Association of Plasma Complement C3 Levels With Primary Angle-Closure Glaucoma in Older Women

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Purpose. The plasma complement component (C)3 concentration and clinical characteristics of primary angle-closure glaucoma (PACG) subjects were analyzed to evaluate whether C3 levels were correlated with PACG severity.

Methods. Peripheral blood samples from subjects with PACG ($n = 237$) and normal controls ($n = 158$) were collected. The plasma levels of C3 were measured by immunoturbidimetry. The assessments of intraocular pressure; vertical cup/disc ratio (VCDR); central corneal thickness; axial length; anterior chamber depth; visual field–derived mean deviation; and mean sensitivity were performed by glaucoma specialists. Based on their sex and age, the PACG subjects were categorized into female (0–49 years, 50–59 years, 60–70 years, and 70+ years) and male (0–49 years, 50–59 years, 60–70 years, and 70+ years) subgroups.

Results. The mean plasma levels of C3 (101.71 ± 21.17 mg/dL) were significantly lower ($P < 0.001$) in PACG subjects compared with controls (110.68 ± 17.63 mg/dL). Plasma C3 concentrations were significantly lower ($P < 0.001$) in female subjects with PACG (100.74 ± 19.83 mg/dL) compared with controls (113.58 ± 18.80 mg/dL). A similar result was observed when plasma levels of C3 were compared between the PACG and control groups with respect to age (≥50 years). The mean plasma levels of C3 were lowest in the severe PACG group followed by moderate PACG and mild PACG, and the differences were significant ($P = 0.003$). Multivariable regression analyses showed that there was a significant correlation between C3 levels and VCDR ($B = -0.310$, $P = 0.047$).

Conclusions. Older women with PACG have lower plasma C3 levels, which were further demonstrated to be negatively associated with PACG severity. These findings suggest the involvement of C3 in the pathomechanisms of PACG in older women.

Keywords: PACG, plasma, complement C3, older women

Primary glaucoma is a chronic ocular neurodegenerative disease and the second leading cause of blindness worldwide. The common characteristic features of primary glaucoma include visual field defects, optic nerve head cupping and elevated intraocular pressure (IOP).[1,2] The precise mechanisms involved in glaucoma are yet to be determined.[3,4] In the past decade, the complement system has become a hot area of research for its involvement in neural degenerative diseases such as glaucoma.[5–7] There has been a gradual accumulation of evidence to suggest that the complement system may play an important role in the pathophysiology of glaucoma.

Component 3 is a central component of the complement system, which interplays with other complement proteins and takes part in the activation of the three pathways of complement activation: the classical, alternative and lectin pathways. Stevens et al.[8] reported on the role of the classical complement cascade in developmental synapse elimination and synapse loss, and found an association with the development of glaucoma. Recent experimental studies have also demonstrated that the concentrations of C3 and C3 split products were significantly elevated in the retinas of glaucoma patients.[6,9,10] Doudevski et al.[11] reported that the levels of complement C3a and soluble C5b-9 were significantly elevated in the aqueous humor of exfoliation glaucoma patients. These studies indicate that C3 activation does occur in glaucoma and is possibly associated with glaucomatous neurodegeneration.[12,13] The exchange of substances between the circulating blood and ocular tissues are governed by two main barriers: the blood–aqueous barrier and the blood–retinal barrier. The blood–retinal barrier is a physiologic barrier that regulates ion, protein, and water flux in and out of the retina. The aqueous humor originates from the peripheral blood via the blood–aqueous barrier and flows out through the trabecular meshwork, draining into the Schlemm's canal and then into the scleral veins. However, it is unknown whether the activation, deposition, and consumption of complement in ocular tissues also result in changes in C3 levels in the peripheral blood of PACG patients. Furthermore, the relation-

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ship between C3 levels and the severity of glaucoma in PACG requires further investigation.

A sex difference also exists among glaucoma patients, with previous studies demonstrating that women are at a higher risk of developing PACG.[14,15] The Blue Mountains Eye Study reported that the prevalence of glaucoma was higher in women, with an odds ratio of 1.5.[16] Anatomic differences (shorter ocular axial length and anterior chamber depth) and endocrinologic differences (hormone levels and hormone therapy,[17] oral contraceptive use,[18] and menopausal status[19]) have been proposed to help explain the discrepancy in the prevalence of glaucoma between the sexes; however, the exact cause behind this disparity in the prevalence and incidence of glaucoma between the sexes remains unclear. It is known that the level of estrogen decreases in postmenopausal women, and estrogens have been hypothesized to mitigate several aspects of the native immune response and play a further role in protection against chronic inflammatory diseases.[20,21] The complement system is the main component of the human native immune system and is involved in inflammatory diseases. Thus, although the mechanism behind the increased risk of PACG in women is unclear, we hypothesize that this could, in part, be mediated by an effect on the activation of the complement and inflammatory systems, which may be heavily involved in the pathogenesis of PACG.

To date, there are no data available regarding the plasma levels of complement C3 in older women with PACG. Additionally, it is not known whether the plasma levels of complement C3 are different between men and women with PACG. Therefore, the objective of the present study was to detect and compare the levels of C3 in the peripheral blood of PACG subjects and to investigate a possible association between plasma complement C3 levels with PACG in older women, which may further shed light on the role of the complement system in PACG development.

Materials and Methods

Patients

This study was approved by the ethics committee of the Eye & ENT Hospital of Fudan University, Shanghai, China. The study was conducted in accordance with the tenets of the Declaration of Helsinki. Subjects with PACG were consecutively recruited into this study between January 2013 and June 2016 from the Department of Ophthalmology & Visual Science, Eye & ENT Hospital, Fudan University. Normal controls were consecutively recruited from subjects who participated in yearly health screenings during the study period. Informed consent was acquired from the PACG subjects and normal controls, and the participants were informed of the nature and possible consequences of this study. Each subject underwent a standardized ophthalmic examination, which included assessments of refractive status, slit-lamp biomicroscopy; fundus examination; IOP; central corneal thickness (CCT); axial length (AL); anterior chamber depth (ACD); visual field examination; and gonioscopy, performed by glaucoma specialists. Mean deviation (MD) and mean sensitivity (MS) were measured using an automated perimeter (Octopus; Haag-Streit AG, Koeniz, Switzerland). We measured IOP using Goldmann applanation tonometry. Fundus photography was performed with a retinal camera (TRC-NW200, Topcon Corp., Tokyo, Japan). We used an A-scan ultrasound (A-Scan Pachymeter; Ultrasonic, Exton, PA, USA) to measure AL, ACD, and CCT. In addition, medical examinations were performed by the respective specialty physicians for all subjects at the Eye & ENT Hospital of Fudan University.

Given the potentially increased risk of blindness from PACG in women,[14–16] the PACG subjects were categorized into female and male subgroups to investigate the role of the complement system in PACG development between the sexes, which is also supported by previous studies demonstrating that menopausal status was associated with the pathomechanisms of glaucoma.[17–19] Several studies have also reported that the prevalence of angle-closure glaucoma increases with advancing age, especially in China.[1,22] Therefore, a subgroup analysis by sex of different age groups (0–49, 50–59, 60–70, and 70+ years) was performed to shed light on the possible role of complement C3 in PACG development with increasing age.

Diagnostic and Inclusion Criteria

Primary angle-closure glaucoma was diagnosed on the basis of narrow anterior chamber angles with glaucomatous optic neuropathy and corresponding visual field loss. This was defined as a glaucoma hemifield test outside normal limits including a cluster of three or more nonedge, contiguous points on the pattern deviation plot, not crossing the horizontal meridian with a $<5\%$ probability of being present in age-matched normals (one of which was $<1\%$); an abnormal pattern standard deviation with $P < 5\%$ occurring in the normal population; and fulfilling the test reliability criteria (fixation losses $<20\%$, false positives $<33\%$ and/or false negatives $<33\%$). Primary angle-closure glaucoma was diagnosed in eyes with narrow angles; with elevated IOP (IOP >21 mm Hg); at least 180° of angle-closure obliterating the pigmented segment of the trabecular meshwork, whether synechial or appositional, segmented or continuous; and in eyes in which the degree of peripheral anterior synechiae was too extensive to be managed by laserperipheral iridotomy.[23,24] Subjects who used glaucoma medications were also included. Glaucoma severity was classified based on the vertical cup/disc ratio (VCDR): mild (VCDR ≤ 0.64); moderate (VCDR ≤ 0.84); and severe (VCDR ≥ 0.85).[25–27]

Subjects with PACG and normal controls were required to have no other ocular diseases or any previous eye surgery—and no systemic diseases such as diabetes, hypertension, cardiac disease, autoimmune disease, cancer or acute infective disease. Control subjects were also excluded if there was any family history of glaucoma or other systemic diseases.

Collection and Detection of the Blood Samples

Blood samples for biochemical measurements were obtained in the morning after subjects had fasted for 8 hours via standard venipuncture of the veins in the antecubital fossae (anterior elbow veins). The blood was prevented from coagulating with the use of EDTA. Tubes were centrifuged for 10 minutes at 1700*g*, and the plasma was collected and stored at -80°C within an hour of collection. Plasma levels of C3 were measured using a commercially available kit (602 951-01, Roche Diagnostics GmbH, Mannheim, Germany). The Normal standardized values for each component were 90 to 207 mg/dL for C3. Internal controls were analyzed daily over the 3-year period, with typical monthly coefficients of variation (CVs) of 2% to 4% and no significant changes in the values.

Data Analysis

The data was analyzed using statistical software (SPSS, version 13.0; SPSS, Inc., Chicago, IL, USA). Results are presented as mean ± SD. Normality was assessed using the Kolmogorov-Smirnoff test. The independent Student's *t*-test, Mann-Whitney *U* test, and χ^2 tests were used for comparison of patient characteristics between the groups. The 1-way ANOVA test was

Table 1. Demographics and C3 Levels of Subjects With PACG

Factors	PACG Group	Control Group	*t* Value	*P* Value
Age	58.78 ± 11.35	58.25 ± 10.49	0.472	0.637
Sex, M/F	84/153	58/100	0.066	0.797
C3 (mg/dL)	101.71 ± 21.17	110.68 ± 17.63	4.403	<0.001

Data are expressed as mean ± SD. Independent-samples *t*- and χ^2 tests were used.

used to compare the levels of plasma C3 and ocular parameters among the three groups. The associations between C3 and ocular parameters in PACG were analyzed using a Pearson correlation. After Pearson correlation, multivariate linear regression analyses were performed to evaluate the association between C3 and ocular parameters. A value of $P < 0.05$ was considered statistically significant.

Results

Characteristics of the Study Patients

A total of 237 subjects with PACG (females = 153, males = 84) and 158 normal controls (females = 100, males = 58) were enrolled in this study. Only one eye was selected randomly if both eyes suffered from PACG. There were a total of 237 eyes in the PACG group. There was no statistical difference in the mean age or sex distribution between PACG and control subjects ($P > 0.05$). The mean plasma levels of C3 were significantly lower in PACG subjects compared with controls ($P < 0.001$). Table 1 summarizes the demographics and C3 levels of the PACG and control groups.

Comparison of C3 Levels and Ocular Parameters in Subjects With PACG, Stratified According to Sex and Age

Based on their sex, the PACG subjects were categorized into female and male subgroups. In the female subgroup, the mean plasma levels of C3 in the PACG group (100.74 ± 19.83 mg/dL) were significantly lower ($P < 0.001$) compared with the control group (113.58 ± 18.80 mg/dL). Based on their age, the PACG subjects were categorized into subgroups (50–59, 60–70, and 70+ years). A similar result was also observed when plasma levels of C3 were compared between the PACG and control groups in older female subjects (age ≥50 years), with significantly lower C3 levels in PACG subjects compared with controls (Table 2). However, in the male subjects, the mean level of C3 was not significantly different between the PACG (103.48 ± 23.42 mg/dL) and control (105.68 ± 14.23 mg/dL) groups ($P = 0.488$), and there was also no significant difference in C3 levels between the PACG and control groups with respect to age ($P > 0.05$).

Table 3. Comparison of Ocular Parameters in Subjects With PACG Between Sexes

Factors	Male Subgroup	Female Subgroup	*P* Value
Age, y	58.48 ± 12.46	58.95 ± 10.74	0.770
IOP, mm Hg	34.12 ± 14.25	29.16 ± 13.49	0.008
VCDR	0.70 ± 0.24	0.58 ± 0.25	<0.001
CCT, mm	548.11 ± 39.59	546.27 ± 50.71	0.785
ACD, mm	2.19 ± 0.62	2.16 ± 1.06	0.851
AL, mm	22.57 ± 1.38	22.13 ± 0.93	0.016
MD, dB	18.02 ± 8.57	15.14 ± 8.74	0.086
MS, dB	10.74 ± 8.16	11.94 ± 8.22	0.522

Data are expressed as mean ± SD. Independent-samples *t*-test was used.

A total of 84 male subjects and 153 female subjects with PACG were enrolled in this study. There was no statistical difference in the mean age between sexes ($P = 0.770$). The male subjects with PACG had a longer AL (22.57 ± 1.38 mm) than female subjects (22.13 ± 0.93 mm, $P = 0.016$; Table 3).

Comparison of C3 Levels and Ocular Parameters in Female Subjects With PACG, Stratified According to Severity

We recruited 153 female subjects with PACG: 76 were categorized as having mild, 42 as moderate, and 35 as severe PACG based on VCDR. Comparison of C3 levels and ocular parameters in female subjects with PACG, stratified according to severity, are shown in Table 4. The mean plasma levels of C3 were lowest in the severe PACG group (92.39 ± 18.86 mg/dL), followed by moderate PACG (96.12 ± 17.02 mg/dL) and mild PACG (105.05 ± 20.05 mg/dL); the differences were significant ($P = 0.003$; Fig. 1). There was no statistical difference in the mean age between the severity groups ($P = 0.728$). The intraocular pressures ($P = 0.034$), VCDRs ($P < 0.001$), and MDs ($P < 0.001$) were greatest in the severe PACG group. The mean sensitivity was smallest in the severe PACG group ($P < 0.001$).

Based on the concentration of C3, the 153 female subjects with PACG were divided into a lower C3 level group (C3 ≤100.74 mg/dL, $n = 77$) and a higher C3 level group (C3 >100.74 mg/dL, $n = 76$). In the lower C3 level group, 38 subjects were categorized as having mild PACG, 20 as having moderate, and 19 as having severe PACG based on VCDR. In

Table 2. Comparison of C3 Levels in Subjects With PACG, Stratified According to Sex and Age*

Subgroup	PACG Group	Control Group	*t* Value	*P* Value (95% CI)
Males	103.48 ± 23.42 ($n = 84$)	105.68 ± 14.23 ($n = 58$)	0.696	0.488 (−4.06 to 8.46)
35–49 y	106.42 ± 21.95 ($n = 24$)	100.23 ± 15.80 ($n = 14$)	0.923	0.362 (−19.80 to 7.41)
50–59 y	110.11 ± 25.52 ($n = 18$)	107.70 ± 12.98 ($n = 21$)	0.380	0.706 (−15.27 to −10.44)
60–70 y	101.30 ± 26.04 ($n = 25$)	108.22 ± 11.18 ($n = 13$)	1.162	0.253 (−7.21 to −23.61)
70+ y	95.53 ± 17.47 ($n = 17$)	102.09 ± 16.30 ($n = 10$)	0.964	0.344 (−5.69 to 22.91)
Females	100.74 ± 19.83 ($n = 153$)	113.58 ± 18.80 ($n = 80$)	5.137	<0.001 (7.92 to 17.76)
33–49 y	106.50 ± 17.06 ($n = 32$)	105.65 ± 20.03 ($n = 27$)	0.171	0.865 (−7.88 to 11.19)
50–59 y	101.80 ± 20.42 ($n = 42$)	113.33 ± 15.98 ($n = 38$)	2.792	0.007 (1.52 to 18.00)
60–70 y	97.78 ± 21.52 ($n = 57$)	120.04 ± 16.68 ($n = 20$)	4.196	<0.001 (11.69 to 32.81)
70+ y	98.35 ± 17.92 ($n = 22$)	119.89 ± 21.86 ($n = 15$)	3.284	0.002 (8.22 to 34.86)

CI, confidence interval.

* Data are expressed as mean ± SD. Independent-samples *t*- and Mann-Whitney *U* tests were used.

Table 4. Comparison of Demographics, C3 Levels and Ocular Parameters in Female Subjects With PACG, Stratified According to Glaucoma Severity

Factors	Mild PACG, $n = 76$	Moderate PACG, $n = 42$	Severe PACG, $n = 35$	*P* Value
Age, y	60.86 ± 9.42	62.38 ± 12.50	60.42 ± 8.58	0.728
IOP, mm Hg	24.07 ± 13.21	31.40 ± 14.26	28.97 ± 14.31	0.034*†
VCDR	0.40 ± 0.11	0.73 ± 0.06	0.93 ± 0.05	<0.001*†‡
CCT, mm	554.54 ± 52.96	545.84 ± 55.86	537.86 ± 41.55	0.389
ACD, mm	2.16 ± 0.97	2.43 ± 1.22	2.33 ± 1.48	0.601
AL, mm	22.10 ± 0.92	22.19 ± 0.53	22.23 ± 0.91	0.763
MD, dB	10.38 ± 6.77	9.17 ± 4.64	23.31 ± 4.45	<0.001*‡
MS, dB	16.57 ± 7.24	17.83 ± 4.40	4.21 ± 4.99	<0.001*‡
C3, mg/dL	105.05 ± 20.05	96.12 ± 17.02	92.39 ± 18.86	0.003*†

Data are expressed as mean ± SD. We used χ^2 test and 1-way ANOVA.
* $P < 0.05$ for the difference between mild and severe PACG (1-way ANOVA with the least significant difference [LSD] post hoc test).
† $P < 0.05$ for the difference between mild and moderate PACG (1-way ANOVA with the LSD post hoc test).
‡ $P < 0.05$ for the difference between moderate and severe PACG (1-way ANOVA with the LSD post hoc test).

the higher C3 level group, 52 subjects were categorized as having mild PACG, 14 as having moderate, and 10 as having severe PACG based on VCDR. The proportion of females with mild, moderate, and severe PACG between the higher and lower C3 groups was significantly different ($P = 0.048$). The proportion of females with moderate PACG in the lower C3 group was higher ($P = 0.098$) than that in the higher C3 group (Fig. 2). The proportion of women with severe PACG in the lower C3 group was significantly higher ($P = 0.029$) than that in the higher C3 group (Fig. 2).

Pearson Correlation and Multiple Linear Regressions for Associations Between C3 Levels and Ocular Parameters in Women With PACG

There was a statistically significant negative correlation between C3 levels and VCDR ($r = -0.215$, $P = 0.007$). In the 50 to 59 years age group of female PACG subjects, there was a statistically significant positive correlation between C3 levels and AL ($r = 0.406$, $P = 0.009$). In the 60 to 69 years age group of female PACG subjects, there was a statistically significant negative correlation between C3 levels and VCDR ($r = -0.290$, $P = 0.029$).

Table 5 demonstrates the multiple linear regressions of C3 levels with the ocular parameters. In multivariable regression analyses adjusting for age, there was a significant correlation between C3 levels and VCDR in the PACG group ($B = -0.310$, $P = 0.047$) and the 60 to 69 years age group ($B = -0.728$, $P = 0.024$). Moreover, there was a significant correlation between C3 levels and AL in the PACG ($B = 0.426$, $P = 0.012$); 50 to 59 years ($B = 0.605$, $P = 0.001$); and 60 to 69 years groups ($B = 0.817$, $P = 0.007$).

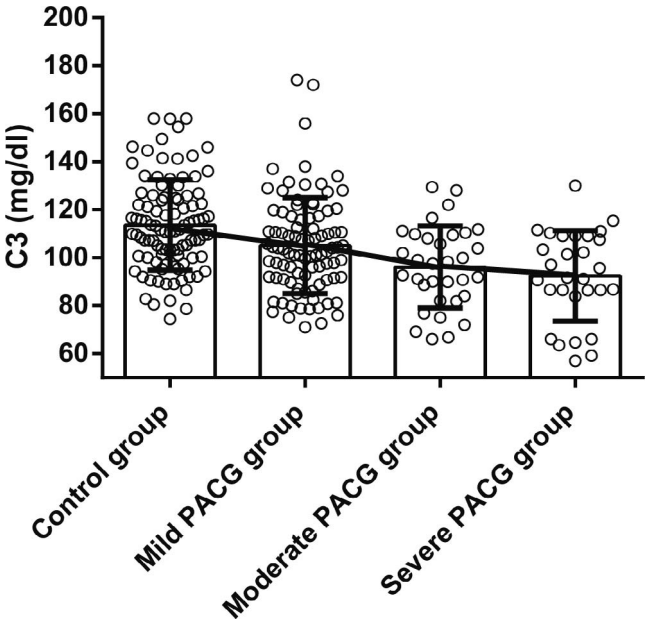

Figure 1. Comparison of plasma levels of C3 in older women with mild, moderate, and severe PACG and the control group. Each data point represents one subject. *Top* of the box plot represents the mean and the *bar* of each box represents the standard deviation.

Discussion

The complement system is an important element of the innate immune response. The innate immune response can eliminate microorganisms, abnormal host cells, and cell debris, and modify molecules via activation of the complement system to maintain homeostasis. Some of the major players of the complement system, such as C3, C5, factor H and factor B, have long been known to be associated with age-related macular degeneration,[28] atypical hemolytic uremic syndrome,[29] familial Mediterranean fever,[30] schizophrenia,[31] and paroxysmal nocturnal hemoglobinuria.[32]

To date, there has been increasing evidence to suggest that the complement system may be involved in the pathogenesis of glaucoma.[33,34] Several studies have investigated complement levels in the aqueous humor of patients with glaucoma,[11] as well as the expression of complement in ocular tissues.[35] Doudevski et al.[11] reported higher levels of C3a in the aqueous humor of patients with exfoliation glaucoma, which provides compelling evidence for the activation of the complement system in exfoliation glaucoma. A better understanding of the peripheral blood levels of C3 and its possible role in PACG may be clinically useful in the management of the disease. To the best of our knowledge, this is the first study focused on the evaluation of the plasma levels of C3 and its relationship with PACG and disease severity.

Nørgaard et al.[36] reported that plasma C3 concentrations were 113 mg/dL in subjects enrolled in the Copenhagen General Population Study and Reynolds et al.[37] also found that plasma C3 concentrations were 110 mg/dL in normal control subjects. In the present study, the mean plasma level of C3 in normal controls was 110.68 mg/dL, which was similar to the results in the normal populations reported by Nørgaard et al.[36] and Reynolds et al.[37] Therefore, our results of normal plasma C3 concentrations are comparable with previous studies, and

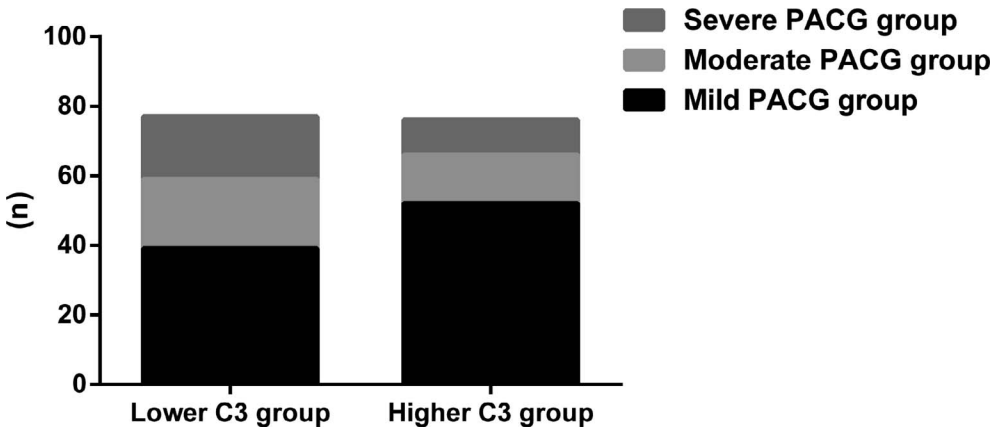

FIGURE 2. The proportion of women with different PACG severities in the lower and higher level C3 groups. The proportion of women with moderate PACG in the lower C3 group was significantly higher than that in the higher C3 group. The proportion of women with severe PACG in the lower C3 group was significantly higher than that in the higher C3 group.

can be reliably used as a normal comparator to PACG subjects in the present study.

It is well known that C3 is the most abundant component in the complement system and is also essential in a number of critical steps in the complement pathway. We found that the mean plasma levels of C3 were significantly lower in PACG subjects when compared with control subjects. Based on sex, the mean plasma levels of C3 were significantly lower ($P < 0.001$) in older female subjects with PACG (100.74 ± 19.83 mg/dL) in comparison with the normal control subjects (113.58 ± 18.80 mg/dL). However, the mean level of C3 was not significantly different between male subjects with PACG (103.48 ± 23.42 mg/dL) and control (105.68 ± 14.23 mg/dL) groups ($P = 0.488$). Our results suggest that the complement system appears to be disordered in older female subjects with PACG; and the role of the complement system may be different between males and females with PACG.

From our results, the following two questions arise: First, why is the plasma concentration of complement C3 lower in older female subjects with PACG? Second, what could be the reason behind the difference in the plasma levels of complement C3 between male and female subjects with PACG when compared with the control groups? Limited data are available in the literature regarding the association of serum complement C3 levels with PACG in women. However, recent observations have supported the existence of ocular autoantibodies that can be detected in the serum[38–41] and the retinas[42] of glaucoma patients. Wax et al.[38] found that autoantibodies to extractable nuclear antigens were found more frequently in patients with normal-pressure glaucoma than in control subjects, and suggested that humoral immune mechanisms may have a role in the pathogenesis of optic neuropathy in patients with normal-pressure glaucoma. Joachim et al.[41] reported that complex IgG autoantibody patterns against human optic nerve antigens existed in the serum of patients with glaucoma. Gramlich et al.[42] also found that retinal IgG autoantibodies were at least twice as high in glaucoma patients as they were in healthy subjects and CD27(+) cells and CD27(+)/IgG(+) plasma cells were observed in all glaucomatous subjects, but not in healthy subjects. Given the fact that IgG can activate the complement system,[43] the activation of ocular autoantibodies in glaucoma may contribute to the depletion of C3. Thus, we speculate that the activation of ocular autoantibodies may be an indispensable factor, which is likely to cause a systemic decrease in C3. Gene mutations of C3 may be another factor that is likely to cause a systemic decrease in C3. Following this study, our laboratory also explored the association between the single nucleotide polymorphisms (SNPs) of the C3 gene and the plasma levels of C3 in females. Unpublished data showed that the SNPs of C3 were not significantly correlated with lower serum C3 levels.

As one of the key components in humoral immunity, C3 depletion might predispose patients to more frequent infections. In our study, PACG subjects with acute infectious diseases were excluded from our study; however, chronic subclinical inflammation in PACG subjects was usually more difficult to detect through medical screenings. There is existing evidence to support this claim, with Ram et al.[44] reporting that patients with lower levels of C3 were recognized to be more susceptible to infection. Astafurov et al.[45] reported that patients with glaucoma had higher oral bacterial counts compared with control subjects and low-dose lipopolysaccharide administration in glaucoma animal models resulted in enhancement of axonal degeneration and neuronal loss. Moreover, a number of studies reported that infection with *Helicobacter pylori*, a bacterium responsible for chronic inflammation of the gastric mucosa, has a statistically significant association with glaucoma.[46–48] Therefore, we hypothesized that PACG patients who had lower levels of C3 were likely to be more susceptible to infection as well which can in turn cause a further decrease in C3 levels.

The difference seen in plasma C3 concentrations between older female subjects with PACG in comparison with the control subjects but not in male subjects may be explained by

TABLE 5. Multiple Linear Regressions for Associations Between C3 Levels and Ocular Parameters in Women With PACG

Factors	IOP B (*P* value)	VCDR B (*P* value)	CCT B (*P* value)	ACD B (*P* value)	AL B (*P* value)	MD B (*P* value)	MS B (*P* value)
C3 (overall)	−0.065 (0.658)	−0.310 (0.047)	−0.086 (0.556)	−0.062 (0.708)	0.167 (0.307)	−0.036 (0.812)	−0.170 (0.239)
C3 (50–59 y)	−0.106 (0.544)	0.019 (0.907)	0.062 (0.696)	−0.119 (0.455)	0.605 (0.001)	0.165 (0.648)	−0.008 (0.969)
C3 (60–70 y)	−0.010 (0.964)	−0.728 (0.024)	−0.015 (0.943)	0.036 (0.899)	0.817 (0.007)	−2.010 (0.737)	−1.912 (0.748)
C3 (70+ y)	0.164 (0.658)	−0.328 (0.345)	−0.389 (0.267)	−0.086 (0.838)	−0.245 (0.536)	−0.072 (0.834)	0.164 (0.651)

endocrinologic and anatomic differences between men and women—which have also previously been described as possible glaucoma risk factors.[17–19] Female eyes are anatomically shorter in axial length and anterior chamber depth than male eyes.[49] Our present study also found that male subjects with PACG had a longer AL (22.57 ± 1.38 mm) than female subjects (22.13 ± 0.93 mm) ($P = 0.016$). Endocrinologic differences between men and women include hormone levels[17] and menopausal status,[19] with the levels of sex hormones decreasing in postmenopausal women. In this study, most of the older female subjects (age ≥50 years) were postmenopausal. Therefore, the levels of sex hormones were lower in the older female subjects with PACG in our study. The metabolic actions of estrogens have been studied extensively and there is also accumulating evidence to suggest that estrogen levels can influence the complement system.[50] Yilmazer et al.[51] reported that healthy postmenopausal women taking oral conjugated equine estrogen had significantly higher mean values of C3 compared with untreated healthy postmenopausal women. Liu et al.[52] also reported that postmenopausal women taking a sex hormone replacement therapy regimen had significantly higher levels of C3 than normal controls. This indicates that plasma C3 concentrations are positively and strongly correlated with sex hormone concentrations.

The possible mechanism behind the influence of estrogens on the complement system is via their anti-inflammatory properties.[50,52] The inflammation modulatory properties of estrogens are their capacity to influence nuclear factor-κB activity, which is a central regulator of a variety of proinflammatory genes.[50] Maggioli et al.[53] found that susceptibility to chronic inflammatory diseases was increased in postmenopausal women. Therefore, lower levels of estrogen in postmenopausal women will result in a higher susceptibility to chronic inflammatory diseases. Overall, in conjunction with the results of previous studies, our findings suggest that activation of the complement system may affect the risk of PACG in older women.

We also explored the association between complement C3 levels with PACG severity in older female subjects. We found that the mean plasma levels of C3 were lower in the mild PACG group when compared with the control group, and that the mean plasma levels of C3 were lower in the moderate PACG group when compared with the mild PACG group. However, the mean plasma levels of C3 were not statistically different between the moderate and severe PACG groups. This suggests that the complement system may play an important role in the early and middle stages of the disease process in older females with PACG, but that the role of the complement system may decrease in the more advanced stages of the disease. Moreover, we found that there was a significantly higher proportion of female subjects with moderate/severe PACG in the lower C3 level group than in the higher C3 level group (Fig. 2), and that there was a significant negative correlation between C3 levels and VCDR ($r = -0.215$, $P = 0.007$) using Pearson correlation analyses. In multivariable regression analyses adjusting for age, there was also a significant negative correlation between C3 levels and VCDR. These findings, along with the above results, suggested that C3 levels are related to glaucoma severity in older women with PACG. Secondary neurodegeneration was thought to play an important role in the pathophysiology of neurodegenerative disease. A recently proposed mechanism, which may help to explain this, was the finding that C3-dependent microglial priming confers susceptibility in multiple sclerosis, resulting in microglial overactivation in response to secondary insults.[54] Microglial activation was reported to play a role in secondary neurodegeneration in glaucoma,[55] and as secondary degenerative events occurred over a greater time frame than primary degenerative events, [56] this may help explain the patterns of plasma C3 levels observed in this study.

To the best of our knowledge, this is the first study to asses a potential relationship between C3 levels and PACG in older women. In this preliminary study, we found that older female subjects with PACG have lower plasma C3 concentrations, which has a significant negative correlation with glaucoma severity. This suggests that the complement system may generally affect older women at risk of PACG via inflammatory mechanisms. Further research is needed to better understand the complex relationship and possible mechanism between the complement system and PACG, especially in older female patients at risk of PACG.

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