Research Article

Plasma levels of vitamin B₁₂, epidermal growth factor and tumor necrosis factor alpha in patients with alzheimer dementia

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ABSTRACT

Background: It was previously reported that vitamin B₁₂ (Vit B₁₂) has the regulatory effects on epidermal growth factor (EGF) and tumor necrosis factor alpha (TNF-α). The role of Vit B₁₂, EGF and TNF-α in the pathogenesis of alzheimer dementia has not been elucidated yet. In this study the plasma Vit B₁₂, EGF and TNF-α level were examined in individuals, between 65-99 years old with and without alzheimer dementia (AD).

Methods: The study group comprised 47 patients with AD and 38 cases without dementia. EGF and TNF-α were analyzed by ELISA, and Vit B₁₂ was analyzed by chemiluminescence method.

Results: Vit B₁₂ and EGF levels were significantly lower (p<0.0001), whereas TNF-α levels were significantly higher (p<0.0001) in the AD group in comparison to those without AD.

Conclusions: Our results suggest that Vit B₁₂, EGF and TNF-α may have a role in the pathophysiology of AD.

Keywords: Alzheimer dementia, Vitamin B₁₂, Epidermal growth factor, Tumor necrosis factor alpha

INTRODUCTION

Dementia is a slow-onset clinical condition characterized by cognitive dysfunction, memory impairment, behavioral and personality changes, and poor judgment which later progresses to severe.¹,² The prevalence of dementia rises markedly with age, the rate is about 10% in people between 65-70 years and 20-48% in over 70 years. Alzheimer dementia (AD) and vascular dementia are the two most common types of dementia. AD accounts for 60% of all dementias.³

Various studies reported that vitamin B₁₂ (Vit B₁₂) levels were lower in individuals with dementia than in those without.⁴,⁵ Reports indicated that, cerebral oxidative damage was due to increased oxidation of Vit B₁₂ generated by methylene synthase activity and the resulting disruption of homocysteine metabolism in AD.⁶,⁷ Current data is not adequate to elucidate the exact correlation between the pathogenesis of AD and Vit B₁₂.⁸,⁹ However low levels of Vit B₁₂ were reported in patients with dementia and significant improvement in cognitive functions were observed by Vit B₁₂ replacement therapy.¹⁰

Vit B₁₂ deficiency in rats and humans was associated with a decrease in epidermal growth factor (EGF) levels and an increase in tumor necrosis factor alpha (TNF-α).¹¹ These results suggest a role for EGF and TNF-α in the neuropathologic mechanisms in dementia patients. Vit B₁₂ is a regulator of the balance between TNF-α and EGF in the central nervous system.¹²

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EGF is a major cytokine in neurogenesis and has protective effects on neurons. A change in the EGF levels may be an inflammatory response to the neuron damage due to the disruption of methylation reactions or single-chain fatty acid metabolism.\(^\text{13}\) TNF-\(\alpha\) and EGF levels change in patients with Vit B\(_{12}\) deficiency since Vit B\(_{12}\) has a major role in the transmission of homocysteine to methionine.\(^\text{14}\) Since EGF is essential for Vit B\(_{12}\) effect in the rat central nervous system, it was shown that EGF receptors may change the biologic signaling of Vit B\(_{12}\) both in neurons and in glial cells.\(^\text{14}\)

TNF-\(\alpha\) is a major proinflammatory cytokine with high levels in AD patients.\(^\text{15}\) TNF-\(\alpha\) is an inducer and regulator of the cytokine cascade in the inflammatory response.\(^\text{16, 17}\) TNF-\(\alpha\) has a major role in the termination and localization of the inflammation, and the repair of the damage.\(^\text{15, 17, 18}\) TNF-\(\alpha\) induces proliferation of astrocytes and expression of interleukine-6 (IL-6) by astrocytes.\(^\text{19, 20}\) IL-6 is another proinflammatory and anti-inflammatory cytokine.\(^\text{21}\) TNF-\(\alpha\) increases nitric oxide (NO) concentration in the central nervous system by inducing NO synthesis. NO has a significant role in central nervous system diseases; it may specifically inhibit methionine synthase activity.\(^\text{22-24}\) Increased inflammation is a major factor in the pathogenesis of AD which again may be associated with TNF-\(\alpha\).

The aim of the present study was to determine the plasma levels of Vit B\(_{12}\), EGF and TNF-\(\alpha\) in patients with and without AD.

**METHODS**

This study comprised of 47 patients with AD and 38 patients without dementia (control group) between 65-99 years of age. Dementia and control group members were recruited from nursing homes. The subjects of the AD and control groups did not have a history of depression, cerebral ischemia and Vit B\(_{12}\) deficiency. Informed consent for participation into the study was obtained from all patients.

AD and control groups were evaluated by neurological and psychiatric examinations and neurological tests. Diagnosis of AD was carried out by using mini mental state examination (MMSE),\(^\text{25}\) national institute of neurological and communicative disorders and stroke and the alzheimer disease and related disorders association criteria and DSM IV.\(^\text{26, 27}\) The MMSE scores were under 24 in AD group. AD patients underwent an imaging test (computerized tomography and/or magnetic resonance imaging) and several biochemical tests (serum levels of T3, T4, TSH folic acid, calcium, and tests for syphilis and HIV) for excluding secondary causes of dementia.

EGF and TNF-\(\alpha\) were analyzed by ELISA and Vit B\(_{12}\) was measured by chemiluminescence. Blood samples were collected between 4:00 and 6:00 p.m. in test tubes containing 5 ml EDTA for Vit B\(_{12}\), EGF and TNF-\(\alpha\) measurement. Tubes were centrifuged at 600 g for 10 minutes; plasma samples were separated and were kept at -80 °C until processing.

Data were analyzed statistically by using SPSS V.11.5 computer program. Mann Whitney U test was used for paired comparisons. The linear correlation between the variables was analyzed by Spearman’s correlation test. Covariance analysis was used after adjusting for age to assess the difference in EGF, TNF-\(\alpha\) and Vit B\(_{12}\) levels between the two groups. Data were expressed as mean±standard deviation or median±range. The levels of statistical significance were set at p <0.05.

**RESULTS**

In the AD group, the mean age was 77.44 years (between 65-99); 32 were female and 15 were male. In the control group, the mean age was 68.11 years (between 65-84); 23 were female and 15 were male.

While Vit B\(_{12}\) and EGF levels were significantly lower in the AD group than those in the control group (p<0.0001), TNF-\(\alpha\) levels were higher in the AD group than those in the control patients (p<0.0001) (Table 1, Figures 1-3).

There was no significant correlation of gender among Vit B\(_{12}\), EGF and TNF-\(\alpha\) levels in both groups (p>0.05). The mean ages in the AD and control groups were significantly different (p<0.001, Figure 4). The analysis was repeated after adjusting for age to evaluate the reason for the differences between the two groups for EGF, TNF-\(\alpha\) and Vit B\(_{12}\). The analysis revealed that the low levels of EGF and Vit B\(_{12}\), and the high levels of TNF-\(\alpha\) in AD patients seemed to be independent of age (Table 2).

**Table 1: Comparison of EGF, vitamin B\(_{12}\), and TNF-\(\alpha\) levels between groups.**

<table>
<thead>
<tr>
<th></th>
<th>Control group median value (range)</th>
<th>Group with dementia median value (range)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit B(_{12}) (pg/ml)</td>
<td>179.8 (22.3 - 609.8)</td>
<td>73.35 (12.4 - 236.5)</td>
<td>P &lt;0.0001</td>
</tr>
<tr>
<td>EGF (pg/ml)</td>
<td>157.75 (23.2 - 412.0)</td>
<td>90.2 (10.3 - 215.3)</td>
<td>P &lt;0.0001</td>
</tr>
<tr>
<td>TNF - (\alpha) (pg/ml)</td>
<td>84.105 (23.9 - 170.8)</td>
<td>97.6 (69.3 - 156.4)</td>
<td>P &lt;0.0001</td>
</tr>
</tbody>
</table>

*Mann - Whitney U test; Vit B\(_{12}\)-Vitamin B\(_{12}\); EGF-Epidermal growth factor; TNF – \(\alpha\)- Tumor necrosis factor alpha.
Table 2: Evaluation of the between-group differences of EGF, TNF-α and Vit B₁₂ levels after adjustment for age.

<table>
<thead>
<tr>
<th></th>
<th>Before adjustment*</th>
<th>P - value</th>
<th>After adjustment*</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean±SD)</td>
<td></td>
<td>(mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>EGF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>95.3±46.3</td>
<td>&lt;0.001</td>
<td>92.3±77.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>174.5±95.1</td>
<td></td>
<td>178.2±78.1</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>98.1±16.7</td>
<td>= 0.005</td>
<td>97.7±21.1</td>
<td>= 0.03</td>
</tr>
<tr>
<td>Control</td>
<td>86.0±22.0</td>
<td></td>
<td>86.6±20.4</td>
<td></td>
</tr>
<tr>
<td>Vit B₁₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dementia</td>
<td>89.8±63.2</td>
<td>&lt;0.001</td>
<td>94.1±130.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>214.8±160.1</td>
<td></td>
<td>209.5±132.2</td>
<td></td>
</tr>
</tbody>
</table>

*Adjustment for age was performed by analysis of covariance; SD: Standard deviation; Vit B₁₂: Vitamin B₁₂; EGF: Epidermal growth Factor; TNF-α: Tumor necrosis factor alpha.

DISCUSSION

This study was the first to examine EGF, Vit B₁₂ and TNF-α concurrently in AD patients. There are several studies researching the cytokines separately for their role in pathophysiology of dementia.

Vit B₁₂ deficiency may be associated with neurological and psychiatric disorders including subacute combined degeneration, multiple sclerosis, depression, dementia, and demyelinating myelopathy. Vit B₁₂ has two main functions; one is in the nucleic acid synthesis and methylation reactions and the other is the inhibition of N-methyl-D-aspartate receptors by its effect on homocysteine consequently leading to neuropsychiatric disorders. High plasma homocysteine levels are a major risk factor for cardiovascular diseases, stroke, and AD. A negative correlation was reported between total homocysteine and plasma Vit B₁₂ levels. The neuron damage in AD was associated with the oxidative stress due to Vit B₁₂ deficiency.
Studies on rats and humans suggested that Vit B12 deficiency decreased EGF levels and increased TNF-α levels.35,36 We found similar results in AD patients with lower plasma Vit B12 and EGF levels and higher plasma TNF-α levels than the controls. Vit B12 was reported to down regulate the expression of TNF-α and EGF genes in addition to its hormone-like and coenzyme functions in the central nervous system.36

Studies suggested that Vit B12 was an essential factor for the signaling pathway of the central nervous system of rats and that VitB12 deficiency with the absence of EGF which is a neurotropic factor, was associated with central neuropathy.37

Vit B12 replacement normalized the EGF levels in the CSF and EGF m-RNA expression in various areas of the central nervous system in rats that underwent total gastrectomy. The findings of this in vivo study showed that the neurotropic function of Vit B12 mediated the stimulation of EGF synthesis in the cerebrospinal fluid (CSF) of gastrectomized rats.36

Unbalanced production and expression of central nervous system cytokines including TNF-α results with neurodegeneration.38 TNF-α and EGF levels are reciprocal in humans with Vit B12 deficiency similar to that in rats; high production of TNF-α causes decreased production of EGF.35 The results of our study regarding plasma Vit B12, EGF and TNF-α levels in AD patients confirmed this relation.

The role of TNF-α in normal brain is not clear but it increases in neuronal cell damage. Increased inflammation is characteristic in the pathogenesis of AD and TNF-α may have a role in this process.18 Significantly increased levels of TNF-α in CSF were reported in AD patients.39

CONCLUSION

In conclusion, the lower plasma levels of EGF and Vit B12 and higher plasma levels of TNF-α in AD patients compared to the controls may suggest a regulatory role for Vit B12 in the pathophysiology of dementia. Further studies on this vitamin and cytokines in larger and more homogenous groups are required to clarify the role of cytokines and Vit B12 in dementia.

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REFERENCES


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