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Original Article

Choroidal Neovascular Membrane in Age-Related Macular Degeneration is Associated with Increased Interleukin-6☆

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SUMMARY

Background: Age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment and blindness among persons aged 60 years and older. Although inflammation has been postulated to have a role in the pathogenesis of AMD, epidemiologic studies have not shown a relationship between systemic inflammation or presence of inflammatory markers at AMD. The aim of our study was to evaluate the differences in various types of cytokines and intracelluler signaling molecules for the onset and progression of AMD.

Materials and methods: There were two groups in our study; Group 1, which acted as the control group ($n = 30$, mean age 67.60 ± 8.32 years), and Group 2, consisting of AMD patients ($n = 22$, mean age 70.10 ± 10.33 years). From serum samples, vascular endothelial growth factor (VEGF) (pg/mL), interleukin-6 (IL-6) and interleukin-1β (IL-1β) (pg/mL), nitrotyrosine (nmol/L) levels were determined by enzyme linked-immuno-sorbent assay method. Nitrite/Nitrate levels were measured by photometric method (μmol/L).

Results: There were no significant differences between the groups with regard to age, VEGF, IL-1β, nitrite/nitrate, and nitrotyrosine. The significant result was the mean IL-6 levels that were higher in the AMD group (55.03 ± 60.03 pg/mL) than in the control group (16.08 ± 8.24 pg/mL, $p < 0.001$).

Conclusion: IL-6 induces an ocular inflammatory response often accompanied by the breakdown of the blood–ocular barrier. The increased levels of IL-6 can support the hypothesis that AMD may be partially mediated through inflammatory mechanisms.

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment among adults aged 60 years and over[1]. Complications of the disease include choroidal neovascularization (CNV), and subsequent bleeding and exudation of the macula, leading to severe vision loss and blindness[2]. All around the world, the elderly population is growing and the problem of the loss of visual function is also steadily increasing. Treatment options for this pervasive problem (AMD) are limited, and prevention remains the best approach[1].

Inflammation has been postulated to play a role in the pathogenesis of AMD[3–5]. Besides endothelial dysfunction resulting from inflammation and hypertension, cigarette smoking has also been postulated to be involved in the pathogenesis of AMD[6].

An early and important sign of AMD is drusen, which is an extracellular deposit that accumulate in the retinal pigment epithelium[7]. Epidemiological studies have shown that numerous and/or confluent drusen significantly increase the risk for developing AMD[8]. Hageman and associates[4] have shown that drusen contain proteins associated with immune-mediated process and inflammation. Additionally, chronic inflammatory cells have been observed on the outer surface of the Bruch's membrane in eyes with neovascular macular degeneration[3]. These cells may cause atherogenesis and microvascular injury through the direct release of long-acting oxidants as toxic oxygen compounds, and proteolytic enzymes may also damage the Bruch's membrane[9]. The presence of biogenesis, composition of drusen (yellowish deposits) in the macula, has been studied in relation with inflammation. It has been hypothesized that large and soft drusen can confer a higher risk of

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progression[10] and may be an indicator of immune-mediated and local inflammatory events in the eye[4,5,11–13].

However, data from epidemiologic studies have not shown any relationship between systemic inflammation and presence of inflammatory markers at AMD[14–16]. Interleukin-6 (IL-6), a potent proinflammatory and multifunctional cytokine, is suggested to be a risk factor for CNV because of its increased levels in the serum of patients with AMD; however, the role of IL-6 in CNV has not been defined[17]. IL-6 may also indirectly cause an increase of vascular permeability by inducing the expression of vascular endothelial growth factor (VEGF)[17] or may directly increase endothelial permeability[18]. VEGFs have been implicated in the pathogenesis of CNV secondary to AMD. There is, however, evidence that intercellular signaling molecules, such as nitric oxide (NO), are also involved in this process. NO is synthesized via the inducible isoform of NO synthase, which is expressed after induction by cytokines[19].

The levels of VEGF, whose effects can be mediated via other factors/cytokines and different inflammatory mediators/cytokines such as IL-6, interleukin-1β (IL-1β), nitrite/nitrate, and nitrotyrozine, were evaluated in order to study how they contribute in the pathogenesis of AMD. The aim of this study was to evaluate the multiple risk factors for the onset and progression of AMD via the inflammatory process.

2. Material and methods

We designed two groups in our study. The first group was the control group, with 13 females and 17 males ($n = 30$, age range 51–80 years), and the second group consisted of patients with choroidal neovascular membrane in AMD, with eight females and 14 males ($n = 22$, age range 50–90 years). Patients with other ophthalmic conditions (e.g., glaucoma, uveitis, pseudoexfoliation syndrome, progressive other retinal disease) and systemic diseases (e.g., diabetes, arthritis, coronary arterial disease, peripheral vascular disease) were excluded. All patients underwent a comprehensive ophthalmic examination. The CNV was determined by slit-lamp biomicroscopes of the fundi, color fundus photographs, fundus fluorescein angiography, and optical coherence tomography. The control individuals have totally normal ophthalmic condition.

All participants, including controls, filled out a questionnaire giving the following information: age, gender, no smoking habits, no supplements such as vitamins and/or antioxidants. Human Ethics Committee rules were satisfied. Informed consent was obtained from patients.

Blood samples were drawn from patients and controls after overnight fasting, before the operation. Blood samples were centrifuged at 2000×g for 10 minutes at 4 °C, and serum samples were stored in the dark at −70 °C for further analyses.

2.1. Biochemical parameters

From the serum samples, VEGF levels were determined using the enzyme-linked immunosorbent assay (ELISA) method Quantikine Immunoassay VEGF; R&D Systems, Minneapolis, MN 55413, USA, Lot No.: 250498 Cat. No.:RRVOO).

Human IL-6 was determined by a solid-phase sandwich ELISA (BioSource Immunoassay Kit; BioSource International, Inc., Camarillo, CA, USA; Cat No: KHCOO61, Lot No: 064903). Human IL-1β was also determined using the same method (BioSource Immunoassay Kit; BioSource International, Inc., Cat No: KHCOOII, Lot No: 064103).

Nitrite/nitrate levels were determined using the colorimetric method (photometric endpoint determination, Cat. No. 1 746 081, by Roche kit). The assay principle is that nitrate is reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate in the presence of the enzyme nitrate reductase. The nitrite formed reacts with sulfanilamide and *N*-(1-naphtyl)-ethylene-diamine dihydrochloride to give a red-violet diazo dye. The diazo dye is measured on the basis of its absorbance in the visible range at 540 nm[20,21].

Nitrotyrosine levels were also analyzed by ELISA kit (Hycult Biotech, Frontstraat 2a, 5405 PB Uden, the Netherlands, Cat. No.: HK501, Lot No.: 5419K18).

2.2. Statistical analysis

Data were analyzed using the SPSS 8.0 statistic program (SPSS, Chicago, IL, USA). Differences in mean values (means) between the two groups were tested by Mann–Whitney *U*-test. The level of statistical significance was set at $p < 0.05$.

3. Results

The means and standard deviations for the ages and biochemical parameters for the AMD group and the control group are listed in Table 1. There were no significant differences between AMD and control groups with regard to age, VEGF, IL-1β, nitrite/nitrate, and nitrotyrosine. The only significant result was the mean IL-6 levels that were higher in the AMD group (55.03 ± 60.03 pg/mL) compared to the control group (16.08 ± 8.24 pg/mL) ($p < 0.001$) (Table 1).

4. Discussion

AMD is a complex multifactorial disease with an unknown etiology and pathogenesis[22]. Inflammation is also associated with angiogenesis and neovascularization that AMD may represent in chronic, age-related inflammatory disease manifested in the eye and other organs, including the heart and the brain[14]. Both brain and retina have an endogenous immune system depending on the blood–tissue barrier. Moreover, the activation of a complement pathway which occurs in that environment might result as an unregulated chronic inflammation inducing a common self-propagating damaging cycle for AMD[7].

Our findings support the hypothesis that AMD may be partially mediated through inflammatory and immune-related mechanisms. Other basic research had suggested that AMD shares biological pathways similar to those found in other inflammatory diseases, such as Alzheimer disease and atherosclerosis[4,5,11–13]. AMD can be classified as atrophic (dry) or neovascular (wet) forms, both of which can lead to significant visual loss[23]. Also, CNV associated with AMD develops after chronic inflammation to the retinal pigment epithelium[2]. In previous studies of human surgical samples and animal models, VEGF was shown to be a key molecule in the development of CNV[24,25], and VEGF has recently been recognized as a proinflammatory cytokine in the eye[26,27]. In

Table 1
Serum VEGF, IL-6, IL-1β, Nitrit-nitrate, Nitrotyrosine in AMD patients and the control group (mean ± SD).

	Age	VEGF pg/mL	IL-6 (pg/mL)	IL-1β (pg/mL)	Nitrit-nitrate (μmol/L)	Nitrotyrosine (nmol/L)
Control (n = 30)	67.60 ± 8.32	447.33 ± 220.49	16.08 ± 8.24	4.54 ± 0.92	4.10 ± 0.74	41.90 ± 7.00
AMD (n = 22)	70.10 ± 10.33	301.90 ± 134.60	55.03 ± 60.03*	4.48 ± 0.29	4.31 ± 0.76	47.80 ± 32.00

*p = 0.002 compared to control group.