Alzheimer's Disease: Serum Biological Markers in Relation to Disease Severity

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ABSTRACT

Background and Purpose: The diagnosis of Alzheimer's disease (AD) is currently based on clinical and neuropsychological examination. To date, there is no blood test available that can discriminate dementia patients from healthy individuals. In the present study, we measured levels of S100B, interleukin-1 (IL-1) and vitamin E in sera of AD patients to find their relation with disease severity in an attempt for intervention of new prophylactic treatment strategies.

Patients and Methods: S100B, interleukin-1 (IL-1) and vitamin E were measured in 65 patients (30 males and 35 females mean age 71.1±3.2 years) diagnosed as clinically probable AD. Their levels were compared with those of age and sex matched healthy controls. All patients were evaluated clinically using Mini-mental State Examination (MMSE), the Global Deterioration Scale and the Clinical Dementia Rating. Patients were divided into three groups according to disease severity on basis of MMSE. Results: There was significant increase of serum S100B, IL-1 levels among patients than controls, while serum vit. E levels were slightly reduced among patients than controls. When comparing serum levels of S100B, IL-1 and vit. E between the three groups of patients: only S100B showed significantly negative correlation with MMSE. Whereas IL-1 and vit. E levels were significantly different in severe cases only in comparison with mild and moderate patients. Conclusion: the definite statement concerning the role of inflammatory biomarkers in AD needs the use of sensitive assays, large patient number and studies showing improvement of cognitive functions after vit. E supplementation are awaited for with interest.

INTRODUCTION

In recent years, the incidence of dementia as a major public health problem has escalated largely due to an increased life expectancy¹. Alzheimer's disease (AD) is the most common form of senile dementia accounting for 50-60% of all cases, and is considered as the fourth highest cause of disability and death in the elderly². Although its occurrence may be age-related, AD is not an inevitable consequence of the aging process³.

Alzheimer's disease is a heterogenous disease with respect to several possible pathophysiological causes and mechanisms contributing to a common stage of cell death⁴, but the exact nature of the molecular entity triggering dementia and cognitive deficits is still open to debate⁵.

In the ideal situation, early diagnosis should be possible with an early and unexpensive blood test, however, no single peripheral biological marker with specificity for AD has been found⁶.

S100B is predominantly astrocytic protein with a cytokine-like functions. The chronic overdose of S100B in Down's syndrome with its associated progressive neurodegeneration and the resemblance of some clinicopathological features to those of AD have led to the hypothesis that S100B may contribute to disease progression⁷. Interleukin-1 (IL-1), a key molecule in systemic immune responses in health and disease, has analogous roles in the brain, where it may contribute to neurodegeneration⁸. Vitamin E (vit. E) is known as a major chain breaking antioxidant in the brain and has been advocated as a modulator of cognitive performance⁹.

The aim of our study is to measure serum levels of S100B, IL-1 and vit. E, in relation to AD patients, hence early AD stage predictive biomarkers would enable diagnosis of the disease at a premature...
phases opening up the prospect for timely application of corrective therapeutic strategies.

**SUBJECTS AND METHODS**

A total of 65 patients of both sexes were included in our study, diagnosed as AD (clinically probable)-according to the criteria of National Institute of Neurological and Communication Disorders and AD and Related Disorders Association (NINCDS/AD RDA)\(^{10}\) with Hachinski Ischemic Score (HIS)<4\(^{11}\). Patients were recruited from the outpatient clinic of Neurology Department of Zagazig University Hospitals. Twenty healthy subjects age and sex matched served as controls.

All subjects were screened by medical history, general and neurological examination, electrocardiogram and laboratory evaluation of serum electrolytes, glucose, renal and hepatic functions, complete blood count and thyroid function studies.

Subjects with past or present major psychiatric disorders, serious head trauma, hypoxia, neurological diseases other than AD, renal or hepatic diseases or chronic obstructive pulmonary diseases were excluded.

All subjects were given the Mini Mental State Examination (MMSE)\(^{12}\) to assess cognitive functions. The assigned scores ranging from 30-for normal function down to 5 in case of severe dementia. These assessments were further supported by tests of cognitive function in accord with the Global Deterioration Scale (GDS)\(^{13}\) and the Clinical Dementia Rating (CDR)\(^{14}\). CDR stages 1 and 2 include persons in the earlier or mild-moderate stages of dementia, stages 3, 4 and 5 include persons in the later advanced stages of dementia.

Patients were divided into 3 groups regarding severity of dementia according to the MMSE\(^{15}\) as follows:

* Group I: included mild cases where MMSE ranged from >19-23.
* Group II: included moderate cases ranging from 10-19.
* Group III: included severe cases with MMSE < 10.

Magnetic resonance imaging (MRI) scan (0.5-Tesla general Electric SIGMA Contour system) was carried out for all patients to exclude any vascular lesion\(^{16}\).

**Serum S100B measurement:**

Venous blood was withdrawn, sera were separated and stored at 20°C. The assessment was done by the commercially available monoclonal two site immunoluminometric assay and fully automatic LIA-mat system (Byk-Sang Tec diagnostica, Dietzenbach, Germany). The detection limit of this test was 0.02 mg/L. When the S100B level was under the detection limit, the value was recorded as 0.02 mg/L.

**Serum IL-1B:**

Venous blood sample was withdrawn and allowed to clot at room temperature for 30 minutes and after being centrifuged for ten minutes, the obtained serum was stored at -80°C. IL-1B levels in serum samples were quantified by ELISA technique, (DIACIONE) research. A monoclonal antibody specific for IL-1B had been coated into wells and microtitre strips. Smaples were pippted into those wells and then incubated. During incubation, the IL-1B antigen and biotinylated monoclonal antibody specific for IL-1B were simultaneously incubated. After washing, the enzyme (streptaridin-peroxylase) is added.

After incubated and washing to remove all unbound enzyme, a substrate solution which is acting on the bound enzyme is added to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL-1B present in the samples.

**Vit. E measurement:**

Serum vit. E was estimated by HPLC according to Teissier et al.\(^{17}\).

Fifty ml of standard (SIGMA Aldrichchemico, Germany) and samples of both controls and patients were diluted in 850 ml of methanol mixed twice for ten seconds by vortex shaker. Centrifugation for one minute at 13000 rpm then 50 ml of the supernatant were injected directly in HPLC apparatus (pum, Gbc-Lc 1150 Australia) flow rate 1m/min, using fluorescence detector set at 295 nm and 330 nm for excitation and emission respectively. The results were calculated and analyzed by Winchrom software.
using calibration curve of vit. E standard which was constructed by using six different concentrations.

**Statistical analysis:**

Data were analyzed by a Statistical Package for Social Sciences SPSS for Windows version 11. Data were expressed as mean±standard deviation for quantitative variables, number and percentage for qualitative variables. ANOVA, student t-test, chi-square and correlation coefficient were used when appropriate. P-value <0.05 was considered to be statistically significant.

**RESULTS**

Sixty five patients diagnosed as clinically probable AD were included in this study. They were 30 males and 35 females, their ages ranged from 66-77 years (mean±SD, 71.1±3.2). Twenty healthy subjects served as controls (12 males & 8 females), mean age 69.8±3.1.

Table (1) shows demographic data, MMSE and disease duration. MMSE scores were significantly lower among AD patients than controls. These assessments were also supported by tests of cognition in accord with the GDS and the CDR. All clinical evaluations yielded consistent results throughout, therefore only MMSE criteria were used for comparative cognitive and statistical studies.

Patients were divided into three groups regarding disease severity in accord with MMSE.
- **Group I** (mild disease): including 34 patients with MMSE ranging from >19-23.
- **Group II** (moderate disease): including 22 patients with score ranging from 10-19.
- **Group III** (severe cases) including 9 patients scoring less than 10 in MMSE.

Serum levels of S100B, IL-1 and vit. E in patients and controls are shown in Table (2) with S100B and IL-1 levels highly significant increase among patients than controls, whereas serum vit. E levels were slightly reduced in patients than controls with no statistical significant difference.

When comparing serum levels of the three studied biomarkers between the three groups of patients: Table (3) shows increased serum S100B levels with increasing severity of the disease with statistical significant difference between the three groups. Regarding IL-1 and Vit. E serum levels, a statistically significant difference is only detected between group III in comparison to group I and II, with increased levels of IL-1, and decreased levels of vit. E, where no statistical significant difference were found between group I and II.

A significant negative correlation is shown between MMSE and serum S100B in the 3 patients groups. Whereas IL-1 and vit. E did not correlate significantly with disease severity judged by MMSE (Table4).

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<thead>
<tr>
<th>Table 1. Demographic and clinical of patient and control groups.</th>
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<tr>
<td><strong>Control</strong> (n=20)</td>
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<td><strong>Age in years</strong></td>
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<td>Mean±SD</td>
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<td><strong>Disease duration</strong></td>
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### DISCUSSION

The leading cause of dementia is AD, which is a progressive neurodegenerative disorder affecting the elderly\(^1\). It is distinguished by a progressive and devastating mental decline that is usually expressed by memory impairment stemming from a disturbance within medial temporal lobe structures such as hippocampus\(^1\). Considering the limited capacity of the central nervous system (CNS) tissue to repair, early intervention in the degenerative processes will be crucial to spare as much tissue as possible\(^2\). Early diagnosis may then help to increase the possibilities for developing and testing new preventive treatment strategies\(^3\).

S100B acts as an astrocyte-derived cytokine with a physiologically beneficial role in promoting neuronal survival and development\(^2\), as well as the synthesis of amyloid precursor protein (APP) in neurons and neurites\(^2\). However, pathologically over expressed S100B induces dystrophic changes in neurons and neurites, which correlate with the transformation of benign amyloid deposits into neuritic plaques responsible for cortical atrophy in AD\(^7\).

In our study, the serum content of S100B was significantly elevated among AD patients than controls. A finding that is supported by previous two reports\(^5,23\). We were not able to compare our results with most of earlier studies for two reasons: firstly to our knowledge almost all previous studies\(^7,23,24,25\) had measure S100B in cerebro-spinal fluid (CSF) not in serum. Secondly, they had used patients suffering neurological diseases other than dementia as control subjects so the results could have diagnostic value only for discrimination of AD patients from other CNS patients. We have found a significant relation between serum S100B levels and disease severity where S100B levels correlated negatively with the MMSE scores, a finding that lend further support to the suggestion that S100B is an important, early acting pathogenic agent in the...
progression of the neuropathophysiological changes underlying the clinical manifestation of AD. In contrast to our results, previous study did not show significant differences in S100B levels in relation to disease severity. This may be referred to the broad range of clinical dementia severity and relatively larger number of AD patient samples examined in our study.

A major inducer of astrocyte activation and S100B expression is the immunomodulatory cytokine IL-1. IL-1 is markedly expressed by activated microglia in AD. In the current study, there was a significantly higher levels of serum IL-1 in AD patients in comparison to control subjects, which was also reported by another study. Where as most studies that investigated serum showed no difference in serum IL-1 levels between AD patients and controls. This may be regarded to the low level of IL-1 present in serum of normal controls in combination with the low sensitivity of the ELISA systems used. So, differences in mean concentrations can only be measured when a large group of AD patients is included, consequently an increase in IL-1 concentration in serum may have very limited diagnostic value for single patients.

When comparing serum IL-1 levels between the three groups of our patients, only the severe AD patients (group III) revealed significantly higher levels when compared with the other two groups. This may be explained by the fact that IL-1 is not able to cross the intact blood brain barrier (BBB), and only with progression of the disease to severity stage and disruption of BBB to permit significant peripheral increase of the level of IL-1 to be detected. In such case, the altered serum level in the mild and moderate cases may not be due to increased flow of inflammation related proteins from the brain when the BBB is intact. It might be possible that a whole body damage in concentrations of interleukins occurs, which affects the brain tissue specifically. Alternatively, damage to brain tissue may be one of the various effects of an increased peripheral concentration of interleukins.

Vitamin E is considered as an important antioxidant whose level can be influenced by dietary habits. It is especially important for the brain, considering the high lipid content and a relatively high proportion of polyunsaturated fatty acids of the brain.

When comparing serum vit. E levels between patients and controls, there was no significant difference with decreased levels among all AD patients under normal dietary circumstances i.e. without any supplementation. Decreased concentrations of vitamins A, C and E in serum of patients with dementia have been reported.

Regarding the relation between disease severity and vitamin E concentration, there was a significant decrease of vit. E levels among group III patients compared to group I & II. However, no correlation was found between serum vit. E and disease severity. A low vitamin status may give an indication of the susceptibility of an individual to oxidative damage. Nevertheless, the causality in the relation between cognitive impairment and vitamin status is not known. As vitamin deficiency is common in older people due to insufficient intake, reduced intestinal absorption. Thus dietary intake or supplementation can influence the levels of this vitamin.

In summary, S100B plays an important role in initiation and progression of AD, though the definite statements concerning inflammation biomarker differences between control and AD patients require the use of sensitive assays, and large patient groups. This is imperative not only for measuring very low concentrations of S100B or IL-1 but all possible AD markers. Studies showing improvement of cognitive functions after vit. E. supplementation is awaited.

REFERENCES


ملخص العربي

الدلالات البيوكيميائية في مصل الدم لمرضى الزهايمر وعلاقتها بشدة المرض

بعد العينة من المشاكل الصحية الشائعة بسبب زيادة في معدل الأعمار ويعتبر مرض الزهايمر من أشهر أسباب عنة الشيخوخة حيث يمثل حوالي 50-60% من أسباب العنة لدى المسنين.

الهدف من الدراسة: قياس بعض الدلالات البيوكيميائية (S100B، البتانولين، فيتامين هـ) في مرضى الزهايمر وعلاقة هذه الدلالات بشدة المرض.

وقد تمثلت هذه الدراسة على 65 مريضاً بمرض الزهايمر تم تقسيم المرضى طبقاً لمواصفات المقياس العالمي للأمراض العصبية ومجموعة مرضى الزهايمر والأمراض المتعلقة بها، والسكينة الدماغية بالإضافة إلى 20 شخص من الأصحاء كمجموعة مقدمة، وقد أجريت لجميع المرضى والمجموعة الضابطة الفحوصات التالية: فحص طبي عام، فحص عصبي دقيق بالإضافة إلى الفحوصات العملية الزوتينية، كما تم عمل أشعة بالرنين المغناطيسي على المخ لجميع المرضى، تم قياس قدرات المرضى المعرفية باستخدام مقياس حالة الذهنية المصغر، مقياس التدهور الشامل وكذلك مقياس العنة الإكلينكي وتم تقسيم المرضى إلى ثلاث مجموعات طبقاً لأنماط في مقياس حالة الذهنية المصغر.

تم قياس مستوى S100B، البتانولين 1، فيتامين H في مصل الدم لكل من المرضى والمجموعات الضابطة، وأظهرت الدراسة النتائج التالية: تراوحت أعمار المرضى من 66-77 عام، وكان أداء المراض أسوأ في مقياس حالة الذهنية المصغر عن أداء المجموعة الضابطة وكانت النتيجة ذات دالة إحصائية. وقد أرتفع مستوى S100B، البتانولين 1 في مصل الدم عن المرضى عن المجموعة الضابطة بمجرد ذات دالة إحصائية، أما بالنسبة لمستويات فيتامين H في مصل الدم فلم يكن هناك فروق ذو دالة إحصائية في المرضى عن المجموعة الضابطة. كما أظهرت النتائج أيضاً، كما زادت شدة المرض ازداد مستوى S100B في مصل الدم وكانت العلاقة ذات دالة إحصائية.
الخلاصة: نستنتج من هذه الدراسة أن زيادة مستوى S100B في مصل الدم لمرضى الزهايمر لها علاقة مباشرة بشدة المرض ولذلك فإن التدخل المبكر لعلاج هؤلاء المرضى يمكن أن يمنحهم فرصة أكبر للتحسين وعدم تدهور قدراتهم المعرفية ويحتاج ذلك لدراسات مستقبلية على عدد أكبر من المرضى.