Association Between Aqueous Flare and Epiretinal Membrane in Retinitis Pigmentosa

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PURPOSE. Epiretinal membrane (ERM) is a frequent macular complication in patients with retinitis pigmentosa (RP). The etiology of ERM formation in RP is largely unknown. The purpose of this study was to investigate the association between aqueous flare, a surrogate index of intraocular inflammation, and ERM secondary to RP.

METHODS. We retrospectively studied a total of 206 eyes of 117 patients who were diagnosed with typical RP. Aqueous flare values were measured consecutively in 2012 and 2013 using a laser flare cell meter. Spectral-domain optical coherence tomography images and fundus photographs taken on the same day of the aqueous flare measurements were analyzed for ERM detection.

RESULTS. The mean values of aqueous flare, age, and frequency of male sex were significantly higher in the RP patients with ERM compared with the RP patients without ERM ($P < 0.0001$, $P = 0.007$, and $P = 0.004$, respectively). After adjustment for age and sex, the eyes in the highest quartile of aqueous flare had significantly higher odds of having ERM than those in the lowest quartile (odds ratio [OR], 2.68; 95% confidence interval [CI], 1.04–6.93), and the linear trend across flare levels was significant ($P = 0.005$). In addition, each 1-log-transformed increase in flare values was associated with an elevation of the likelihood of having ERM (OR, 2.59; 95% CI, 1.33–5.06).

CONCLUSIONS. Our analysis demonstrated that elevated aqueous flare is associated with ERM secondary to RP, suggesting that inflammation may be implicated in the pathogenesis of ERM formation in RP.

Keywords: retinitis pigmentosa, epiretinal membrane, aqueous flare, intraocular inflammation, optical coherence tomography

Retinitis pigmentosa (RP) is a group of inherited retinal degeneration diseases resulting from photoreceptor cell death, and more than 1.5 million individuals have RP conditions.1 Along with progressive rod and cone degeneration, cases of RP are frequently associated with macular complications such as epiretinal membrane (ERM) and cystoid macular edema (CME),2–5 which debilitate central vision.6,7 Aqueous flare has been used as a sensitive marker of blood–retina barrier breakdown and intraocular inflammation in ocular diseases such as diabetic retinopathy, age-related macular degeneration, and proliferative vitreoretinopathy, as well as for the disease activity in these conditions.8–12 We recently demonstrated that (1) aqueous flare values are increased in patients with RP and (2) increased aqueous flare is correlated with worse central visual function.13 Together with our previous findings that inflammatory cells and proinflammatory cytokines are substantially increased in the vitreous of RP patients,14 these findings suggest that chronic inflammation may play a role in the pathology of RP.

Advances in optical coherence tomography (OCT) imaging have enabled precise and sensitive evaluations of macular diseases. A recent study using spectral-domain (SD) OCT reported that the prevalence of ERM in RP patients is relatively high (19.8%)7 compared with earlier studies using time-domain OCT (1.2%) or fundus examination by ophthalmoscopy (0.8%).6,15 Secondary ERM, such as that from uveitis or retinal detachment, is associated with abundant inflammatory cell infiltration, indicating that an inflammatory response is implicated in its formation.16 However, the mechanisms of ERM formation in RP remain unclear. In the present study, we identified an association between ERM and aqueous flare in RP patients, suggesting a role of chronic inflammation in the development of ERM in RP.

METHODS

Study Design and Ethics Statement

We retrospectively reviewed the records of patients with RP and obtained examination results including aqueous flare values, OCT findings, and visual and systemic parameters. The aqueous flare was consecutively measured in RP patients who were referred to the Kyushu University Hospital in 2012 and 2013. Optical coherence tomography images taken on the
same day of the aqueous flare measurements were analyzed for ERM detection.

This study was approved by the Institutional Review Board of Kyushu University Hospital (Fukuoka, Japan) and was conducted in accord with the tenets of the Declaration of Helsinki on Biomedical Research Involving Human Subjects. The review board waived the need for written informed consent because the study design comprised a retrospective chart review.

Patients

Patients were recruited from Kyushu University Hospital in 2012 and 2013: 244 patients with a diagnosis of typical RP underwent an ophthalmic examination including the measurement of aqueous flare. The eyes of patients who had a history of other ocular diseases or intraocular surgery (e.g., cataract surgery), those who had undergone antiglaucoma treatment (e.g., topical prostaglandin analogues, topical beta blockers, and topical dorzolamide or oral acetazolamide), and those who had cystoid macular edema were excluded. After these exclusions, a total of 117 of the original 244 patients were enrolled. The methods for comprehensive eye examination were previously described.13

The diagnosis of typical RP was based on a history of night blindness, visual field constriction and/or ring scotoma, and markedly reduced or nonrecordable a- and b-wave amplitudes on electroretinography testing, in addition to ophthalmoscopic findings (e.g., bone spicule-like pigment clumping in the midperipheral and peripheral retina and attenuation of retinal vessels).

Laser Flare Photometry

The aqueous flare was measured with a Kowa FM-600 laser flare meter (Kowa Co., Nagoya, Japan).17–19 Flare values were obtained 30 minutes after pupilary dilation with 0.5% tropicamide and 5% phenylephrine hydrochloride. Five measurements were taken and averaged in each eye. The results are expressed as photon counts per millisecond.

Definition of Epiretinal Membrane

Epiretinal membrane was detected by fundus examination and by SD-OCT (Cirrus; Carl-Zeiss Meditec, Dublin, CA, USA). Fundus photographs and OCT images taken on the same day of the aqueous flare measurements were analyzed in the present study. The diagnosis of ERM using SD-OCT is based on the presence of a hyperreflective line or band over the retinal surface, frequently associated with wavy changes in the underlying retina (Fig.).7,20 Epiretinal membrane was diagnosed when it was detected on both fundus photographs and OCT images.

Statistical Analysis

Mean values were compared using the Student’s t-test, and differences in frequencies were compared using the χ² test. A linear relationship between aqueous flare and other variables was examined by dividing the eyes of the patients into four groups based on the quartile level of aqueous flare: quartile 1, flare <5.5 pc/ms; quartile 2, flare 5.5–7.5 pc/ms; quartile 3, flare 7.6–10.9 pc/ms; and quartile 4, flare >10.9 pc/ms.21 We analyzed the linear trend for mean values by performing a linear regression model, and we used a logistic regression model to analyze the linear trend for frequency. Thereafter, the aqueous flare values were treated as a continuous variable and were transformed into logarithms to improve the skewed distribution. We estimated the crude and age- and sex-adjusted odds ratio (ORs) and 95% confidence interval (CIs) for the development of ERM by performing a logistic regression analysis.
Aqueous Flare and ERM in RP

TABLE 1. Characteristics of Eyes With RP by Status of ERM

<table>
<thead>
<tr>
<th>Variable</th>
<th>Eyes of RP Without ERM (eyes = 133)</th>
<th>Eyes of RP With ERM (eyes = 73)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45.9 ± 15</td>
<td>51.8 ± 15</td>
<td>0.007</td>
</tr>
<tr>
<td>Sex, male, %</td>
<td>44</td>
<td>55</td>
<td>0.006</td>
</tr>
<tr>
<td>Flare, pc/ms</td>
<td>7.03 (2.64–18.73)</td>
<td>9.68 (3.49–26.82)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VA, logMAR</td>
<td>0.50 ± 0.5</td>
<td>0.35 ± 0.5</td>
<td>0.574</td>
</tr>
</tbody>
</table>

Inheritance mode, n

| AD                  | 21 (15.8%)                           | 8 (11.0%)                       | 0.457   |
| AR                  | 20 (15.0%)                           | 6 (8.2%)                        | 0.234   |
| X-linked            | 0 (0.0%)                             | 0 (0.0%)                        | 0.100   |
| Spontaneous         | 92 (69.2%)                           | 59 (80.8%)                      |         |

Values are given as means ± SD or numbers. Flares are shown by geometric means and 95% CIs due to the skewed distribution. Comparison of mean values between eyes of RP without ERM and with ERM was done by the Student’s t-test and frequencies by the χ² test. AD, autosomal dominant; AR, autosomal recessive; logMAR, logarithm of the minimal angle of resolution; pc/ms, photon counts per millisecond; VA, visual acuity.

All of the statistical analyses were performed with SAS software, ver. 9.3 (SAS Institute, Cary, NC, USA). Two-sided P < 0.05 was considered significant.

RESULTS

Using the total of 206 eyes of 117 patients with RP, we compared the demographic data between the patients with and without ERM. The mean values of age, frequency of male sex, and aqueous flare were significantly higher for the RP patients with ERM compared with those without ERM (P = 0.007, P = 0.004, and P < 0.0001, respectively; Table 1).

We divided the data of the patients’ eyes into quartiles based on the aqueous flare values (Table 2). The mean values of age, visual acuity, and frequencies of male sex in each quartile of aqueous flare were significantly increased as the quartile level rose (P < 0.001, P = 0.001, and P < 0.03, respectively; Table 2). Given the association of ERM with age and sex, we adjusted for these variables to exclude the confounding effects.

The eyes in the highest quartile of aqueous flare had significantly higher odds of having ERM than those in the lowest quartile, after adjustment for age and sex (OR, 2.68; 95% CI, 1.04–6.93; Table 3). The OR of ERM significantly increased as the flare quartile levels were elevated (P = 0.009; Table 3). When the aqueous flare values were assessed continuously, each 1-log–transformed increase in flare levels was associated with an elevation of the likelihood of having ERM after age and sex adjustment (OR, 2.59; 95% CI, 1.33–5.06; Table 3).

DISCUSSION

To our knowledge, this is the first study to investigate the association between aqueous flare and ERM in RP patients. Our findings demonstrated that the presence of ERM is significantly correlated with elevated aqueous flare independent of age and sex, suggesting the involvement of inflammation in the formation of ERM in RP.

Previous studies showed that there are apparent histologic differences between idiopathic ERM and secondary ERM such as that from uveitis or rhegmatogenous retinal detachment (RRD).15,22–27 Idiopathic ERM is composed predominantly of glial cells with minimal inflammatory cell infiltration, and it has been postulated that the traction on the retinal surface in the process of posterior vitreous detachment or due to vitreomacular adhesions may induce Müller cell gliosis.28 On the other hand, secondary ERM contains abundant inflammatory cells, including lymphocytes and macrophages, which may contribute to its development.15,17

In the vitreous of patients with uveitis or RRD, there is a substantial increase of proinflammatory cytokines/chemokines such as IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1).29,30 In line with these findings, we previously showed that these inflammatory factors are elevated in the vitreous of RP patients with secondary ERM compared with that of patients with idiopathic ERM,13 supporting the association between inflammation and ERM in RP. However, because strong vitreomacular adhesion is frequently observed in RP patients, chronic macular traction may also contribute to the ERM formation in RP.31 Further studies including histologic investigation are needed to clarify the pathogenesis of ERM secondary to RP.

Advanced ERM results in a reduction of visual acuity accompanied by metamorphopsia and diplopia. We and others reported the long-term surgical outcomes of pars plana vitrectomy (PPV) against ERM in RP patients.6,32,33 The results showed that PPV with internal limiting membrane peeling improves macular morphology, although it does not provide a significant improvement of visual acuity or metamorphopsia. The prevention of ERM formation or progression will thus be required to prevent vision loss due to macular complications in RP.

The results of our study suggest that the inflammatory process may be a potential target for the treatment of ERM in RP. Because long-term uses of a steroid (e.g., repeated intravitreal or subtenon injection of triamcinolone acetonide) carries considerable side effects such as cataract and glaucoma,34,35 targeting key molecules involved in chronic inflammation in RP—although not identified—may be a better approach to therapy. Such interventions will have broader clinical impacts in the treatment of RP because inflammation is associated not only with ERM but also with the degenerative processes.36

Our present analysis revealed that the prevalence of ERM in RP patients, as assessed by SD-OCT, was 35.7%. This is comparable to the prevalence reported by Triolo et al.

TABLE 2. Characteristics of Patients According to Flare Quartile Levels in RP Patients

<table>
<thead>
<tr>
<th>Flare Quartile Level, pc/ms</th>
<th>Quartile 1, &lt;5.5</th>
<th>Quartile 2, 5.5–7.5</th>
<th>Quartile 3, 7.6–10.9</th>
<th>Quartile 4, &gt;10.9</th>
<th>P for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes</td>
<td>52</td>
<td>51</td>
<td>52</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>40 ± 15</td>
<td>46 ± 15</td>
<td>50 ± 14</td>
<td>56 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, male, %</td>
<td>31</td>
<td>37</td>
<td>48</td>
<td>49</td>
<td>0.03</td>
</tr>
<tr>
<td>VA, logMAR</td>
<td>0.16 ± 0.4</td>
<td>0.20 ± 0.4</td>
<td>0.53 ± 0.7</td>
<td>0.40 ± 0.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are given as means ± SD or numbers.
(35.5%),37 but higher than that described by Testa et al. (19.8%); both of those studies were of Italian RP patients and used SD-OCT for ERM detection. The reasons for these differences in the prevalence of ERM could be the differences in genetic backgrounds or the methods of diagnosis. In the present study, we defined even a subtle hyperreflective lesion on the retinal surface as ERM, regardless of the presence of wavy inner retinal changes. Our study may thus include earlier stages of ERM. Another possibility to explain the differences in the prevalence of ERM between studies is the age distribution. The mean age of RP patients in the present study is higher than those of the previous studies, which may have led to the higher prevalence of ERM in our population.

In the present series, there were 13 cases (18%) with a subtle hyperreflective lesion on the retinal surface without the presence of wavy inner retinal changes. We included these cases for the analysis because we hypothesized that inflammation may be widely related to the pathology of ERM from its formation to progression in RP patients. An additional analysis excluding these 13 cases demonstrated that the association between aqueous flare and ERM remained significant (age- and sex-adjusted OR, 2.65; 95% CI: 1.30–5.40). These results indicate that the inclusion of the earlier ERM cases did not significantly affect the association between aqueous flare and ERM.

Our quartile analysis showed that the OR of ERM increased in Q3 (aqueous flare values >7.6 pc/ms) and Q4 (aqueous flare values >10.9 pc/ms). These findings suggest the possibility that an aqueous flare count >7.6 or >10.9 pc/ms is the threshold for the development of ERM. To investigate whether these scores can be applied as a marker to predict ERM formation in RP, we calculated the sensitivity, specificity, and positive predictive value (PPV) of ERM, as follows: >7.6 pc/ms, 69.9% sensitivity, 60.9% specificity, 49.5% PPV; >10.9 pc/ms, 37.0% sensitivity, 82.0% specificity, 52.9% PPV. Although the specificity of aqueous flare values >10.9 pc/ms was relatively high at 82.0%, the sensitivity and PPVs were not satisfactory due to the large variation of flare values.

This study is significant because of its relatively large sample size. Nevertheless, there are some limitations that should be discussed. First, although potential confounders, i.e., age and sex, were included in our analyses, we cannot rule out the possibility of unknown confounding factors for aqueous flare and ERM. Second, because of the cross-sectional nature of our study, it is difficult to define the causal relationship between aqueous flare and ERM. However, because ERM is formed in a restricted area of the macula, it is less likely that ERM itself significantly affects the aqueous flare values. We therefore propose that chronic intraocular inflammation may contribute to ERM formation, at least in part.

In conclusion, the results of our analysis demonstrated that elevated aqueous flare is associated with ERM secondary to RP, suggesting that inflammation may be implicated in the pathogenesis of ERM formation in RP.

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