Association Between Serum Complement C3 Levels and Age-Related Cataract

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PURPOSE. The serum complement component 3 (C3) concentration and clinical characteristics of age-related cataract (ARC) subjects were analyzed to evaluate whether serum C3 levels are correlated with ARC.

METHODS. A total of 492 ARC patients and 466 normal subjects from the Department of Ophthalmology and Visual Science, Eye and ENT Hospital of Fudan University, Shanghai, China, were consecutively recruited into this study between June 2014 and May 2017. Immunoturbidimetry was used to measure the serum C3 levels. Clinical information (intraocular pressure, visual acuity, central corneal thickness, axial length, vertical cup/disc ratio) and demographic information were obtained. Based on sex and age, the ARC subjects were categorized into female (50–60 years, 61–70 years, and 71+ years) and male (50–60 years, 61–70 years, and 71+ years) subgroups.

RESULTS. The mean serum C3 level was significantly lower in the ARC group (100.97 ± 18.22 mg/dL) in comparison to the control group (123.27 ± 22.51 mg/dL) (t = 16.888, P < 0.001). A similar result was also observed when the serum C3 levels were compared between the ARC and control groups with respect to sex and age. The mean serum C3 level was lowest in the nuclear ARC subgroup (98.63 ± 18.76 mg/dL) followed by the cortical ARC subgroup (102.26 ± 18.04 mg/dL) and the posterior subcapsular ARC subgroup (103.80 ± 17.20 mg/dL), and the differences were significant (P = 0.057). Logistic regression analyses revealed that complement C3 (odds ratio [OR] = 0.924, 95% confidence interval [CI] = 0.913–0.935) was associated with ARC.

CONCLUSIONS. ARC patients have lower serum C3 levels, which were further demonstrated to be correlated with the onset/development of ARC. These findings suggest the involvement of C3 in the pathomechanisms of ARC.

Keywords: age-related cataract, complement component 3, serum

Age-related cataract (ARC) is one of the leading causes of blindness among the elderly, worldwide, and the prevalence of ARC is increasing rapidly.1,2 There are three types of ARC based on the location of the opacity within the lens: cortical, nuclear, and posterior subcapsular.3 Although surgical extraction is the only available and effective treatment, ARC is associated with great personal and societal medical costs and inevitable risks for patients, especially in developing countries.4,5 The precise mechanisms involved in ARC are yet to be completely understood. However, recently, several studies have suggested that the complement system may play an important role in the pathophysiology of ARC.6,7

The complement system is the main component of the human native immune system, and it is involved in eye diseases. For example, Li et al.5 reported that people with primary angle-closure glaucoma have lower plasma complement component 3 (C3) levels, which were further demonstrated to be negatively associated with the severity of this condition; moreover, Yates et al.7 found that complement C3 is important in the pathogenesis of age-related macular degeneration. Complement C3 has the highest content and is the central component of the complement system, which interplays with other complement proteins and participates in the activation of the three pathways of complement activation: the classical, alternative, and lectin pathways.8 Several factors are associated with activation of the complement system, such as aging,10 ultraviolet light,11 smoking,12 abdominal obesity,12 and viral infection,13 which have been associated with increased risk of ARC. To our knowledge, no previous study has investigated the serum levels of complement C3 in patients with ARC, and it is not known whether the serum levels of complement C3 are different between men and women with ARC and among cortical, nuclear, and posterior subcapsular ARC.

To meet this need, we conducted a large case-control study to detect and compare the serum C3 levels of ARC subjects and to investigate the possible association between serum complement C3 levels and ARC.
**Materials and Methods**

**Patients**

The study was approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University, Shanghai, China (EENT20150111). It was conducted in accordance with the Declaration of Helsinki. Informed consent was acquired from the ARC subjects and normal controls for the use of any clinical data and blood in the research study. ARC patients from the Department of Ophthalmology and Visual Science, Eye and ENT Hospital of Fudan University were consecutively recruited into this study between June 2014 and May 2017. Both newly diagnosed ARC patients and referral ARC patients were included. Age-matched and sex-matched normal controls were consecutively recruited from subjects who participated in yearly health screenings during the study period.

**Examination**

Medical examinations were performed for all subjects at the Eye and ENT Hospital of Fudan University. This included assessments of electrocardiograms, X-rays, liver function, renal function, infectious disease, blood pressure, heart rate, and body mass index (BMI). Each ARC patient also underwent a standardized ophthalmic examination performed by cataract specialists. Intraocular pressure (IOP) was measured using Goldmann applanation tonometry. A visual acuity measurement was obtained for each patient based on the International Standard Visual Acuity Chart. A-scan ultrasound (A-Scan Pachymeter; Ultrasonic, Exton, PA, USA) was used to measure central corneal thickness (CCT) and axial length (AL). Fundus photography was used to capture photographs of the back of the eye via a retinal camera (TRC-NW200; Topcon, Tokyo, Itabashi-ku, Japan). Moreover, other clinical and demographic information (economic situation, education level, life habits, tobacco and alcohol intake, drug history, and disease history) was obtained from the medical data platform of the Eye and ENT Hospital of Fudan University by trained staff (SL and MS) using standardized data collection and quality control procedures, resulting in reliable data for analysis. The methods used to detect the C3 levels have been previously described in detail.8

**Inclusion Criteria**

**ARC Subjects.** A total of 1268 cataract subjects were recruited, of whom 776 patients (metabolic cataract = 21, traumatic cataract = 13, congenital cataract = 65, secondary cataract = 578, cancer = 12, heart disease = 17, hyperlipidemia = 5, renal disease = 4, glaucoma = 6, gout = 6, fundus lesions = 11, other diseases = 38) were later excluded from the study based on the inclusion criteria. The final sample consisted of 492 ARC patients who met the following criteria: (1) hospital inpatients: age ≥ 18 years; (2) ARC recorded using the Lens Opacities Classification System III (LOCS III)13; (3) ARC15 defined as the presence of any type of ARC (cortical, nuclear, or posterior subcapsular) or a history of previous cataract surgery (pseudophakia or aphakia eyes) in either eye; (4) no history of secondary cataracts, congenital cataracts, or any other eye diseases; (5) no history of systemic diseases, such as acute infectious diseases, kidney disease, autoimmune disease, cancer.

**Control Subjects.** A total of 600 subjects were recruited, of whom 134 normal subjects were later excluded from the study based on the inclusion criteria. The final sample consisted of 466 control subjects who met the following criteria: (1) age ≥ 18 years; (2) no history of any type of cataract, secondary cataract, congenital cataract, or any other eye disease; (3) no history of systemic diseases, such as acute infectious diseases, kidney disease, autoimmune disease, cancer.

**Subgroup Analysis.** The prevalence of ARC in China is higher in females than in males (37.2% in men and 39.0% in women [Tang et al.15]). Moreover, in our study, there were a greater number of female ARC subjects than male ARC subjects (290 vs. 202). Therefore, the subjects were categorized into sex subgroups.

Tang et al.15 also reported that the percentage of cataracts increased with age for all types of cataracts by eye and by sex (in nuclear, cortical, and posterior subcapsular cataracts). Therefore, within the male and female subgroups, the ARC patients were further subdivided into three groups based on age: a 50–60 subgroup, a 61–70 subgroup, and a 71+ subgroup.

**Statistical Analyses.** The study’s results are presented as mean ± standard deviation (SD). Normality was assessed using the Kolmogorov-Smirnoff test. The independent Student’s t-test and χ² test were used to compare the subject characteristics between the groups. A 1-way ANOVA test was used to compare the C3 levels and ocular parameters among the three groups (cortical, nuclear, and posterior subcapsular). Receiver operating characteristics (ROC) analysis was performed to demonstrate the sensitivity and specificity of the admission C3 and the optimal cutoff value for predicting ARC. Upon verification of the predicted value of the C3 as a continuous variable, we further investigated the relevance of the C3 level in categorizing subjects among five groups by assigning the subjects into two subgroups based on the best cutoff value (C3 ≤ 109.455 mg/dL, C3 > 109.455 mg/dL). A 2-sided P < 0.05 was considered to be statistically significant. Logistic regression analyses were performed to identify the association between C3 levels and ARC (control group = 0, ARC = 1; male = 0, female = 1). Odds ratios (ORs) with 95% confidence intervals (95%CIs) were estimated by logistic regression analyses. All analyses were performed using SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) and a spreadsheet program (Microsoft Excel 2007; Redmond, WA, USA).

**Results**

**Characteristics of the Study Patients**

A total of 492 subjects with ARC (males = 202, females = 290) and 466 normal controls (males = 200, females = 266) were eligible for the study from June 2014 to May 2017, which was conducted at the Eye and ENT Hospital of Fudan University. Only one eye was selected randomly if both eyes suffered from ARC. There were a total of 492 eyes in the ARC group. There was no statistical difference in the mean age and sex between the ARC and control subjects (P > 0.05). However, there was a statistical difference in the mean BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension between the ARC and control subjects (P < 0.05). The mean serum C3 level was significantly lower in the ARC group (100.97 ± 18.22 mg/dL) in comparison to the control group (123.27 ± 22.51 mg/dL) (t = 16.888, P < 0.001) (Table 1; Fig. 1). Table 1 presents a summary of the demographics, ocular parameters, and serum C3 levels of the ARC and control groups.

**Comparison of the Serum C3 Levels in the ARC Subjects Stratified by Sex and Age**

The ARC subjects were categorized into sex-based female and male subgroups. In both the female and male subgroups, the
TABLE 1. Demographic Parameters, Ocular Parameters, and Serum C3 Level Between the ARC and Control Groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ARC Group, n = 492</th>
<th>Control Group, n = 466</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>68.45 ± 7.64</td>
<td>67.73 ± 7.76</td>
<td>1.442</td>
<td>0.150</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>202/290</td>
<td>200/266</td>
<td>0.341</td>
<td>0.560</td>
</tr>
<tr>
<td>Duration of disease, y</td>
<td>2.89 ± 2.88</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>14.01 ± 3.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vision</td>
<td>0.34 ± 0.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VCDR, mm</td>
<td>0.34 ± 0.10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CCT, µm</td>
<td>532.98 ± 34.64</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AL, mm</td>
<td>23.67 ± 2.60</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.73 ± 10.35</td>
<td>21.50 ± 2.80</td>
<td>3.890</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>132.63 ± 16.24</td>
<td>130.22 ± 5.61</td>
<td>3.035</td>
<td>0.004</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>74.47 ± 9.38</td>
<td>76.29 ± 5.42</td>
<td>3.528</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking, yes/no</td>
<td>3/489</td>
<td>2/464</td>
<td>0.150</td>
<td>0.698</td>
</tr>
<tr>
<td>Drinking, yes/no</td>
<td>5/487</td>
<td>2/464</td>
<td>1.137</td>
<td>0.286</td>
</tr>
<tr>
<td>Diabetes, yes/no</td>
<td>34/458</td>
<td>20/446</td>
<td>3.086</td>
<td>0.079</td>
</tr>
<tr>
<td>Hypertension, yes/no</td>
<td>167/325</td>
<td>80/388</td>
<td>35.653</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C3, mg/dL</td>
<td>100.97 ± 18.22</td>
<td>123.27 ± 22.51</td>
<td>16.888</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

VCDR, vertical cup/disc ratio.

mean serum C3 level was significantly lower (female [105.22 ± 18.02 vs. 130.08 ± 22.13 mg/dL]), male [94.88 ± 16.75 vs.114.25 ± 19.70 mg/dL]) (P both < 0.001) in the ARC group in comparison to the control group. The ARC subjects were further categorized into age-related subgroups, 50–60 year, 61–70 year, and 71+ year subgroups based on sex. In the all age subgroups (50–60 years, 61–70 years, and 71+ years), a similar result was also observed when the serum C3 levels between the ARC subjects and the control group were compared. The mean serum C3 level was significantly lower in the ARC group in comparison to the control group, regardless of age (50–60 years, 61–70 years, or 71+ years) or sex (Table 2).

Comparison of Serum C3 Levels and Ocular Parameters in Subjects With Different Types of ARC

Of the 492 ARC patients, 186 had nuclear cataracts, 165 had cortical cataracts, and 114 had posterior subcapsular cataracts. Twenty-seven of the subjects with nuclear cataract were excluded as they could not be age-matched and sex-matched to the posterior subcapsular cataract and cortical cataract groups. A comparison of the serum C3 levels and the ocular parameters in the ARC subjects is shown in Table 3 and Figure 1. No statistical difference in the demographic and ocular parameters was found between the ARC and control groups (P > 0.05). However, the mean serum C3 level was lowest in the nuclear cataract group (98.65 ± 18.76 mg/dL) followed by the cortical cataract group (102.26 ± 18.04 mg/dL) and the posterior subcapsular cataract group (103.80 ± 17.20 mg/dL), and the differences were statistically significant (P = 0.037).

ROC Analyses of the Studied Variables

The ROC analyses of the studied variables are shown in Figure 2. The results show that the area under the ROC (AUROC) value of the C3 to distinguish ARC patients from the normal control subjects was 0.784 (P < 0.001, 95%CI = 75.50%–81.30%). The best cutoff value was 109.455, with a sensitivity of 75.48% and a specificity of 70.12%.

Based on the best cutoff value of C3, the ARC patients were divided into a lower C3 level group (C3 ≤ 109.455 mg/dL, n = 344) and a higher C3 level group (C3 > 109.455 mg/dL, n = 148) (Table 4). The lower C3 level group consisted of 344 ARC subjects; of these, 141 had nuclear cataracts, 110 had cortical cataracts, and 72 had posterior subcapsular cataracts. The higher C3 level group consisted of 148 ARC subjects; of these, 55 had cortical cataracts, 45 had nuclear cataracts, and 42 had posterior subcapsular cataracts. In the control group, 114 subjects were placed in the lower C3 level group and 352 subjects were placed in the higher C3 level group. The proportion of ARC patients in the lower C3 level group was higher (P < 0.001) than that in the control group (Fig. 3). The proportion of the three types of ARC patients (nuclear cataract, posterior subcapsular cataract, and cortical cataract) between the higher and lower C3 level groups was significantly different (P = 0.043) (Fig. 3).
1.215, 95%CI = 0.784, best cutoff value C3, mg/dL = 98.63
AL, mm = 23.71
VCDR = 0.34
Vision = 0.32
IOP, mm Hg = 13.74
70.12%
Duration of disease, y = 2.76
DBP, mm Hg = 74.63
SBP, mm Hg = 132.05
complement C3 in predicting age-related cataract. Area under the ROC
FIGURE 2.
2.881), and C3 levels (OR = 1.002–1.030), hypertension (OR = 1.882, 95%CI = 1.229-2.881), and C3 levels (OR = 0.924, 95%CI = 0.913-0.935) were associated with ARC after adjusting for age and other ocular parameters.

LOGISTIC REGRESSION ANALYSIS OF THE ASSOCIATION BETWEEN SERUM C3 LEVELS AND ARC IN THE ARC AND CONTROL SUBJECTS

Logistic regression analyses were performed to identify the association between serum C3 levels and ARC in comparison to the control subjects (Table 5). Logistic regression analyses revealed that sex (OR = 5.811, 95%CI = 3.802-8.882), BMI (OR = 1.215, 95%CI = 1.145-1.288), SBP (OR = 1.016, 95%CI = 1.002-1.030), hypertension (OR = 1.882, 95%CI = 1.229-2.881), and C3 levels (OR = 0.924, 95%CI = 0.913-0.935) were associated with ARC after adjusting for age and other ocular parameters.

DISCUSSION

In this cross-sectional, case-control study, we investigated the association between serum C3 levels and ARC in China, and we report that patients with ARC have decreased serum C3 levels in comparison to normal controls. In addition, a similar result was also observed when the serum C3 levels were compared between the ARC and control groups with respect to sex and age. Furthermore, ROC analysis showed that the AUROC value of C3, which distinguished the ARC subjects from the control subjects, was found to be 0.784, with best cutoff values of 109.455. Logistic regression analyses revealed that C3 was correlated with the onset/development of ARC. These findings suggest the involvement of C3 in the pathomechanisms of ARC.

To the best of our knowledge, no previous study has examined the association between serum C3 levels and ARC. To date, there has been increasing evidence to suggest that the immune system may be involved in the pathogenesis of cataracts. Tanemoto et al. 17 reported that posterior subcapsular cataracts developed in Wistar rats that were immunized with a bovine lens membrane protein and adjuvant. Moreover, Kempen et al. 18 reported that patients with acquired immunodeficiency syndrome and cytomegalovirus retinitis have a higher risk of cataracts than the general population. The complement system is an important element of the immune response; it can eliminate microorganisms, abnormal host cells, cell debris, and so on. Using microarray analysis, Medvedovic et al. 19 reported that several members of the complement system are upregulated during the early steps of lens regeneration, which is also characterized by epithelial-mesenchymal transition. Moreover, complement C3a is an important mediator of glomerular and tubulointerstitial injury and it can induce tubular epithelial-mesenchymal transition. Therefore, lens cells may be damaged by the activation of the complement system, causing the development of ARC.

From our results, we questioned why the serum concentration of complement C3 was lower in patients with ARC. To our knowledge, limited data are available in the literature regarding the association between serum complement C3 levels and ARC. However, recent observations have supported the assumption that viral infection may play a role in the development and/or progression of cataracts. For example, Yoshida et al. 21 found that patients with ARC had significantly higher seropositivity for the hepatitis C virus than an age-matched general population (18.3% vs. 7.1%). Emre et al. 22 reported that a transfusion-transmitted virus was detected in
the serum, tear, and aqueous humor in most ARC patients. Moreover, several studies have reported that the herpes simplex virus-1 and rubella virus were associated with congenital cataracts. Viral infection can lead to the production of antibodies. Given the fact that immunoglobulin G can activate the complement system, we speculate that the activation of ocular autoantibodies may be an indispensable factor that is likely to cause a systemic decrease in the level of C3. Recently, Suetugu-Maki et al. suggested that administration of a C5aR antagonist in C57BL/6J mice decreases the epithelial–mesenchymal transition, as evidenced by z-smooth muscle actin expression and cell proliferation. These results suggest that an antagonist to C3 receptors plays a possible therapeutic role in preventing ARC. C3 gene mutations may be another factor likely to cause a systemic decrease in C3, but limited data are available to determine the association between C3 gene mutations and the risk of ARC.

The aqueous humor comes from peripheral blood through the blood–aqueous barrier and the blood–retinal barrier. Ocular inflammation, whether because of local factors or as part of systemic inflammatory conditions, also appears to increase the risk of ARC. Several studies reported that the levels of aqueous humor inflammatory factor increased in cataract patients. Ram et al. reported that patients with lower levels of C3 are recognized to be more susceptible to infection. The C3-mediated immune response may be a major trigger of systemic inflammation and systemic inflammation is involved in the progression of ARC; we hypothesized that ARC patients tend to be more susceptible to infection, which can cause a further decrease in serum C3. Schaumberg et al. reported that baseline C-reactive protein was significantly higher among men who later developed cataract than levels among those who remained free of cataract, and indicated that systemic inflammation may play a role in the pathogenesis of ARC. Moreover, Klein et al. suggested that interleukin-6 and intracellular adhesion molecule-1 were associated significantly with prevalent nuclear cataract. On the basis of this evidence and our results, we hypothesized that C3 might influence the onset of the different cataract types by mediated immune response.

We also explored the association between complement C3 levels and three types of ARC: cortical, nuclear, and posterior subcapsular cataracts. We found that the mean serum C3 level was lowest in the nuclear cataract group (98.63 ± 18.76 mg/dL) followed by the cortical cataract group (102.26 ± 18.04 mg/dL) and the posterior subcapsular cataract group (103.80 ± 17.20 mg/dL), and the differences were statistically significant (P = 0.037). One reason for this result may be that the course was shorter in the posterior subcapsular cataract group followed by the cortical cataract and nuclear cataract groups, so C3 would be more active with a prolonged course of ARC. Another reason may be that people with nuclear cataract suffer more cumulative sunlight exposure than the others. Moreover, the proportion of ARC patients in the lower C3 level group was higher (P < 0.001) than that in the control group (Fig. 3). The proportion of the three types of ARC patients (nuclear, posterior subcapsular, cortical cataracts) between the higher and lower C3 level groups was statistically significantly different (P = 0.045) (Fig. 3). These findings, along with the above results, suggest that C3 levels are related to different types of ARC.

Although this is the first study exploring the association between serum C3 levels and ARC, we acknowledge that our present study has some limitations. First, this was a cross-sectional, case–control study, which limited our ability to explore the exact mechanisms underlying the associations between C3 and ARC, and the exact logic for cause and effect between C3 and ARC. Therefore, the C3 concentration in the aqueous humor and retina in ARC patients should be tested, and animal experiments should be conducted in future research. Second, the data were collected at a single eye center, which may limit the generalizability of the results. Therefore, a forward-looking, multicenter study with a larger sample size should be conducted.

In this preliminary study, we found that the mean serum C3 level was significantly decreased in the ARC patients, and logistic regression analyses indicated that C3 may be correlated with the onset/development of ARC. These findings suggest an association between C3 and the development of ARC, as well
as the involvement of the complement system in the pathogenesis of ARC.

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