

Plasma beta amyloid and cytokine profile in women with Alzheimer's disease

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Abstract

Alzheimer's disease (AD) belongs to a group of neurodegenerative disorders. It is characterized by irreversible and progressive memory loss accompanied with decline in other cognitive functions. At a microscopic level, the typical neuropathologic features, senile plaques and neurofibrillary lesions are found. The pathological processes lead to neuronal loss, synaptic dysfunction and inappropriate activity of neurotransmitters. The major constituent of senile plaques is abnormally aggregated beta amyloid protein. Beta amyloid (Abeta) is a short (40-42 amino acid) product of proteolysis of the transmembrane amyloid precursor protein (APP). Extracellular depositions of Abeta 1-42 may initiate a wide range of pathological processes including glia activation, neuroinflammation and neuronal apoptosis.

There is convincing evidence that inflammatory response to accumulation of beta amyloid plays a pivotal role in the progression of neuropathological changes found in AD. Current research was directed at assessing beta amyloid, cytokines (IL-6, IL-10 and TNF alpha) plasma levels in women with AD.

Hundred and twenty four women, aged between 59 to 86 years, were enrolled in the study. Amongst them 57 were diagnosed with AD (29 subjects in early stage and 28 subjects with moderate to severe stadium of disease) and 67 women without dementia were investigated as a control group.

The lowest values of Abeta 1-42 were found in AD subjects in moderate to severe stage of disease as compared with the early stage of AD ($p < 0.05$) and the control group ($p < 0.01$). Change in IL-6 values was significantly different between groups with the lowest values found in women without dementia. Both subset of AD patients demonstrated statistically enhanced IL-6 levels when compared with the control group ($p < 0.001$, $p < 0.01$ respectively for early and moderate/severe stage of AD). Moreover, our study revealed a trend to increase in TNF alfa and IL-10 values in AD. However, those differences were not statistically significant. In addition, we did not detect any correlations between plasma beta amyloid and investigated cytokines.

We conclude that plasma beta amyloid and cytokine concentrations are altered in Alzheimer's disease. Further studies are needed to establish the ideal biomarkers for diagnosis of AD as it is multifactor and heterogeneous disease.

INTRODUCTION

Alzheimer's disease (AD) belongs to a group of neurodegenerative disorders. It is characterized by irreversible and progressive memory loss accompanied with decline in other cognitive functions. At a microscopic level, the typical neuropathologic features, senile plaques and neurofibrillary lesions are found. The pathological processes lead to neuronal loss, synaptic dysfunction and inappropriate activity of neurotransmitters, including acetylcholine and serotonin. The diffuse brain atrophy with predilection to temporal and parietal lobes is also present [1].

The major constituent of senile plaques is abnormally aggregated beta amyloid protein. Beta amyloid (Abeta) is a short (40-42 amino acid) product of proteolysis of the transmembrane amyloid precursor protein (APP). The process of APP proteolysis depends on the activity of different enzymatic complexes called secretases [2]. The production of beta amyloid 1-42, more hydrophobic than Abeta 1-40 protein with higher ability to aggregation, is under control of β and γ – secretases [3]. An imbalance between the production and clearance of Abeta in the brain leads to "amyloid cascade" and in consequence to neuronal degeneration and dementia [4]. Extracellular depositions of Abeta 1-42 may initiate a wide range of pathological processes including glia activation, neuroinflammation and neuronal apoptosis.

It has been reported that activated microglial cells in response to pathological factors release several substances such as reactive oxygen intermediates and species, reactive nitric intermediates, proteases and mediators of inflammation [5]. A chronic inflammatory process might contribute to the neurodegeneration associated with AD, by over expression of cytokines and other inflammatory molecules in activated microglia surrounding senile plaques [6].

Interleukin 6 (IL-6) is a cytokine produced in the central nervous system by microglia and astrocytes as well as neuronal and endothelial cells. In the acute phase of inflammation it enhances the activity of immune system and protects cells against harmful conditions. However, in the prolonged inflammation it shows proinflammatory properties. Besides, other cytokines like Tumor Necrosis Factor alpha (TNF alpha) or Interleukin 10 (IL-10) may also modulate immunological response to neurodegeneration in the course of AD [5]. There is convincing evidence that inflammatory response to accumulation of beta amyloid plays a pivotal role in the progression of neuropathological changes found in AD [7].

Current research was directed at assessing beta amyloid, cytokines (IL-6, IL-10 and TNF alpha) plasma levels in women with AD.

SUBJECTS AND METHODS

Subjects

Hundred and twenty four women, aged between 59 to 86 years, were enrolled in the study. Amongst them 57 were diagnosed with AD (mean age 73.19 yrs \pm 4.09) and 67 women without dementia were investigated as a control group (mean age 72.85 \pm 5.64). According to the results of Mini Mental State Examination (MMSE) the group of AD women was divided into subgroups:

- 1) 29 subjects in early stage of disease (mean age 72.86 \pm 4.24; MMSE 21.17 \pm 3.04)
- 2) 28 subjects with moderate to severe stadium of disease (mean age 73.54 \pm 3.98; MMSE 10.75 \pm 4.18)

The diagnosis of AD was confirmed clinically with neurological examination, neuropsychological assessment and brain CT or MRI scan.

The protocol was approved by the Local Ethics Committee. All subjects and/or their caregivers were informed and the written consent was obtained.

PROTOCOL

All study participants attended one outpatient-type visit during which the medical history was taken and clinical examination was performed to exclude the presence of inflammatory process.

Blood samples were taken after at least 6 hours of fasting. Immediately after collection, the plasma was separated by centrifugation and, after being divided into 1 ml lots, was stored at -70°C for further evaluation. Test-tubes with the protease inhibitors, aprotinin and EDTA, were used in the study protocol. Additionally, the samples were not further aliquoted nor were they repeatedly frozen and thawed.

Methods

Plasma beta amyloid, IL-6, IL-10 and TNF alpha levels were estimated using ELISA methods. In detail, beta amyloid was evaluated with BioSource sandwich-ELISA kit (Human β Amyloid HS 1-42) and the sensitivity of the assay was 1.0 pg/ml with an intra-assay and inter-assay coefficient of variation (cv) of less than 10%. For cytokine measurement commercial kits from Endogen were used. The sensitivity of the assays was as follows: IL-6 1 pg/ml, IL-10 3 pg/ml and TNF alpha 2 pg/ml, respectively. Intra assays cv and inter assays cv in all of the above measurements were less than 10 %.

Statistical analyses

Statistical analyses were made using the ANOVA test followed by the unpaired t-Student test. In order to study the possible correlations between beta amyloid and cytokine levels the Pearson test was performed. All results are presented as mean \pm SEM. Statistical significance was accepted at $p < 0.05$.

RESULTS

Our study revealed differences in plasma beta amyloid levels between the investigated groups. The lowest values were found in AD subjects in moderate to severe stage of disease as compared with the early stage of AD ($p < 0.05$) and the control group ($p < 0.01$). However, we did not observed a significant difference in this particular parameter between patients in early stage of disease and controls. The results are shown in Figure 1.

To investigate the effects of neurodegenerative processes occurring in Alzheimer's disease on immunological status, we performed the analysis of plasma pro-inflammatory and anti-inflammatory cytokines. The results are shown in Table 1 and Table 2.

Change in IL-6 values was significantly different between groups with the lowest values found in women without dementia. Both subset of AD patients demonstrated a statistically enhanced IL-6 levels when compared with the control group ($p < 0.001$, $p < 0.01$ respectively for early and moderate/severe stage of AD). Despite the presence of tendency to the highest results of IL-6 in the group of women in early stage of disease, there was not a significant difference in comparison with subjects with advanced form of AD. Moreover, our study revealed a trend to increase in TNF alpha and IL-10 values in AD. However, those differences were not statistically significant.

In addition, we did not detect any correlations between plasma beta amyloid and investigated cytokines.

DISCUSSION

The present study focused on assessing plasma markers in Alzheimer's disease. Beta amyloid, a short peptide derived from the longer precursor protein, is known to accumulate in the brain tissues of AD patients. Although the process of production of Abeta is well established, the exact mechanism underlying the initial phenomenon is still undiscovered. Our study resulted in findings that plasma Abeta 1-42 levels differ between subjects diagnosed with Alzheimer's disease and the control group. The highest values were obtained in women without dementia, and the lowest were found in patients with moderate/severe form of the disease. The significant difference was also observed between two subgroups of patients suffering from AD. Our results are in agreement with data published by Pesaresi et al [8]. However, other studies concerning Abeta plasma

Table 1. Pro-inflammatory cytokines levels in controls and AD

	Control group	AD – Early stage	AD – Moderate to severe
Number of subjects	67	28	29
IL-6 - mean values (pg/ml)	2.27	7.51	6.63
SEM	0.20	1.70	0.95
TNF alpha – mean values (pg/ml)	4.80	16.26	24.56
SEM	0.62	3.27	5.35

Table 2. Anti-inflammatory cytokine IL-10 levels in controls and AD

	Control group	AD – Early stage	AD – Moderate to severe
Number of subjects	67	28	29
IL-10 - mean values (pg/ml)	4.80	16.26	24.56
SEM	0.62	3.27	5.35

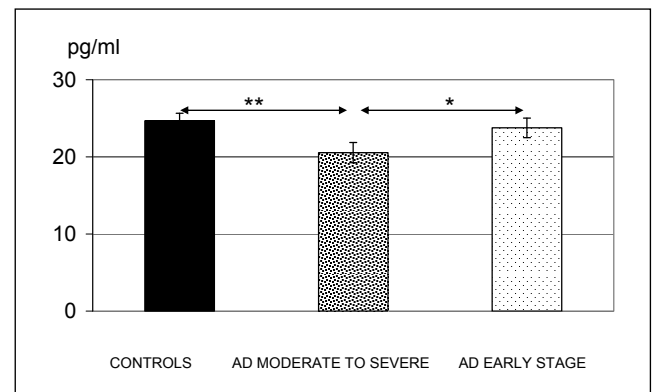


Figure 1. Serum beta amyloid levels in controls and AD subjects
* $p < 0.05$; ** $p < 0.01$

concentration remain equivocal. Mayeux et al. found the elevated Abeta 1-42 values in AD with the highest outcome at the beginning of disease [9]. On the contrary, some authors do not report any significant differences in this parameter between AD and the controls [10]. Moreover, previous studies have indicated that only subjects with mild cognitive impairment (MCI) showed an increase in plasma Abeta 1-42 values [11]. Interestingly, it has been also suggested that enhanced Abeta 1-42 levels in patients without dementia may be a risk factor for transition to AD in future [9, 12]. Besides, results from the cohort, prospective study conducted by van Oijen et al. did not confirm this hypothesis [13].

Therefore, an extensive research is necessary to explain the role of plasma Aβ₁₋₄₂ concentration changes in the course of AD.

An increase in activity of immunological system in the neurodegenerative processes occurring in AD is indisputable. Broad spectrum of substances being produced in response to beta amyloid accumulation causes a focus of local inflammation and enhances the pathological processes [14]. Our data indicated that Alzheimer's disease is associated with changes in plasma cytokine profile. We found significant differences in IL-6 plasma concentration between subjects with and without AD. The highest values were shown in women in early stage of the disease but the differences between early and advanced stage were not statistically significant. These findings support the data of other researchers [15, 16]. However, in literature there are controversial opinions on IL-6 levels as other studies did not reveal these differences [17]. Moreover, it has been reported that a polymorphism in the IL-6 gene is not related to the risk of AD [18]. Nevertheless, recent studies have found that an increase in IL-6 concentration correlates with worse results in cognitive tests and may be a marker of acceleration of dementia [19, 20]. On the other hand, it has been also established that IL-6 may play a dual role in the central nervous system as it can be a mediator of neuroinflammation or, in certain conditions, may act like a neuroprotective factor [21, 22]. Similarly to IL-6, TNF alpha is recognized as a pro-inflammatory cytokine and is synthesized by activated microglia. In addition, a positive feedback loop between TNF alpha and microglia has been suggested [23]. As a result of our study we showed the tendency to increase in TNF alpha concentration in women diagnosed with AD. The trend to the highest values in the group of women in moderate/severe stage of disease was present, although the differences between examined groups were not statistically significant. Alvarez et co-workers [24] demonstrated an increase in TNF alpha concentration in AD subjects in comparison with healthy controls, while Yasutake et al. failed to establish this correlation [25]. Besides, recent studies by Zuliani et al. indicated that increase in plasma TNF alpha, altogether with higher values of IL-1 beta, may support the hypothesis of low-grade systemic inflammation in older patients with late onset Alzheimer's disease (LOAD) [26]. Contrary to its pro-inflammatory properties TNF alpha may be considered as a mediator of neuroprotection. Data from *in vitro* studies implicated that TNF alpha protects neurons against beta amyloid neurotoxicity. It has been suggested that TNF alpha modulates kappa B-binding factor and attenuates peroxide and Ca²⁺ accumulation [27]. Additionally, TNF alpha may prevent neuronal cell death by decreasing the activity of kinases involved in neurodegeneration [28]. It has been known that IL-10 is a potent suppressor of TNFα, IL-1α, IL-1β and IL-6 [29]. *In vitro* studies have shown that IL-10 suppresses amyloid β peptide and/or lipopolysaccharide – induced inflam-

matory cytokines production in rat and murine microglia [30, 31]. Data from research conducted by Ma et al. demonstrated that polymorphism of IL-10 gene, which resulted in lower IL-10 serum concentrations, is related to higher AD risk [32]. However, in other study no significant differences in the allelic distribution of the IL-10 gene polymorphisms have been found between AD patients and controls [33].

Controversial opinions presented above suggest that laboratory methods used nowadays for evaluating potential biomarkers of AD in serum/plasma are insufficient. It is worth to notice that cerebrospinal fluid (CSF), compared to other body fluids, has the advantage of close proximity to the brain, hence, pathological processes are more likely to be reflected in CSF. Therefore, changes in beta amyloid levels in CSF show good sensitivity and specificity for AD [34].

We conclude that plasma beta amyloid and cytokine concentrations are altered in Alzheimer's disease. Further studies are needed to establish the ideal biomarkers in plasma or serum for diagnosis of AD as it is multifactor and heterogeneous disease.

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