Vascular Endothelial Growth Factor and Angiogenin in Alzheimer's Disease

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Abstract

Background: Alzheimer's disease (AD) is the most common neurodegenerative disorders, yet its pathogenesis is not well established. Recently pathological angiogenesis become an attractive hypothesis to explain the evolution of Alzheimer's disease.

Objective: To assess the serum levels of angiogenin (ANG) and vascular endothelial growth factor (VEGF) in AD patients and its relation to disease severity.

Methods: Nineteen patients with AD, thirteen patients with mild cognitive impairment (MCI) and twelve healthy control were subjected to full medical, neurological examinations and the mini mental state examination test. MCI and AD patients were evaluated using Clinical dementia rating scale (CDR).Venous blood samples were collected from all subjects after overnight fasting for routine biochemical analysis and measurement of serum ANG and VEGF with ELISA.

Results: Serum levels of ANG and VEGF were significantly lower in patients with MCI and AD than healthy controls. The low serum levels of such cytokines were significantly negative correlated with CDR scores.

Conclusion: Low serum levels of ANG and VEGF indicating that pathological angiogenesis is one of the important mechanism that play a role in development and progression of AD. Monitoring of these factors may be considered as a marker for both diagnosis and progression of AD.

Keywords: Alzheimer's Disease; Angiogenin; Vascular Endothelial Growth Factor; Clinical Dementia Rating Scale (CDR)

Introduction

One of the emerging health problem worldwide is Alzheimer's disease (AD), yet its pathological basis still unclear [1]. The proposed mechanisms for AD include amyloid cascade hypothesis, cerebral hypo-perfusion, inflammation and gene polymorphisms [2]. Insufficient angiogenesis in the AD brain might represent another pathological mechanism involved in the disease development and progression [3,4]. on the other hand, impaired cerebral blood flow (CBF) may results in a compensatory increase of pathogenic angiogenesis in AD [5].

Vascular endothelial growth factor (VEGF) is one of the most important cytokine involved in blood vessels formation, and considered as key player in AD pathogenesis via modulating vascular permeability, and induction of BBB hyper-permeability [6]. On the other hand Angiogenin (ANG) is a member of the ribonuclease A superfamily, it's important for cellular proliferation induced by other angiogenic cytokines including VEGF, but its role in AD not well established [7]. In this study, we investigate the serum levels of ANG and VEGF in AD and its relation to disease severity.

Subject and Methods

This study includes nineteen patients (11 males and 8 females) diagnosed as AD, their mean age was 72.5 ± 7.3 years. Thirteen patients (7 males and 6 females) with amnestic mild cognitive impairment (MCI) their mean age was 69.3 ± 3.1 years (They were diagnosed according to Major and mild neurocognitive disorders diagnostic criteria, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) [8], and twelve healthy control subjects (7 males and 5 females) their mean age was 68.1 ± 4.2 years. All patients were recruited from Neuropsychiatry Department, Tanta University Hospital during the period from February 2017 till August 2017. All participants were subjected to full medical and neurological assessment, and Mini Mental State Examination (MMSE) [9]. Patients with AD and MCI were subjected to Clinical Dementia Rating (CDR) scale [10] to categorize severity of dementia in a five-point scale in which CDR-0 connotes no cognitive impairment and the remaining four points ranging from very mild dementia CDR-0.5 to severe one CDR-3. Patients with previous stroke, head trauma, cardiac, renal, hepatic, neoplastic disorders or intracranial infections were excluded from the study. Venous blood samples were collected after an overnight fasting (about 12 hr.) Aliquots of these samples were used for routine biochemical analysis immediately (fasting, 2 hr. post prandial blood glucose, serum cholesterol, triglycerides, HDL and LDL cholesterol), but aliquots for serum ANG and VEGF were stored at -20°C until tested. Serum ANG was measured by the commercially available enzyme linked immunosorbent assay (ELISA) kit (Ray Biotech.) as described by the company. Whereas VEGF was measured by enzyme immunoassay technique as described by the manufacturer's protocol supplied by (ALPCO Diagnostics).Informed consent was obtained from all cases from their 1st degree relatives and control subjects, and the protocol of the study was approved by the faculty of medicine ethical committee.

Statistical Analysis

In this study we use the Chi-square test, one way Anova and Spearman's linear correlation test, apart from SPSS statistical package

Results

Table 1 summarize the demographic and clinical data of the study participants, and shows no significant difference among them regarding age, sex, and the vascular risk factors (hypertension, diabetes mellitus and dyslipidemia).

	MCI N = 13	AD N = 19	CS N = 12	P value
Age	69.3 ± 09	72.5 ± 12	68.5 ± 11	0.314
Male %	53.8%	57.8%	58%	0.723
Smoking %	53.8%	52.6%	50%	0.888
Patients with hypertension	46.1%	47.3%	50%	0.568
Patients with diabetes mellitus	38.4%	42.1%	41.6%	0.561
Patients with dyslipidemia	30.7%	31.5%	33.3%	0.645

Table 1: *Demographic and Clinical characteristics of the studied subjects.*
MCI: Mild Cognitive Impairment; AD: Alzheimer's Disease; CS: Control Subjects

There was non-significant difference regarding years of education among the three groups. While there were significant differences among the studied group regarding MMSE scores. And 73.6% of AD patients had moderate to severe dementia according to CDR scale (Table 2).

	MCI N = 13	AD N = 19	CS N = 12	P value
Educations (years)	12.7 ± 2.6	13.2 ± 3.1	11.5 ± 2.8	0.085
MMSE score	23.1 ± 3.5	16.4 ± 4.9	25.3 ± 3.1	0.001
CDR %				
CDR-0	0.0	0.0	100.0	
CDR-0.5	100%	10.5	0.0	
CDR-1	0.0	15.7	0.0	
CDR-2	0.0	31.5	0.0	
CDR-3	0.0	42.1	0.0	

Table 2: Psychometric tests among the studied subjects.

MCI: Mild Cognitive Impairment; AD: Alzheimer's Disease; CS: Control Subjects; MMSE: Mini-Mental State Examination; CDR: Clinical Dementia Rating Scale.

The mean level of serum ANG in AD patients was significantly lower than that of MCI and control groups. Also, there was significantly lower serum level of VEGF in AD than MCI and CS groups (Table 3).

	MCI N = 13	AD N = 19	CS N = 12	p-value
Serum ANG (ng/ml)	278.23 ± 29.74	208.74 ± 19.84	378.50 ± 45.07	0.001
Serum VEGF (pg/ml)	280.23 ± 20.47	199.16 ± 15.20	381.08 ± 14.8	0.001

Table 3: Serum levels of ANG and VEGF among three groups.

MCI: Mild Cognitive Impairment; AD: Alzheimer's Disease; CS: Control Subjects; CDR: Clinical Dementia Rating Scale; ANG: Angiogenin; VEGF: Vascular Endothelial Growth Factor.

There was significant negative correlation between serum levels of ANG and VEGF and CDR scales (r = 0.925, P = 0.001), (r = 0.947, P = 0.001) respectively (Figure 1 and 2).
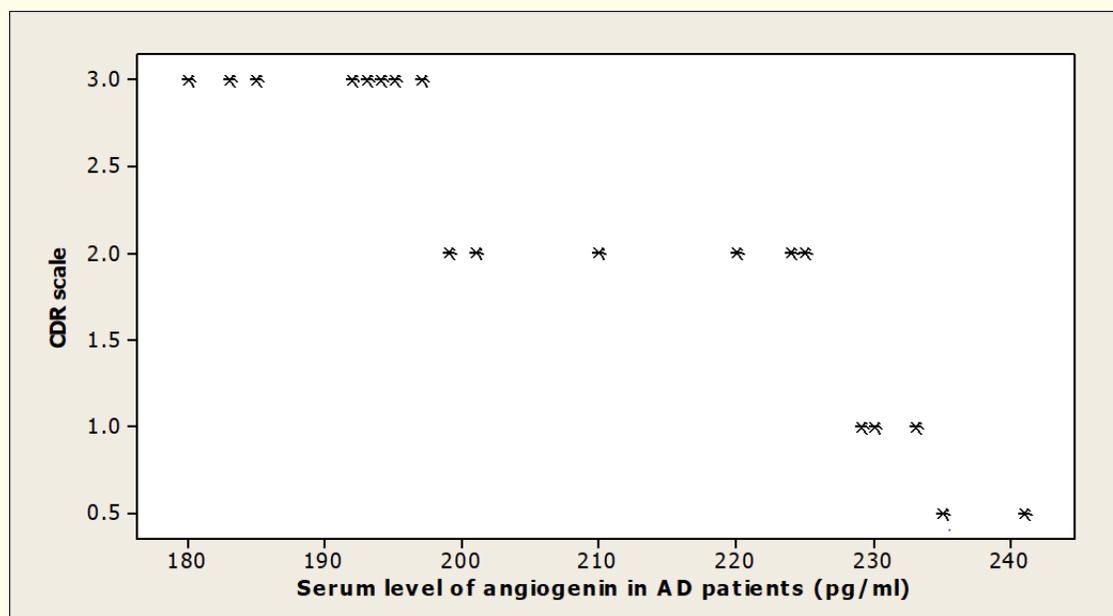
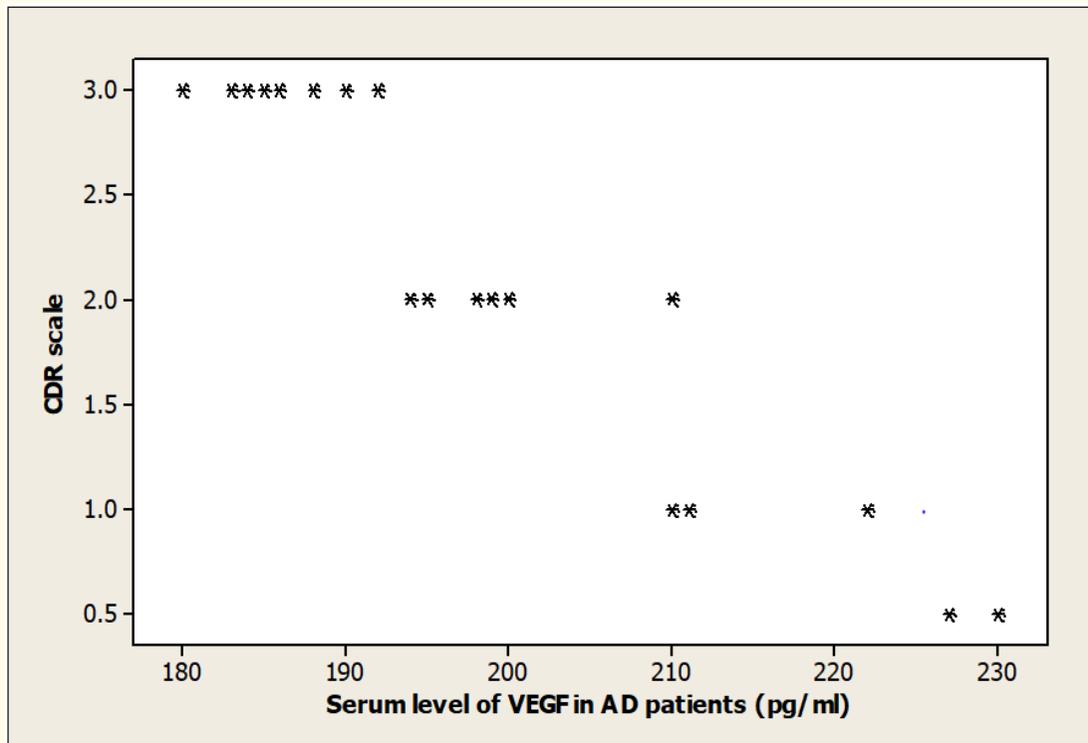*Figure 1: Correlation between serum level of ANG in AD patients and CDR scale.*

Figure 2: Correlation between serum level of VEGF in AD patients and CDR scale.

Discussion

The expected number of AD patients in 2040 will be more than 80 million, so it's essential to establish a reliable markers for diagnosis and prognosis of AD [11]. The relationship between vascular risk factors and AD is evident, as carotid atherosclerosis and elevated cholesterol level are associated with more cognitive decline, also, cerebral blood flow changes predict AD progression [12] vascular theory emerged as new concept to explain the pathophysiology of AD, this hypothesis assume that; decreased micro-vascular density, increased capillary irregularity, and the associated hypoxia results in induction of β secretase activity, decrease α secretase and increase reactive oxygen species with secondary formation of either neurofibrillary tangles or amyloid plaques [13].

Recent studies have reported that, beta amyloid (Aβ) causes increased angiogenesis in AD patients associated with BBB disruption leading to disease progression through promoting more amyloid aggregation [12,14]. Mixed aggregates of both β amyloid and VEGF were discovered in AD brain autopsy, and postmortem study has reported increased vascular density in the hippocampus of AD patients [15]. Both ANG and VEGF are involved in regulation of physiological and pathological neovascularization.

In this study we found decrease serum level of both ANG and VEGF factors in AD and MCI groups compared to normal subjects. Low level of ANG results in endothelial cell dysfunction and impairment of angiogenesis, as it's important for cell growth and survival, and abnormal ANG levels were observed in a variety of disorders, suggesting that ANG may be a valuable diagnostic or prognostic marker for certain diseases including AD, in spite of it unrecognized role in neurodegenerative process [7].

The low serum level of VEGF may be due to accumulation and colonization of these factor with amyloid plaques in the brain and with continuous deposition, resulting in decrease in available VEGF and impaired angiogenesis, more hypoxia and more progression and wors-

ening of the disease. Meanwhile VEGF loaded nano-sphere implanted in a mouse model of AD, enhanced hippocampal neurogenesis and increased clearance of amyloid plaques [16]. Also low CSF level of VEGF were present in AD when compared with control subject [17].

On the contrary to our results Qin., *et al.* [18] reported significant elevated serum level of ANG in AD Patients. Another study by Kim and Kim [19] reported increased serum level of VEGF associated with lower ANG level in AD patient when compared to control. While Mateo., *et al.* [20] reported significant decrease of VEGF in AD patients than control. To explain these different results we can hypothesis that; genetic and racial factors may play a role in expression of these angiogenic factors, in addition to the other comorbid medical which affect pathogenic angiogenesis as hypertension, diabetes mellitus [7].

Our study results confirm the two-hit vascular hypothesis of AD pathogenesis. Cerebral blood flow dysregulation, BBB damage, disruption of neuronal function and accumulation of Aβ in the brain (hit 1), followed by Aβ-mediated vascular dysfunction (hit 2) leading independently and/or synergistically to neuronal and synaptic dysfunction, neurodegeneration and cognitive impairment [21].

Conclusion

The findings of low serum levels of angiogenin and VEGF indicating that angiogenesis is one of the important mechanism that play a role in development and progression of AD. Monitoring of such factors though a less invasive method may be play a role in the future as a marker for diagnosis and progression of AD.

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Conflict of Interest

The authors have no conflict of interest to be disclosed.

Compliance with Ethical Standards

Funding: The authors hadn't received any fund.

Informed Consent: Informed consent was obtained from all cases from their 1st degree relatives and control subjects.

The protocol of the study was approved by the faculty of medicine ethical committee.

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