The level of IL-1α, IL-10 and IL-17A in Alzheimer's disease patients: Comparative study

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Received 8, September, 2013
Accepted 4, December, 2013

Abstract:
The objective of this study is to evaluate the level of cytokines IL-1α, IL-10 and IL-17A in the serum of patients with Alzheimer's disease (AD), vascular dementia (VD) and down syndrome (DS). The results showed that Serum level of IL-1α was significantly increased in AD patients (3.79 ± 0.26 pg/ml) as compared with DS patients (2.78 ± 0.39 pg/ml) or controls (2.78 ± 0.22 pg/ml), while no significant difference was observed between AD and VD (3.25 ± 0.20 pg/ml) patients or between VD patients, DS patients and controls. The serum level of IL-10 was approximated in VD and DS patients and controls (3.39 ± 0.24, 2.77 ± 0.39 and 3.41 ± 0.35 pg/ml, respectively), but was significantly (P ≤ 0.05) increased in AD patients (5.73 ± 0.55 pg/ml) as compared to these groups. The serum level IL-17A was significantly increased in AD and VD patients (6.28 ± 0.35 and 5.32 ± 0.42 pg/ml, respectively) as compared with DS patients (3.75 ± 0.40 pg/ml) or controls (4.05 ± 0.28 pg/ml).IL-10 is important to differentiation between AD and VD.

Key words: Alzheimer's disease, IL-1α, IL-10, IL-17A cytokines

Introduction:
Alzheimer's disease (AD) is an age-related heterogeneous neurodegenerative disorder associated with progressive functional decline, dementia and neuronal loss. And it is considered as a major public health problem with a huge associated impact on individuals, families, healthcare system and society [1]. Senile plaques are composed primarily of the protein fragment β-amyloid (Aβ), and are generally thought to be formed extracellularly. Although there is also from murine suggested models that the process of oligomerization and subsequent deposition begins in intracellular compartments [2]. These effects are achieved through both autocrine stimulation (i.e., affecting the same cell that secreted it) and paracrine (i.e., affecting a target cell in close proximity) activities, and can also exert systemic or endocrine activities [3]. The main function of cytokines is the regulation of T-cell differentiation from undifferentiated cells to T-helper 1 and 2, regulatory T cells, and T-helper 17 cells. These regulatory proteins include interleukins (ILs), interferons (IFNs), colony stimulating factors (CSFs), tumor necrosis factors (TNFs), and certain growth factors [3]. Cytokines are induced in response to specific stimuli; for instance, bacterial lipopolysaccharides, flagellin, or other bacterial products, through the ligation of cell adhesion molecules or through the recognition of foreign antigens by host lymphocytes. Many of these cytokines have been shown to be produced by neurons or glia and there are a number of reports indicating changes in their levels in AD brain, blood and cerebrospinal fluid [4].

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Levels of IL-1α, IL-1β, IL-6, TNF-α and IFN-α have been reported to be increased in AD patients, and a number of interactions between cytokines and components of the AD senile plaques have also been reported suggesting that a vicious circle might be generated [5]. Thus, the Aβ protein of the plaques has been suggested to potentiate the secretion of several interleukins by activated astrocytoma cells; moreover, synergistic effects may also occur between cytokines and Aβ. For example, IFN-γ has been shown to synergize with Aβ to cause the release of TNF-α and reactive nitrogen species that are toxic to neurons, and IL-1 is reported to increase the toxicity of Aβ in PC12 cell line [6]. In the present study, three cytokines were investigated: IL-1α, IL-10 and IL-17A.

Materials and Methods:
Three groups of subjects were enrolled in the present study during the period November 2011 - May 2012. The first included 30 cases of Alzheimer's disease (AD), with an age range of 38-100 years. These cases were ascertained through Psychiatric Private Clinics distributed in Baghdad and surrounding governorates, in which the diagnosis was made. The diagnosis was based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (now known as the Alzheimer's Association) work-group criteria (NINCDS-ADRDA). This diagnostic tool specifies eight cognitive domains that may be impaired in AD: memory, language, perceptual skills, attention, constructive abilities, orientation, problem solving and functional abilities [2]. In addition, all patients were evaluated using Magnetic Resonance Imaging (MRI) to reach the most probable clinical diagnosis of AD. The second group included 28 patients (age range: 61-92 years), who had vascular dementia (VD), and they were ascertained from the same clinics. Both groups of patients were subjected to a personal interview using a designed questionnaire. A third group included 10 Down’s syndrome (DS) cases with an age range of 8-15 years. A fourth group of age (age range: 44-90 years), gender and ethnicity (Arab Muslims) matched controls were also enrolled. They were 20 individuals who had no history of any cognitive difficulties (apparently healthy).

Cytokines (IL-1α, IL-10 and IL-17A)
The sera of patients and controls were assessed for the level of three cytokines, which were IL-1α, IL-10 and IL-17A by means of ELISA that were based on similar principles, the standard curves were as the following.

Fig. 1-1: Standard curve of IL-1α

Fig. 1-2: Standard curve of IL-10.
Statistical Analysis:

Data were presented as either percentage frequencies or means ± standard errors (S.E.). Significant differences between percentage frequencies were assessed by Pearson’s Chi-square test, while such differences between means were assessed by ANOVA (analysis of variance) followed by Duncan test, in which probability (P) ≤ 0.05 was considered significant. In both cases, the computer package SPSS version 16 was used to carry out such analysis. In further analyses, significant differences between proportions were assessed by Z test. Odds ratio was also assessed in some cases. The latter two assessments were carried out using the computer package PEPI version 4.

Results and Discussion:

Interleukin-1α

Serum level of IL-1α was significantly increased in AD patients (3.79 ± 0.26 pg/ml) as compared with DS patients (2.78 ± 0.39 pg/ml) or controls (2.78 ± 0.22 pg/ml), while no significant difference was observed between AD and VD (3.25 ± 0.20 pg/ml) patients or between VD patients, DS patients and controls (Table 1-1). In addition, distributing the subjects of the four investigated groups according to gender, revealed no significant differences between males and females.

The present results suggest a role for IL-1α in the pathogenesis of AD. With respect to AD, over-expression of IL-1α in Alzheimer brain was demonstrated, and such over-expression was evident both immunohistochemically, as a 6-fold increase in the numbers of IL-1α-immunoreactive microglia, and biochemically, as elevated tissue levels of IL-1α. These IL-1α-overexpressing microglia in Alzheimer brain were frequently associated with Aβ plaques, and the pattern of distribution of these microglia across brain regions correlated with the distribution of Aβ plaques [7]. Such over-expressing microglia further suggests a role for IL-1α in the initiation and progression of neuritic and neuronal injury in AD. This association appeared to commence early in plaque formation, to wax and wane with neuritic pathology within the plaques (and with the conversion of diffuse Aβ deposits into compact form), and ultimately to disappear in the end-stage “burnt-out” plaques that are devoid of injured neuritic elements [8]. In AD, even the early, diffuse (non-fibrillar, and nonneuritic) ‘pre-amyloid’ deposits were found to contain activated microglia that over-expressing IL-1α. This is in contrast to a lack of microglia in the similar diffuse Aβ deposits sometimes found in nondemented elderly individuals; an observation that suggests that activated microglia may be important in the initiation of plaque progression and of the neuritic pathology that is central to the initiation and progression of AD [8]. The transformation of the presumably benign diffuse deposits of Aβ protein into the diagnostic neuritic plaques of AD was found to be accompanied by increases in the number, size, and IL-1α immunoreactivity of plaque-associated microglia, and this was accompanied...
by progressive condensation of diffuse Aβ deposits to form congophilic amyloid [9]. Due to such role of IL-1α in AD, the studies have also been extended to shed light on the association between IL-1α genetic polymorphisms, and several authors have reported a significant association between some variants of IL-1α gene and AD [10-13]. These studies strongly correlated between the serum level of IL-1α and its genetic polymorphism and AD, and an implication of such cytokine in the progression of AD can not be ignored.

Table 1-1: Serum level of cytokines in Alzheimer's, vascular dementia and Down's syndrome patients and controls.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Serum Level of cytokines (pg/ml)</th>
<th>Mean ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IL-1α</td>
<td>IL-10</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>30</td>
<td>3.79 ± 0.26&lt;sup&gt;A&lt;/sup&gt;</td>
<td>5.73±0.55&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>28</td>
<td>3.25 ± 0.20&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.39±0.24&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Down's syndrome</td>
<td>10</td>
<td>2.78 ± 0.39&lt;sup&gt;B&lt;/sup&gt;</td>
<td>2.77±0.39&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>2.78 ± 0.22&lt;sup&gt;B&lt;/sup&gt;</td>
<td>3.41±0.35&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Different letters: Significant difference (P ≤ 0.05) between means.

**Interleukin-10**

The serum level of IL-10 was approximated in VD and DS patients and controls (3.39 ± 0.24, 2.77 ± 0.39 and 3.41 ± 0.35 pg/ml, respectively), but was significantly (P ≤ 0.05) increased in AD patients (5.73 ± 0.55 pg/ml) as compared to these groups (Table 1-1). In addition, distributing the subjects of the four investigated groups according to gender, revealed no significant differences between males and females.

These results suggest that IL-10 (anti-inflammatory and regulatory cytokine) may play a role in the pathogenesis of AD. In agreement with such suggestion, [14] reported that level of IL-10 is elevated in the serum of patients with dementia but these levels do not discriminate between different types of dementia, and one of the mechanisms attributed to the role of IL-10 in reducing inflammation in AD is suppression of pro-inflammatory cytokines. Such increase has also been correlated with Aβ and following immunization with full length Aβ and a corresponding reduction in plaque load, Tg2576 mice displayed elevated IL-10 plasma levels. Similarly, mice expressing mutant APP and human presenilin 1 (PS1), immunized with an adenovirus vector encoding repeats of Aβ, showed increased IL-10 in blood plasma following treatment [15], while a treatment of the mice with granulocyte colony stimulating factor (GM-CSF) reduced plasma levels of several cytokines, including IL-10 [16]. Accordingly, monitoring serum level of IL-10 in AD patients may have therapeutic benefits, but studies of serum cytokines in AD patients thus far do not have the consistency necessary for a biomarker, and these preclinical studies suggest that inflammatory markers; for instance IL-10, may have utility as indicators of therapeutic efficacy [17]. Interleukin-10 has also been suggested to play an important role in neuronal homeostasis and cell survival, and mediates its effect on cells by interacting with specific cell surface receptors (IL-10Rs), present on glial cell populations in the brain, and it limits inflammation by reducing the synthesis of pro-inflammatory cytokines such as IL-1α by suppressing cytokine receptor expression and by inhibiting receptor activation in the brain [18]. The
regulatory role of IL-10 in AD (and its correlation with Aβ) has also recently been documented in vitro after challenging mononuclear cells obtained from AD patients with Aβ. The results revealed that IL-10 is produced by Aβ-specific T helper cells and highlight the T-cell-mediated nature of the observed regulatory polarization of the immune response in Alzheimer patients [19].

**Interleukin-17A**

The serum level IL-17A was significantly increased in AD and VD patients (6.28 ± 0.35 and 5.32 ± 0.42 pg/ml, respectively) as compared with DS patients (3.75 ± 0.40 pg/ml) or controls (4.05 ± 0.28 pg/ml) (Table 1-1). In addition, distributing the subjects of the four investigated groups according to gender, revealed no significant differences between males and females. Interleukin-17A is pro-inflammatory cytokine secreted by activated T cells, but recent investigations demonstrated that IL-17A can also be secreted by innate immune cells such as macrophages, dendritic cells, and NK cells, and such cytokine emerged as critical players in the pathophysiology of immune-mediated chronic inflammatory diseases [19]. Its relation with AD or VD has not well been investigated, although the present results may suggest a role in both morbidities. However, [19] analyzed the TH17 response in wild-type mice after vaccination with Aβ, and described for the first time of a TH17 immune response after Aβ peptide immunization. A direct role for TH17 cells as effector cells causing neuronal dysfunction and neuroinflammation has recently been described by in vivo imaging. Serum Level of IL-17A (pg/ml) experiments in an EAE mouse model [20], and it is possible that Aβ specific TH17 cells might have been involved in the occurrence of the meningoencephalitis in AD patients; however, further studies are certainly required to define the role of IL-17A in AD.

**Reference:**

Expression of cytokine IL-1α and S100β in different types of plaques in Alzheimer's disease. Zhonghua Bing Li Xue Za Zhi ;40(9):581-4.
تقيم مستوى الحركيات الخلوية (IL-1α, IL-10, IL-17α) في مرضى الزهايمر: دراسة مقارنة

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الخلاصة:
تهدف الدراسة الحالية إلى تقييم مستوى الحركيات الخلوية (IL-1α, IL-10, IL-17α) في مصل الدم للمرضى المصابين بمرض الزهايمر ومقارنتها بمستوياتها في مصل مرضى الخرف الناشئ عن الجلطة ومرضى متلازمة داون.

أظهرت هذه الدراسة أن مستوى (IL-1α) اظهر زيادة معنوية (3.79 ± 0.26 pg/ml) في مرضى الزهايمر مقارنة مع مرضى متلازمة داون وعينات السيطرة، بينما لم يلاحظ فرق معنوي مع مرضى الخرف الناشئ عن الجلطة، وإن مستوى (IL-10) كان متقارب في كل من متلازمة داون ومرضى الخرف الناشئ عن الجلطة (3.39 ± 0.39 pg/ml) ، بينما أظهر زيادة معنوية في مرضى الزهايمر أما مستوى (IL-17α) فقد أظهر زيادة معنوية في مرضى الزهايمر ومرضى الخرف الناشئ عن الجلطة (6.28 ± 0.35 and 5.32 ± 0.42 pg/ml) مقارنة مع عينات السيطرة.

نستنتج من الدراسة الحالية إن الحركيات الخلوية الثلاث (IL-1α, IL-10, IL-17α) ترتبط في مرضى الزهايمر ومرضى الخرف الناشئ عن الجلطة الدماغية من خلال

(10).