

## Plasma amyloid $\beta$ levels in Alzheimer's disease and cognitively normal controls in Syrian population

Rafah Manafikhi<sup>\*1</sup> , M. Bassam Haik<sup>2</sup>, Raghda Lahdo<sup>3</sup>, Faizeh AlQuobaili<sup>1</sup>

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### Abstract

**Background:** The pathogenesis of Alzheimer's disease (AD) is believed to be occurred by the production of neurotic plaques of the beta-amyloid peptide ( $A\beta$ ) and deposition of them. Therefore, biomarkers of abnormal  $A\beta$  processing may represent before the AD clinical biomarkers, which could be benefit for a successful disease management that may prevent the AD development. The aim of this study is to investigate of plasma  $A\beta_{40,42}$  levels in Alzheimer's patients in Syria and thus determine whether they may have a potential role as biomarker for identifying and predicting AD.

**Methods:** In this cross-sectional study, the plasma levels of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  were investigated in two groups represent Syrian population, AD group; clinically diagnosed AD patients ( $n=50$ ) and CN group; cognitively normal participants ( $n=33$ ). This study first determined the reference interval of plasma  $A\beta_{1-40}$  and  $A\beta_{1-42}$  for cognitively normal Syrian. Results were analyzed using SPSS, 24, depending on independent-samples t test, considering that the value of  $p < 0.05$  is statistically significant.

**Results:** The results showed that the plasma levels of  $A\beta_{1-40}$  ( $p < 0.001$ , OR=1.031, 95%CI: 1.012-1.051) and  $A\beta_{1-42}$  ( $p < 0.001$ , OR=1.306, 95%CI: 1.145-1.490) were significantly higher in AD patients than in cognitively normal participants, and no significant association was shown between both of education and sex with plasma  $A\beta$  levels.

**Conclusion:** The plasma levels of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  could be potential biomarkers for identifying and predicting AD.

**Keywords:** Alzheimer disease,  $A\beta_{40,42}$ , Biomarkers, Syria

**Conflicts of Interest:** There is no conflict of interest

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### Introduction

According to the World Health Organization (WHO), worldwide, around 50 million people have dementia, and there are nearly 10 million new cases every year. The total number of people with dementia is expected to reach 82 million in 2030 and 152 million in 2050. Alzheimer's disease (AD) is the most common form of dementia and could contribute to 60–70 percent of cases. World Health Organization reveals that about 60% of all people living with dementia worldwide are from a low to middle in-

come countries (1).

AD is characterized by a gradual and progressive decline in cognitive functions and the presence of specific neuron and synapse loss in addition to the senile plaques and neurofibrillary tangles. For diagnosis of AD, the presence of neurotic plaques (composed of highly insoluble amyloid- $\beta$  ( $A\beta$ ) peptide in large part) in the brain parenchyma is needed. This concept suggests that a plaque deposition occurs as a result of chronic imbalance between

**Corresponding author:** Rafah Manafikhi, [rafahmanafikhi@gmail.com](mailto:rafahmanafikhi@gmail.com)

<sup>1</sup> Department of Biochemistry and Microbiology, Faculty of Pharmacy, University of Damascus, Syria

<sup>2</sup> General Manager of the General Committee of Ibn Khaldun Hospital, Aleppo, Syria

<sup>3</sup> Department of Biochemistry and Microbiology, Faculty of Pharmacy, University of Aleppo, Syria

#### ↑What is "already known" in this topic:

$A\beta$  is a biomarker that plays an important role in AD diagnosis, where it accumulates in the brain and forms plaques that lead to neurons damage. There are many studies, in several countries, that investigate  $A\beta$  plasma levels in AD patients and there is a contradiction in their results.

#### →What this article adds:

In Syria, there are no studies on the normal and pathological plasma values of  $A\beta$ . It is the first study on plasma levels of  $A\beta$  in AD patients in Syrian population.

production the amyloid-beta peptide and clearance of it (2).

The beneficial roles of amyloid- $\beta$  involve repairing leaks in the blood-brain barrier (BBB), contributing in healing the injury, protecting the body from infections and controlling synaptic perform. Evidence for these putative roles comes from in vivo and in vitro studies, which showed that amyloid- $\beta$  generation increases rapidly in a physiological challenge response and usually reduces once healing (3).

A biomarker or biological marker, assesses as an index of normal biological functions, pathogenic conditions or responses to a therapeutic procedures. A biological marker can evaluate as an indicator of healthiness and illness (4).

Amyloid precursor protein (APP) gene is expressed in the brain and other parts of the body tissues. The molecular mechanism implicated in APP breakage down and amyloid- $\beta$  synthesis has yet to be determined precisely (5).

Amyloid- $\beta$  peptide is produced by a number of actions of alpha ( $\alpha$ ), beta ( $\beta$ ) and gamma ( $\gamma$ ) secretases on APP. Amyloid- $\beta$  is generated in the endoplasmic reticulum (ER), Golgi apparatus and endosomal compartment by the action of  $\beta$ -secretase and  $\gamma$ -secretase (6). Therefore, several amyloid- $\beta$  pieces have been produced, but those ending at location 40 (A $\beta$ 1-40) are the mos plentiful (approximately 80-90 percent), then those ending at location 42 (A $\beta$ 1-42, approximately 5-10 percent). The somewhat longer species of amyloid- $\beta$ , mainly A $\beta$ 1-42, have more hydrophobicity and fibrillogenesis properties, and they are the mainly pieces positioned into the brain (7).

Taking cerebrospinal fluid (CSF) samples is an annoying procedure with probable side effects, with the difficulties in screening of patients and making follow-up studies of the same patient for many years. So, there is an urgent need to do researches for determining biomarkers in other body fluids such as blood to make an accurate diagnosis of Alzheimer's disease (8).

It is not comprehended yet how the blood molecule level associates with pathological alterations in the brain, particularly in Alzheimer's disease. Therefore, identifying biomarkers in the blood that associate with Alzheimer's disease must initiates with established biomarkers in CSF, such as biomarkers linked to amyloid- $\beta$  and tau protein (4). Furthermore, there is no accepted reference values for amyloid- $\beta$  in the plasma. A study of the reference values of A $\beta$  in the plasma for cognitively normal Korean adults revealed that the 95th percentile reference values for A $\beta$ 1-40 were 127-331 pg/mL and for A $\beta$ 1-42 were 2.31-19.84 pg/mL (9). Another study in Poland showed that the plasma levels of A $\beta$ 1-40 and A $\beta$ 1-42 in control group were 160.1 $\pm$ 15.2 pg/mL and 36.3 $\pm$ 6.3 pg/mL, respectively (10). A study in USA found that the A $\beta$ 40 and A $\beta$ 42 plasma levels in control were 211.4 $\pm$ 74.4, 31.6 $\pm$ 14.0 pg/mL respectively (11). Further researches are needed to identify clear reference interval in other countries.

In Syria, there are no studies on the normal and pathological plasma values of A $\beta$ . So that, there is an urgent need to more studies related to this field. The fact that the pathophysiological alterations associated with Alzheimer's disease begin several years before the appearance of clinical

cognitive complaints (12). Thus, it's necessary to determine biomarkers that help in early AD diagnosis.

## Methods

### Study Subjects

This was cross-sectional study in Syria, it was conducted over a period of two months. Samples were collected from Ibn Khaldoun Psychosocial Support Committee, nursing homes and private clinics in Aleppo city. Patients with diagnosed Alzheimer's disease (AD) (n=50) and cognitively normal subjects (CN) (n=33) aged above 60 years (60-100 years for AD, 60-89 years for CN) are involved in this study.

Exclusion criteria of participants: A history of cardiovascular diseases.

Alzheimers' subjects were included in our study if their disease was diagnosed by neurologists after they received a detailed clinical history, neurological examination in addition to assessment of activities of daily living and the Mini-Mental State Examination (MMSE) criteria (should be below 24 points), and this is the protocol used and approved for diagnosis by neurologists in Syria. Healthy controls were included in our study if no familial or personal cognitive impairment or psychiatric history were reported, neurological examination by neurologists was normal and the MMSE (Arabic version) score was above 26 points (bearing in mind that in the Spanish population, the MMSE cut off value has been illustrated to be <25) (13).

This study was approved by the Ethical Committee, Faculty of Pharmacy, Damascus University. All participants volunteers (or their guardians) were informed by the aim of the study and a permit was obtained from each subject (or his/her guardian) to participate in this study.

### Plasma A $\beta$ quantification

Venous blood samples (5 mL) were collected into tubes containing EDTA as anticoagulant. Plasma samples were aliquoted into polypropylene tubes (two tubes, 250  $\mu$ L in each one) after centrifugation, and stored at -20  $^{\circ}$ C.

Plasma was used to quantify amyloid- $\beta$ 1-40 and amyloid- $\beta$ 1-42 by using Sandwich ELISA method (EURO-IMMUN Medizinische Labordiagnostika AG, Lubeck, Germany) depending on monoclonal anti-beta-amyloid antibodies.

### Statistical analysis

Results are expressed as mean  $\pm$  SD. Results were analyzed using SPSS 24 statistical program, depending on Independent-samples T Test and chi-square ( $\chi^2$ ) test considering that the value of  $p < 0.05$  is statistically significant. Normal distribution was checked using a Shapiro-Wilk test. Odds ratio (OR) was calculated to evaluate the association with AD, the association is considered when  $OR \neq 1$ . The corresponding 95% confidence intervals (CIs) were also calculated.

## Results

### Aβ1-40 and Aβ1-42 levels in AD group and CN group

Plasma Aβ levels were investigated in 83 subjects; AD group (clinically diagnosed AD patients, n=50; 19 men and 31 women, 41 workers and 9 employees) and CN group (control group of cognitively normal subjects, n=33; 15 men and 18 women, 24 workers and 9 employees) (Table 1).

The comparison between both groups showed that the plasma levels of both Aβ1-40 and Aβ1-42 were higher in AD patients (AD group) than in cognitively normal participants (CN group) and this augmentation of Aβ was significant for both Aβ1-40 ( $p<0.001$ , OR=1.031, 95%CI: 1.012-1.051) and Aβ1-42 ( $p<0.001$ , OR=1.306, 95%CI: 1.145-1.490), taking into account that individuals in both groups are of the same age group and that they are

matched in gender and the number of the educated and uneducated, and no significant difference was shown between both groups (AD and CN) with regard to age, education and sex ( $p>0.05$ ) (Table 1).

Figure 1 shows the results of Aβ1-40 levels in AD group and CN group;  $155.95\pm39.08$  pg/mL and  $118.96\pm34.94$  pg/mL, respectively. Also this figure shows the mean values of Aβ1-42 levels in AD group and CN group;  $25.25\pm22.16$  pg/mL and  $8.39\pm6.59$  pg/mL respectively.

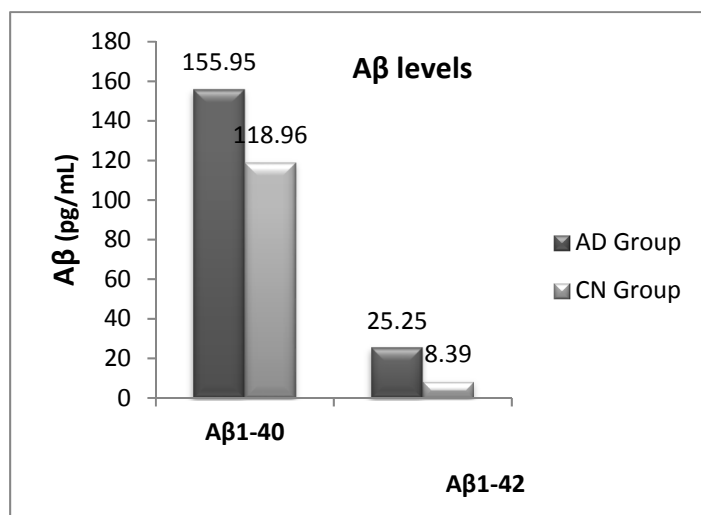
### Levels of Aβ1-40, Aβ1-42 according to gender and Job

Table 2 shows that there is no significant difference in Aβ levels between the educated (employees) and the uneducated (workers) subjects in AD group. Our results show that there is also insignificant difference between Aβ

Table 1. Characteristics of Subjects involved in the study and levels of Aβ1-40, Aβ1-42

Variable		Total	Groups		p
			AD	CN	
N		83	50	33	
Age		> 60 (60-100)	> 60 (60-100)	> 60 (60-89)	0.568
Gender	Female n (%)	49 (59.04%)	31 (62%)	18 (54.55%)	0.499
	Male n (%)	34 (40.96%)	19 (38%)	15 (45.45%)	
Job	Workers n (%) (uneducated)	18 (21.69%)	9 (18%)	9 (27%)	0.502
	Employees n (%) (educated)	65 (78.31%)	41 (82%)	24 (72.73%)	
Aβ1-40 pg/mL	mean ± SD	—	155.95±39.08	118.96±34.94	<0.001
Aβ1-42 pg/mL	mean ± SD	—	25.25 ± 22.16	8.39 ± 6.59	<0.001

AD: Alzheimer Disease, CN: Cognitively Normal, Aβ: Amyloid-β, SD: Std. Deviation



AD: Alzheimer Disease, CN: Cognitively Normal, Aβ: Amyloid-β

Fig. 1. Mean values of Aβ1-40 and Aβ1-42 levels in AD group and CN group

Table 2. Levels of Aβ1-40, Aβ1-42 according to gender and Job

	Sex		p	Job		p
	Female	Male		Workers (uneducated)	Employees (educated)	
AD (n)	31	19		41	9	
Aβ (1-40) pg/mL mean ± SD	152.50± 28.04	161.47± 52.92	0.552	150.00± 19.86	159.00± 4.24	0.550
Aβ (1-42) pg/mL mean ± SD	23.16± 14.81	20.43± 9.70	0.518	15.48± 4.10	16.00± 0.40	0.835

AD: Alzheimer Disease, Aβ: Amyloid-β, SD: Std. Deviation

levels in males and females in AD group.

## Discussion

### A $\beta$ 1-40 and A $\beta$ 1-42 levels in AD group and CN group

The plasma levels of both A $\beta$ 1-40 and A $\beta$ 1-42 in our study are close to other studies (10, 14). Our results are in accordance with a study in Taiwanese cohorts that revealed a significant increase in the plasma levels of A $\beta$ 1-42 in mild cognitive impairment (MCIs) individuals, and in early stages of Alzheimer's disease (15). The A $\beta$ 1-42 is less recurrent than A $\beta$ 1-40. However, it deposits much earlier and accumulates more quickly in comparison with A $\beta$ 1-40 (16).

Our results were consistent with the results of previous clinical studies (10, 14, 17) which had found also that the plasma levels of both A $\beta$ 1-40 and A $\beta$ 1-42 were higher in AD patients than in cognitively normal people. These results may be explained depending on the assumption of other studies which suggested that the circulating amyloid- $\beta$  may inflow via BBB and promote the accumulation of amyloid- $\beta$  in the brain. Since the expression of APP occurs in several tissues in addition to the brain. Therefore, the levels of amyloid- $\beta$  can be detected in the blood (18). Normally, liver and kidneys facilitate the circulating amyloid- $\beta$  systematic clearance through identified transporter. Thus, the circulating amyloid- $\beta$  level increases when the systematic clearance is defective as in Alzheimer's disease patients, in addition to that the free circulating amyloid- $\beta$  would be augmented by the increasing of oxidative damage which happens in Alzheimer's disease damage (18).

A study on rodents showed that the circulating amyloid- $\beta$  may flow through the BBB (19), by identified receptor of advanced glycation end products (RAGE), and a RAGE up-regulation was noticed in Alzheimer's disease human patients.

On the other hand, other studies (20, 21) found that the levels of plasma amyloid- $\beta$  were lower in AD patient than in cognitively normal people. This may be due to the assumption of that the BBB won't clear the amyloid- $\beta$  from CSF into the blood through specific lipoprotein receptor related protein 1 (LRP-1) due to its dysfunction which could happen in Alzheimer's disease, and thus contribute to aggregation of amyloid- $\beta$  in the brain (22).

These conflicting results could be as a result of several factors that can affect the plasma amyloid- $\beta$  peptide level as it has been suggested in some studies (23, 24), for instance, sex, age, levels of plasma proteins, function of kidney, race and stage of disease. Amyloid- $\beta$  peptides in plasma are bound to other plasma proteins easily and broken down in the blood (25). So, the results of plasma amyloid- $\beta$  could be affected by the levels of total protein.

Variances in studies cohort, such as cognitive decline stage, cognitive function estimation (by various examinations), treatment and other illnesses, all of these issues can affect the results of different studies (26). Plasma amyloid- $\beta$  may be increased by alterations in renal function, since plasma amyloid- $\beta$  is normally excreted via the kidneys (27). The traditional technique of quantifying the

plasma amyloid- $\beta$  is sandwich ELISA, but the plasma proteins and lipoproteins could affect immunological detection (28). In addition to that, soluble amyloid- $\beta$  pieces bind to numerous proteins, for instance, albumin as well as other proteins. These complexes could affect ELISA immunoreactions and also influence the results (29).

### Levels of A $\beta$ 1-40, A $\beta$ 1-42 according to gender and Job

Our results are consistent with those of some studies that have not found a significant correlation between amyloid- $\beta$  levels and education (30). But it contrasts with other studies that have shown a statistical association with education (31). Our results show that there is also insignificant difference between A $\beta$  levels in males and females in AD group, and this is consistent with the result of other study on African American that did not find a significant difference between men and women in terms of levels of amyloid beta (32), but it contradicted on the other hand with other clinical studies that showed a significant correlation between sex and levels of amyloid beta (31).

The limitations of this study are the relatively small sample size in addition to that the patients are not classified according to the stages of the disease. So, further researches and studies are recommended with considering the stages of disease, increasing size of sample, and adjusting the study conditions.

## Conclusion

In conclusion, plasma beta-amyloid 40 and 42 may be important biomarkers and could help in Alzheimer's disease diagnosis.

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## Conflict of Interests

The authors declare that they have no competing interests.

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