·Clinical Research·

Brain-derived neurotrophic factor in patients with advanced age-related macular degeneration

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Abstract

• AIM: To investigate the serum level of the brain-derived neurotrophic factor (BDNF) in age-related macular degeneration (AMD) and healthy control subjects. The disruption in the tight balance of neuroinflammatory and neuroprotective processes in an immune-privileged site like retina is proposed to contribute to the pathogenesis of AMD. One of the main neuroprotective mediators in the central nervous system is BDNF with its serum level notably affected in several neurodegenerative disorders.

• METHODS: Thirty-six patients with AMD and 36 age-matched controls were enrolled in this study. The serum level of BDNF was measured using the enzyme-linked immunosorbent assay method. Results were analyzed to compare case and control values. Comparisons were also made between the BDNF level of wet- *vs* dry-AMD, and male *vs* female patients and controls. Analysis of variance (ANOVA) and Student's *t*-test were employed to analyze the data.

• RESULTS: The mean BDNF levels in AMD group were significantly higher than the control group. Furthermore, our analysis revealed greater BDNF values in all AMD subgroups compared to controls (P=0.004, 0.005, 0.001 and 0.02 for male wet-AMD, male dry-AMD, female wet-AMD and female dry-AMD *vs* controls, respectively). The BDNF level however did not vary between wet- and dry-AMD patients (P=0.74). While within-group comparisons in males and females of AMD and control groups did not show any difference in BDNF (P=0.16, 0.64 and 0.85 for wet-AMD, dry-AMD and control groups, respectively), between-group data showed a higher mean BDNF in both male and female AMD subjects than their peer controls.

• CONCLUSION: This study demonstrated that the serum BDNF level is different in patients with AMD as compared to subjects without AMD. Future attempts should be done to unravel beneficial or deleterious effect of this neurotrophin in the pathogenesis of AMD.

• KEYWORDS: age-related macular degeneration; brain-derived neurotrophic factor; serum level; pathogenesis
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INTRODUCTION

Age-related macular degeneration (AMD) is amongst the leading causes for visual acuity loss in elderly with an estimated number of 30 million subjects affected worldwide [1,2]. The role of oxidative stress, inflammation and neurodegenerative cell death in the retinal pigment epithelium (RPE) of retina is well established in the pathogenesis AMD [3]. AMD is typically categorized into 2 types including non-neovascular and neovascular which are referred to as dry- and wet-AMD, respectively[4].

On cellular and molecular level, once the inflammatory and angiogenesis-related pathways are activated in the course of dry-AMD, toxic accumulations composed of intra-cellular waste materials such as lipofuscin and oxidized mitochondria lead to the formation of extracellular drusen. This inflammatory process which is partly promoted by the reactive oxygen species (ROS) and the resultant oxidative stress, contributes to the activation of apoptotic cellular pathways and eventually RPE cell death [5,6]. On the other

hand, the critical vision loss in wet-AMD is secondary to bleeding and choroidal neovascularization (CNV) where the role of the vascular endothelial growth factor (VEGF) is crucial [4,7]. Some patients with slowly progressing AMD may remain asymptomatic for a long time while in advanced forms of AMD, serious decline or loss of vision may occur due to central retinal atrophy or significant neovascular changes in the retina[4,8,9].

Progressive degenerative changes in the retina may be the sign of the AMD, hence given the fact that retina is considered as an outpunching of the central nervous system (CNS) [10], it might be relevant to address AMD as a neurodegenerative process leading to the atrophy and loss of RPE and photoreceptors[11,12].

There have been recent efforts to unravel associations between the underlying pathogenesis of AMD and typical neurodegenerative disorders such as Alzheimer's disease (AD) [3,13-17]. Interestingly, the pathophysiology of AMD is suggested to have common molecular pathways with other neurodegenerative disorders[1,5,11,18,19].

In light of the above findings, it has been hypothesized that the degenerative changes and the resultant atrophy in the retina during the course of AMD might have influences on the CNS on structural and chemical levels and vice versa[1,19].

The brain-derived neurotrophic factor (BDNF) is one of the nerve growth factors which plays a central role in the survival and differentiation of neurons [20,21]. Brain concentration and serum levels of this neuroprotective factor are shown to be altered in some neurodegenerative disorders such as AD[15,22,23].

The expression of BDNF and other neurotrophins however are shown to be time-dependent. In other words, at different points of an insult resulted from the oxidative stress, BDNF levels may increase, remain stable or decrease in distinct areas of the brain and the serum [24-28].

As far as we know, the role of BDNF in the pathophysiology of AMD has not been articulated in the literature. We hypothesized that the serum level of this factor is altered in AMD patients. The current investigation was designed to determine: 1) whether the serum level of BDNF in AMD patients differ from age- and sex-matched healthy controls; 2) if there is any difference between the serum BDNF level between dry- and wet-AMD patients.

SUBJECTS AND METHODS

Thirty-six patients with AMD (through sequential sampling method) and 36 age-matched controls were included in this study. Patients were those who referred to our out-patient clinic at Shiraz University of Medical Science (May 2011 to Dec. 2012) and controls were healthy volunteers. The inclusion criteria were the presence of any geographic atrophy or diffuse RPE and retinal atrophy or more than 2 large drusens ($\geqslant$125 μm) or more than 4 intermediate size drusens ($\geqslant$64 μm) or the presence of wet-AMD (choroidal neovascularization or CNV).

The exclusion criteria for patients and controls were observed CNV without any signs of AMD in subjects younger than 50 years old (idiopathic CNV). In addition, control subjects and patients with any documented neurologic, psychiatric, neurodegenerative or neurosurgical problems over the past recent 5y, those reported any significant ocular/head trauma or surgery in the past recent year and patients who were on any type of neuro-psycho-pharmacotherapy were excluded from the study.

Informed consent was obtained from all patients and control subjects who were willing to participate in the study. Three milliliter peripheral venous blood was drawn from each participant. The specimen were sampled into serum tubes where immediately immersed in melting ice. Samples were centrifuged for 30min, and then stored at -18℃ until future use.

BDNF levels were assessed using a specific enzyme-linked immunosorbent assay (ELISA) kit (Quitikine) according to the procedure described in the manufacturer's manual. This human BDNF Immunoassay kit is used to measure human BDNF in plasma as well as cell culture supernatant.

One-way analysis of variance (ANOVA) for multiple comparisons using the Scheffe method as well as the Student's t-test were employed to analyze BDNF data in AMD and control groups. In this analysis, any P value <0.05 was considered statistically significant.

RESULTS

The study sample comprised 36 patients with AMD and 36 age-matched controls. The mean ±standard error of mean (SEM) of the AMD patients' and controls' age was 69.77±8.45 and 66.5±1.32, respectively (P=0.095). According to the ANOVA results, no significant difference in mean age (mean±SEM) between the dry-AMD (68.68±2.16y, n=16) and wet-AMD (70.65±1.85y, n=20) subgroups was noted [F (2, 33) =1.76, P=0.95].

Comparing the age of wet- and dry-AMD cases with the control subjects (multiple comparison through Scheffe), no significant difference was observed (P=0.45). Based on demographic comparisons, female to male ratio amongst subjects in our subgroups (wet-, dry-AMD and controls) was not significantly different (P=0.4). Given the above, the AMD (including the wet- and dry-AMD subgroups) and control groups were evenly balanced for age and sex.

The binomial two-tailed t-test results indicated a significant difference in the mean BDNF level between the AMD (16.36±5.23 ng/mL) and control (10.33±3.79 ng/mL) groups (P<0.001).

In the AMD group, the mean level of BDNF in wet- (n=20) and dry- (n=16) AMD cases were 17.18±1.22 ng/mL and 15.39±1.87 ng/mL, respectively, with no significant difference as per t-test result (P=0.74).

Table 1 Within-group comparison of the serum BDNF level between male and female subjects in AMD subgroups and controls mean±SEM (ng/mL)

Study groups	Males		Females		Two-tailed *t*-test
Wet AMD	*n*=12	15.73±1.49	*n*=8	18.63±0.97	*P*=0.16
Dry AMD	*n*=5	14.60±1.64	*n*=11	16.18±2.11	*P*=0.64
Control	*n*=21	10.40± 0.75	*n*=15	10.21±1.12	*P*=0.85

Based on the two-tailed *t*-test results, gender did not affect BDNF levels across groups. AMD: Age-related macular degeneration; SEM: Standard error of mean; ng/mL: Nanogram per milliliter.

Multiple comparison using the ANOVA Scheffe method, indicated a significant difference in the serum BDNF level of wet-AMD *vs* control group [mean difference=6.56±1.26 ng/mL (95% CI: 3.36-9.76); P<0.001)], and dry AMD *vs* control group [mean difference=5.35±1.37 ng/mL (95%CI: 1.92-8.8); P <0.001)]. The analysis however did not reveal any meaningful difference in the serum BDNF levels of wet- *vs* dry-AMD cases [mean difference=1.2±1.53 (95%CI: -2.64 to 5.04; P=0.73)].

Two-tailed paired *t*-test revealed no significant difference in the serum BDNF level of male *vs* female subjects in wet-AMD, dry-AMD and control groups. These results are summarized in Table 1.

Further analysis demonstrated a significant difference in the mean serum BDNF level of male subjects (15.40±4.7 ng/mL, n =17) in AMD group as compared to the control male subjects (10.40±0.75 ng/mL, n=21), P<0.01.

Similarly, a significant difference in the serum BDNF level of female AMD patients (17.22±5.64 ng/mL, n =19) and female subjects in the control group (10.21±1.12 ng/mL, n=15) was observed, P<0.01.

Further between-group comparison of data using the ANOVA Scheffe method showed marked differences between the serum BDNF levels of the AMD patient subgroups and control subjects. The above comparison outcome is summarized in Table 2.

Table 2 Between-group comparison of the serum BDNF levels based on gender

Gender	Between-group comparison	F (2, 33)	*P*
Male	Wet-AMD *vs* Control	7.92	0.004
	Dry-AMD *vs* Control	6.79	0.005
Female	Wet-AMD *vs* Control	8.39	0.001
	Dry-AMD *vs* Control	5.32	0.020

Multiple comparison using ANOVA, Scheffe method. Results demonstrate significant differences when the mean serum BDNF levels in wet- or dry-AMD subgroups were compared to those of control group both in male and female subjects.

In summary, our findings indicated a significantly higher mean serum BDNF levels in AMD group as compared to the control group. In addition, the mean serum BDNF level in both AMD subgroups (dry- and wet-AMD) were shown to be significantly higher than that of the control group. The BDNF levels however did not vary between dry- and wet-AMD subgroups. Since the case and control groups were matched for age, the effect of this variable on our comparative results was eliminated. The current study also separately evaluated the serum BDNF levels in males and females both in AMD and control groups. Results showed significantly higher mean BDNF values in male and female patient subgroups than their peer controls, whereas no significant difference in the level of serum BDNF in male and female subgroups was noted.

DISCUSSION

AMD is a neurodegenerative disorder of retina in which retinal nerve cells become degenerated [5,8]. This condition results in a possibly progressive visual loss in elderly people. Cumulating evidence suggest that various neuroinflammatory processes involving pro-apoptotic mechanisms such as interleukin-1, tumor necrosis factor-alpha (TNFα)- or TNF-related apoptosis-inducing ligand (TRAIL)- and FAS-L pathways are involved in the pathogenesis of AMD leading to RPE and photoreceptor cell loss in the retina [1,11,19]. On the other hand, neuroprotective biomarkers such as the BDNF[29] and neural growth factor-1 (NGF-1)[30] counteract the cell death process in the face of oxidative or nitrative stress and play a crucial role in cellular resilience and neuronal survival. Moreover, the level of antioxidative enzymes including superoxide dismutase (SOD)[31,32], glutathione (GSH)[33] and catalase [34] are shown to be related to the extent of the oxidative damage resulting from the neuroinflammatory processes.

BDNF is known to play a neuroprotective role in the CNS with its concentrations modulated *via* several factors [20]. BDNF may be down-regulated by chronic stress and up-regulated by physical activity, learning process and dietary restriction[20].

Some recent neuroscience studies on animal models have highlighted BDNF dynamics following induced neurodegeneration [20,35]. For instance, BDNF concentrations are decreased in the serum and parietal cortex in a rat model of cholinergic degeneration[21].

It has been shown that the serum level (circulating concentration) of some neuroprotective biomarkers such as BDNF are surrogates for their concentration within distinct parts of the CNS[28].

On clinical perspective, although the serum level of BDNF is shown to be affected in conditions like AD [15,23], Huntington's disease (HD)[22], depression [36], bipolar mood disorder [37],

schizophrenia [38], conversion- and post-traumatic stress disorder [39]; there exists no evidence on the serum BDNF level in AMD. There might be some fundamental relations between the mechanisms involved in neurodegenerative diseases like AD and AMD. Several reports have explained shared genetic and pathological features between AD and AMD [16]. One report has shown that amyloid-beta (Aβ) is found in drusen from some AMD retinas, but not in drusen from normal retinas [13]. Further investigations examined the effects of AMD immunotherapy with anti-Aβ monoclonal and interestingly showed that when the amounts of Aβ in the retina and brain decreased, the electroretinography deficits in the retina were attenuated. These intriguing results proposed Aβ as a therapeutic target for AMD [14]. Apart from the beneficial effects of neuroinflammatory markers' blockade, the question is whether the administration of neurotrophic factors (such as BDNF) can be sought as a therapeutic approach for AMD? This is the question which is worth in-depth investigations. Although some studies have introduced the BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases[20], there are many unanswered questions here and the role of BDNF in various complex neuroinflammaroy and degenerative conditions needs to be further elucidated.

Since retina is considered as a part of the nervous system[10], we hypothesized that the serum level of BDNF may be changed in the process of retinal degeneration in AMD.

The results of our study showed that the serum level of BDNF was significantly higher in AMD patients compared to controls. Given the well-established degenerative and inflammatory process in the retina during AMD [1,11], before this study, we speculated that the serum level of BDNF ought to be lower in AMD however, our results suggested the opposite.

Some justifications presented for these findings include: 1) increased serum level of BDNF in AMD may be a compensatory phenomenon in response to the degenerative process in the retina, through which CNS has produced more BDNF. This hypothesis must be evaluated in further studies; 2) the confounding factors might have influenced the results of our study. The level of serum BDNF level may be affected by many confounders such as stress, exercise and so on. Although we tried to exclude major confounding factors, some other confounders might have still remained.

Nevertheless, some studies have reported an increased level of BDNF associated with an inflammatory phenomenon and a redox modification, in the plasma of patients undergoing a physical stress such as major surgery [40]. It is necessary to understand whether BDNF has a beneficial or deleterious effect during such tremendous physical stress. This question also implies to what we studied here, the age-related macular degeneration.

The importance of our study was to show a significant variation in the serum BDNF in AMD patients compared to healthy controls.

In conclusion, understanding the dynamics, implications and mechanistic role of neuroinflammatory and neuroprotective biomarkers in AMD would provide more insights into the pathophysiology of this disease. Such insights may lead to utilization of the diagnostic, prognostic and therapeutic value of these biomarkers in the management and follow up of patients with AMD. This study demonstrated that the serum BDNF level is different in patients with AMD as compared to subjects without AMD. Future studies are needed to measure BDNF in retina and RPE in AMD patients *vs* age-matched control subjects. Such evidence would potentially describe the effects of BDNF on the pathogenesis of AMD in the retina.

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