right), \quad (1)$$

where A_o is the amplitude of the distribution ϕ is the concentration of plasma biomarker, $\overline{\phi}$ and σ are the mean value and the standard deviation of the distribution in concentration, respectively.

RESULTS

There were 255 female and 167 male subjects enrolled in this study. The ages of the enrolled subjects ranged from 20 to 89 years. The numbers of enrolled female and male subjects in young adults (20–44 years), middle-aged adults (MA, 45–64 years), and old adults (OA, ≥ 65 years) are listed in Table 1. The percentage of was approximately 10%, MA was almost 50%, and OA was approximately 40% in this study.

Brief demographics is listed in Table 2. The average ages were 60.5 ± 12.2 years for females and 62.4 ± 14.2 years for males. The average age of all subjects was 61.2 ± 13.0 years. The education of each subject is more than 6 years. The clinical dementia ranking score was zero, and the MMSE score was higher than 28 for every individual. Thus, all enrolled subjects were cognitively normal. In addition, there was no movement disorder or language disorder in any subject. Therefore, from the phenotype point of view, all enrolled subjects were nondemented and normal controls. *APOE* genotype is not analyzed in this study.

Table 1
Numbers of enrolled female and male subjects at various age ranges

Group	Female, *n* (%)	Male, *n* (%)	All, *n* (%)
Age (y)			
20–44 (young adults)	23 (9.02)	20 (11.98)	43 (10.19)
45–64 (middle-aged adults)	135 (52.94)	66 (39.52)	201 (47.63)
≥ 65 (old adults)	97 (38.04)	81 (48.50)	178 (42.18)
Total	255	167	422

Table 2
Demographics of enrolled subjects

Group	Female	Male	All
n	255	167	422
Age (y)	60.5 ± 12.2	62.4 ± 14.2	61.2 ± 13.0
Education		≥ 6 y	
CDR	0	0	0
MMSE		≥ 28	
$A\beta_{1\text{-}40}$ (pg/ml)	56.52 ± 11.67	57.41 ± 15.10	56.9 ± 13.1
$A\beta_{1\text{-}42}$ (pg/ml)	15.64 ± 2.21	15.81 ± 2.00	15.71 ± 2.13
T-Tau (pg/ml)	19.37 ± 7.07	20.19 ± 6.23	19.69 ± 6.75
$A\beta_{1\text{-}42}$ x T-Tau (pg^2/ml^2)	309.9 ± 145.5	327.7 ± 148.0	316.9 ± 146.6
$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$	0.288 ± 0.080	0.291 ± 0.087	0.289 ± 0.083

CDR, the global score of Clinical Dementia Rating; MMSE, Mini-Mental State Examination; Aβ, amyloid-β; T-Tau, total tau protein.

The mean measured $A\beta_{1\text{-}40}$ concentrations in plasma for females were 56.52 ± 11.67 pg/ml, 57.41 ± 15.10 pg/ml for males, and 56.9 ± 13.1 pg/ml for all subjects. There was no significant difference in plasma $A\beta_{1\text{-}40}$ levels between females and males ($p > 0.05$). Regarding plasma $A\beta_{1\text{-}42}$, females had 15.64 ± 2.21 pg/ml, males had 15.81 ± 2.00 pg/ml, and all subjects had 15.71 ± 2.13 pg/ml. Through *t*-test analysis, the *p* value between females and males for plasma $A\beta_{1\text{-}42}$ levels was higher than 0.05. This reveals that the plasma $A\beta_{1\text{-}42}$ level is sex independent. Notably, the average plasma $A\beta_{1\text{-}42}$ concentrations in females and males were lower than the cutoff level (= 16.33–17.22 pg/ml) in discriminating amnesic mild cognitive impairment (aMCI) and AD [31, 32, 38]. This evidence indicates that

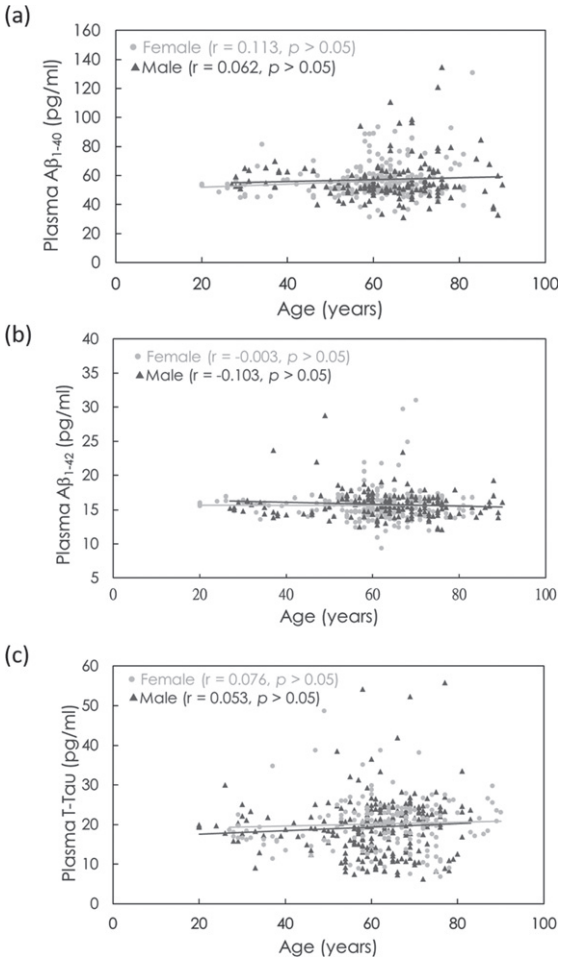

Fig. 1. Age-dependent concentrations of plasma (a) $A\beta_{1\text{-}40}$, (b) $A\beta_{1\text{-}42}$, and (c) T-Tau in females (gray dots) and males (dark gray dots).

the recruited cohort in this study was a normal control. However, some individuals have plasma $A\beta_{1\text{-}42}$ concentrations higher than the cutoff level.

For plasma T-Tau concentrations, the female was 19.37 ± 7.07 pg/ml, the male was 20.19 ± 6.23 pg/ml, and all subjects had 19.69 ± 6.75 pg/ml. Similar to $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, there was no significant difference in plasma T-Tau levels between females and males ($p > 0.05$). According to reported papers, the cutoff level of differentiating aMCI and AD from normal controls is 21.3–25.41 pg/ml [31, 32, 38, 39]. The cutoff level was obviously higher than the averaged values in females and males. This also shows that the enrolled cohort is a normal control.

The biomarker concentrations of $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau as functions of age are shown in Fig. 1a–c. The Pearson correlation r and *t*-test *p* value between the biomarker concentration and age were analyzed, as labeled in Fig. 1a–c. In both females and males, there was no significant correlation between age and plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, or T-Tau ($r = -0.103$–0.113; $p > 0.05$). This implies that the concentrations of plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau in cognitively normal controls are age independent from 20 to 89 years. This is consistent with the results reported in a previous paper [40].

Figure 1b and 1c show that there are some subjects showing higher concentrations of plasma $A\beta_{1\text{-}42}$ or T-Tau. According to reported papers [27, 31, 32, 38, 39], the levels of plasma $A\beta_{1\text{-}42}$ and T-Tau measured with IMR are elevated in aMCI and AD patients compared with normal controls. The levels of plasma $A\beta_{1\text{-}42}$ x T-Tau have been demonstrated to be an index for assessing the risk of aMCI and AD and have been approved in clinical uses in Europe and Taiwan. The cutoff value of plasma $A\beta_{1\text{-}42}$ x T-Tau to discriminate aMCI and AD from normal controls was demonstrated to range from 382 to 455 pg^2/ml^2 [31, 32, 38, 39]. The difference in the cutoff levels among reports might be due to several possible causes, such as AD severity of enrolled subjects and subjective comments on neuropsychological tests.

All subjects were enrolled at 11 hospitals in Taiwan. The enrolled numbers of subject are not consistent among hospitals. For example, there were 151 subjects enrolled at National Taiwan University Hospital (NTNU), but only 11 subjects at Taipei City Hospital-Ren-Ai Branch. Instead of 11 hospitals, we divided all sites into three parties: NTNU, hospitals in north Taiwan (North, 6 hospitals), and hospitals in middle and south Taiwan (Middle-to-South, 4 hospitals). The enrolled subjects are 151 in NTNU, 141 in North and 130 in Middle-to-South. The enrolled numbers of subjects are close to each other among NTNU, North and Middle-to-South. The ages, levels of plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, T-Tau, and $A\beta_{1\text{-}42}$ x T-Tau are listed in Table 3.

Through one-way ANOVA analysis, significant differences in age, plasma $A\beta_{1\text{-}40}$ concentrations and $A\beta_{1\text{-}42}$ concentrations among NTNU, North and Middle-to-South. Plasma T-Tau and $A\beta_{1\text{-}42}$ x T-Tau concentrations show no significant difference among NTNU, North and Middle-to-South. Thus, plasma $A\beta_{1\text{-}42}$ x T-Tau was used as a parameter to assess the risk of AD in this study.

The percentages of HRAD and subhealth in females and males in young adults (YA, 20–44 years), middle-aged adults (MA, 45–64 years), and old adults (OA, $\geq$ 65 years) were analyzed.

Table 3
Measured concentrations of plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau in three territories of 11 hospitals joining this study

Item	NTUH	North*	Middle-to-South^{+}	*p*
n (female%)	151 (55.6%)	141 (59.6%)	130 (66.9%)	–
Age (y)	62.9 ± 15.1	64.5 ± 7.1	55.7 ± 13.8	<0.0001
$A\beta_{1\text{-}40}$ (pg/ml)	61.50 ± 16.39	55.74 ± 13.05	54.11 ± 7.99	<0.0001
$A\beta_{1\text{-}42}$ (pg/ml)	15.22 ± 1.86	15.81 ± 1.50	16.17 ± 2.80	<0.001
T-Tau (pg/ml)	19.52 ± 1.86	20.25 ± 5.93	19.28 ± 7.71	>0.05
$A\beta_{1\text{-}42}$ x T-Tau (pg^2/ml^2)	302.5 ± 124.9	324.1 ± 113.5	325.9 ± 193.9	>0.05

Aβ amyloid-β; T-Tau, total tau protein; NTNU, National Taiwan University Hospital; *including 6 hospitals in north Taiwan; $^{+}$including 4 hospitals in middle or south Taiwan.

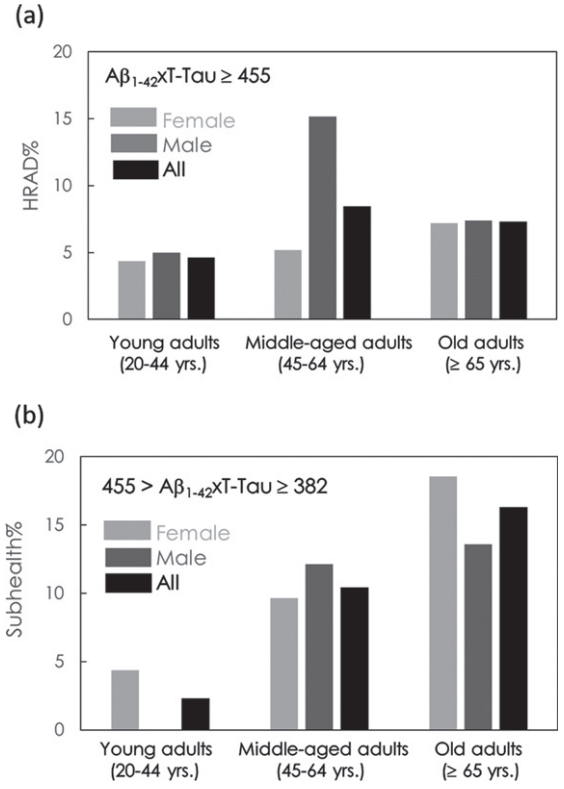

Fig. 2. Percentage of (a) high risk for amnesic mild impairment or AD (HRAD) and (b) subhealth in cognitively normal subjects in young adults, middle-aged adults and old adults.

As shown in Fig. 2a, 4.35% of YA, 5.19% of MA, and 7.22% of OA among females showed HRAD. Among males, 5.00% of YA, 15.2% of MA, and 7.41% of OA showed HRAD. Overall, 4.65% of YA, 8.46% of MA, and 7.30% of OA in all subjects showed HRAD. The percentage of HRAD in MA was 1.86 times that in YA and in OA was 1.56 times that in YA. A remarkable result is shown in Fig. 2a. MA shows a relatively high percentage for HRAD, especially in males. This group also confers more cardiovascular diseases that result in more AD pathology in midlife and mortality effects on the reduction prevalence of HRAD in male OA. The results in Fig. 2a suggest that attention should be given to preventing cognitive impairment not solely for old adults but also for middle-aged adults.

As mentioned in the Introduction, the prevalence of dementia in the elderly population (≥65 years) is 8% in Taiwan [1–4]. That means 92% of elderly people are nondemented. With the results shown in Fig. 2a, 7.3% of cognitively normal aged people have HRAD. As a result, approximately 6.7% of aged population are HRAD, which is close to that of demented patients.

The percentages of subhealthy individuals in YA, MA, and OA are shown in Fig. 2b. A total of 4.35% of YA, 9.63% of MA, and 18.6% of OA in females were subhealthy. In males, none of the YAs, 12.1% of MA, and 13.6% of OA were subhealthy. In all subjects, 2.33% of YA, 10.4% of MA, and 16.3% of OA were subhealthy. The percentage of subhealthy individuals in the MA group was 4.46 times that in the YA group and 7.00 times that in the OA group. The percentages of subhealthy individuals in MA and OA were dramatically higher than those in YA. The results again suggest that prevention from cognitive impairment should be performed for an individual when he/she becomes middle-aged.

DISCUSSION

As shown in Fig. 2, in all subjects, the ratio of subhealth to HRAD in terms of subject numbers in YA was 0.5. The ratio increases to 1.24 in MA and 2.23 in OA. This reveals the fact that the population of subhealthy individuals is higher than that of HRAD individuals in both MA and OA. In OA, the population of subhealth individuals is more than twice that of HRAD patients.

In Fig. 2a, male MA (15.2%) shows a relatively high percentage of HRAD than female MA (5.19%). In Fig. 2b, male MA (12.1%) shows a higher percentage of subhealth than female MA

Table 4
Values of fitting parameters in Equation (1) for plasma biomarkers $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$ and T-Tau

Biomarker	Gender	A_o	$\overline{\phi}$ (pg/ml)	σ (pg/ml)
$A\beta_{1\text{-}40}$	Female	50.7	54.37	5.73
	Male	44.6	55.05	7.04
$A\beta_{1\text{-}42}$	Female	72.7	15.39	1.17
	Male	72.2	15.66	1.22
T-Tau	Female	37.4	19.20	5.71
	Male	45.6	19.82	4.35

Aβ, amyloid-β; T-Tau, total tau protein.

(9.63%). The reasons for the high percentage of HRAD and subhealth in male MA are unknown. However, according to published reports [41–44], comorbidities such as diabetes, obesity, cardiovascular disease, or metabolic syndrome are risk factors for dementia. Many groups in Taiwan have shown that the prevalence of these comorbidities is significantly higher in male than in female in midlife [45–47]. This could be the possible reasons for the high percentage of HRAD in male MA found in this study.

The concentration distributions of plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau in female and male were analyzed via Equation (1), as shown in Fig. 3a–c. The values of parameters A_o, $\overline{\phi}$, and σ in Equation (1) for plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau are listed in Table 4. The coefficients of determination R^2 for all the curves in Fig. 3a-c are higher than 0.9. This reveals the high consistence between the data and the fitted Gaussian distribution. Besides, for a given plasma biomarker, there is no significant deviation in the Gaussian distribution between female and male. Hence, the concentrations of plasma $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$, and T-Tau measured with IMR are gender dependent in cognitively normal subjects.

The results in Table 4 showed that the mean value of $A\beta_{1\text{-}40}$ concentrations in cognitively normal subjects is 54.37 pg/ml in female and is 55.05 pg/ml in male. The mean value of $A\beta_{1\text{-}42}$ concentrations in cognitively normal subjects is 15.39 pg/ml in female and is 15.66 pg/ml in male. The mean value of T-Tau concentrations in cognitively normal subjects is 19.20 pg/ml in female and is 19.82 pg/ml in male. The mean values of plasma concentrations obtained via Equation (1) are close to the values listed in Table 2.

The ratio of σ to $\overline{\phi}$ denotes the im-precession of the plasma biomarker concentration span. The ratio was calculated to be approximately 11% for plasma $A\beta_{1\text{-}40}$, 7.8% for $A\beta_{1\text{-}42}$, and 25% for T-Tau. Thus, narrow spans were found for $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, and a moderated span for plasma T-Tau in cognitively normal subjects.

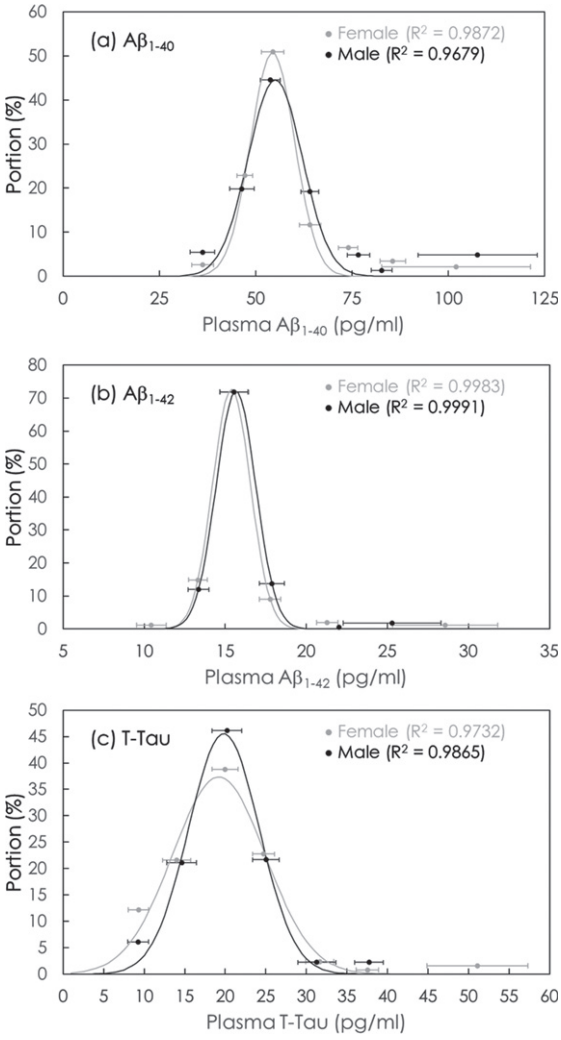

Fig. 3. Gaussian distributions of concentrations of (a) $A\beta_{1\text{-}40}$, (b) $A\beta_{1\text{-}42}$, and (c) T-Tau in plasma in cognitively normal subjects. The solid lines are the fitted Equation (1). The values of fitting parameters are shown in Table 3.

In this study, 422 cognitively normal subjects were enrolled. It would be better to recruit more subjects to validate the percentages of HRAD and subhealth in cognitively normal populations. The results of preliminary study provide direct evidence from the plasma biomarkers that the MA cohort for should be a target of dementia prevention.

CONCLUSION

The exploration of age-dependent AD-related biomarkers in plasma in clinically cognitive normal controls is very rare. In this study, 422 subjects with normal cognition were enrolled in seven cities around

Taiwan. The ages of the enrolled subjects ranged from 20 to 89 years. The results show that the concentrations of plasma $A\beta_{1\text{-}40}$, $A\beta_{\text{-}1\text{-}42\text{-}}$, and T-Tau are age dependent in females and males. There was no significant difference in biomarker levels between females and males. The results reveal that the percentage of subhealth is 13.2% for individuals who become middle-aged ($\geq$ 45 years). The percentage of subjects that are high risk for aMCI or AD (HRAD) is 7.9% for individuals who are middle-aged ($\geq$ 45 years). Thus, the data strongly suggest paying attention to not only old adults but also middle-aged adults to prevent cognitive impairment, especially for the middle-aged male.

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CONFLICT OF INTEREST

SY Yang is an employee and a shareholder of MagQu Co., Ltd. Other authors do not have conflicts of interest.

ETHICAL STANDARDS

The Institutional Review Board of Shuang Ho Hospital, National Taiwan University Hospital, National Cheng Kung University Hospital, Taipei Veterans General Hospital, Show Chwan Memorial Hospital, Tri-Service General Hospital, Linkou Chang Gung Memorial Hospital, China Medical University Hospital, Cardinal Tien Hospital, Taipei City Hospital, Kaohsiung Chang Gung Memorial Hospital, approved this study. Written informed consent was obtained from all patients or their legal guardians.

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