Article

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Correlation between Anatomical and Functional Outcomes in Patients with Idiopathic Epiretinal Membrane after Vitrectomy

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Background: Idiopathic epiretinal membrane (iERM) is a common disorder of the vitreomacular interface characterized by decreased visual acuity and metamorphopsia. This study aimed to analyze the association between the anatomical change of the retina and functional outcomes in iERM patients so as to derive the prognostic factors of visual acuity (VA) and metamorphopsia.
Methods: Forty-five patients (one eye per patient; 45 eyes in total) who underwent pars plana vitrectomy and membrane peeling for iERM by a single surgeon were enrolled in this retrospective study. The results on best-corrected visual acuity (BCVA) and metamorphopsia as well as retinal images were obtained before the surgery and 1, 3, 6 months after the surgery. The BCVA and retinal microstructure, including central retinal thickness (CRT), ganglion cell layer (GCL) thickness, inner nuclear layer (INL) and outer nuclear layer + outer plexiform layer (ONL+OPL), and continuity of photoreceptor inner/outer segment (IS/OS) junction before and after iERM surgery were compared using paired samples *t*-test (continuous variables) or Chi-square test (categorical variables). Multiple regression analysis was carried out to explore the association among BCVA, M-score, and the parameters derived from optical coherence tomography.
Results: Compared with preoperative data, a significant improvement in BCVA was observed 1, 3, and 6 months postoperatively (t = 5.37, $p < 0.0001$; t = 7.29, $p < 0.0001$; t = 6.44, $p < 0.0001$ for 1, 3, and 6 months postoperatively, respectively), whereas the M-score only decreased significantly 3 and 6 months after the surgery (t = 2.36, $p = 0.02$; t = 5.00, $p < 0.0001$, respectively). In comparison with the baseline, the CRT, INL, and ONL+OPL thickness showed a significant decrease at each postoperative follow-up time, and GCL thickness (t = 3.86, $p = 0.0002$) and IS/OS disruption ratio ($\chi^2 = 4.86$, $p = 0.027$) were markedly reduced only 6 months postoperatively. Six-month postoperative BCVA was considerably associated with preoperative CRT and ONL+OPL thickness, as well as postoperative CRT, ONL+OPL thickness, and severity of IS/OS disruption. Moreover, the M-score after surgery was markedly correlated with both the preoperative and postoperative INL and CRT thickness.
Conclusions: Both VA and M-score in iERM patients were significantly improved after vitrectomy. Pre- and post-operative CRT was significantly associated with both postoperative BCVA and M-score. Besides, pre- and post-operative INL thickness was correlated to postoperative metamorphopsia, and postoperative BCVA was associated with postoperative ONL+OPL thickness and IS/OS integrity.

Keywords: idiopathic epiretinal membrane; metamorphopsia; internal limiting membrane

Introduction

Characterized by avascular fibrocellular proliferation over the internal surface of the retina, idiopathic epiretinal membrane (iERM) is a common retinal disorder that results in visual disturbances such as metamorphopsia and reduced visual acuity (VA) [1]. iERM affects 7% of the general population but the prevalence increases to approximately 20% in the population aged over 75 years old [2]. The primary treatment for iERM is pars plana vitrectomy (PPV) and peeling of the ERM, which was first described by Machemer in 1978 [3]. Internal limiting membrane (ILM) is a proliferative scaffold over the residual glial cells in recurrent iERM patients, and at present, ILM stripping has been broadly applied in combination with ERM removal to prevent recurrence and ensure complete removal of iERM fragments [4]. However, it has been demonstrated that visual disturbances and metamorphopsia will remain for a long period, even if ERM has been successfully and completely removed [5]. Since the improvement of VA and metamorphopsia significantly are correlated with the quality of life in ERM patients, evaluating the prognostic factors of VA and metamorphopsia is of great clinical significance.

Two common methods have been applied to detect and assess metamorphopsia: the Amsler charts and M-charts (Inami Co., Tokyo, Japan). Amsler charts are used to determine the degree of image distortion on the basis of a subjective self-description. In contrast, M-charts (Inami Co, Tokyo, Japan) can be used to give a quantitative assessment of metamorphopsia severity resulting from maculopathy by determining whether the line is distorted [6,7]. For example, if the patient recognized a straight line as straight, the metamorphopsia score was 0. When the patient reported a dotted line as straight, the visual angle was recorded as the metamorphopsia score. Meanwhile, due to the rapid scanning speed and high resolution, spectral-domain optical coherence tomography (SD-OCT) has been generally applied in evaluating and diagnosing retinal conditions [8–11]. Additionally, the intraretinal anatomical structure, including ganglion cell layer (GCL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and photoreceptor inner/outer segment (IS/OS) junction, can be clearly visualized with SD-OCT [8]. On that account, the relationship between metamorphopsia and the architectural morphology of the retina can be assessed using SD-OCT.

Previous studies revealed that impaired IS/OS [12–14], thickening of central macula [14–16] and outer layer of the retina [17,18], and thickening of INL and GCL [19,20] were correlated with poor VA improvement. While several studies also surveyed the association between metamorphopsia and optical coherence tomography (OCT) findings, which demonstrated that INL thickness [20–22], disruption of the IS/OS [23,24], and central macular thickness [22,25] were associated with the severity of metamorphopsia. However, the conclusions mentioned above are controversial. Besides, the studies focusing on the relationship between metamorphopsia and microstructural change of macula are scarce and not informative. Therefore, we performed this study to assess the relationship between the intraretinal architectural morphology and the parameters of VA and metamorphopsia in iERM patients.

Subjects and Methods

In this retrospective study, we enrolled 45 iERM patients (one eye per patient; 45 eyes in total) hospitalized in our department from April 2018 to May 2020. The diagnosis of iERM was made according to the clinical diagnostic criteria [26]. Patients with myopia above 6 diopters, a history of vitreoretinal surgery, any other retinal macular disorders, and secondary ERM caused by trauma, retinal rupture, uveitis and retinal vascular disease were excluded. PPV (25-gauge) combined with ERM and ILM peeling assisted by indocyanine green angiography were performed on all subjects. The len was removed, if necessary, via phacoemulsification, and an artificial len was implanted followed by vitrectomy. All surgeries were performed by the same ophthalmologist. The research was approved by the Institutional Review Boards of Affiliated People's Hospital, Jiangsu University, and the study was conducted in adherence with the principle of the Declaration of Helsinki. All patients gave their informed consent prior to participating in the study.

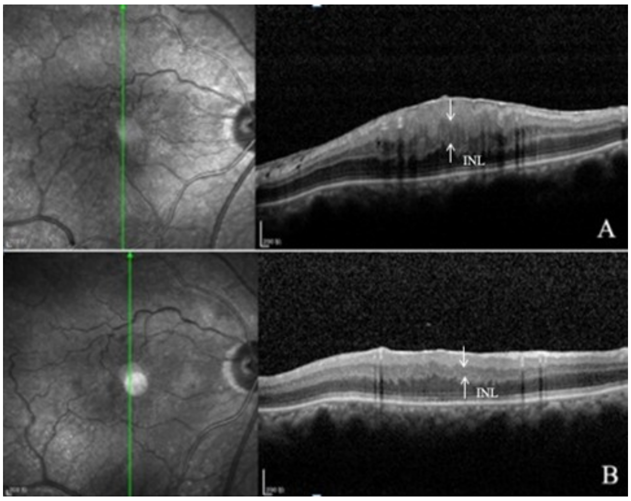

Fig. 1. Spectral-domain optical coherence tomography (SD-OCT) images of the right eye from a 62-year-old woman with idiopathic epiretinal membrane (iERM). (A) Preoperative SD-OCT image. (B) Postoperative SD-OCT image. INL, inner nuclear layer.

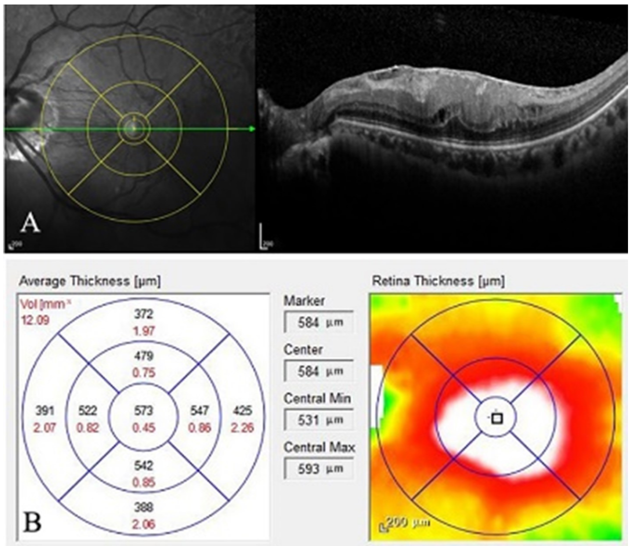

Fig. 2. Optical coherence tomography (OCT) parameters for pre and postoperative morphologic analysis of patients undergoing vitrectomy. (A) Mean central retinal thickness (CRT) corresponding to the vertical distance between the outer surface of retinal pigment epithelium and the inner surface of the retinal neuroepithelial layer of the central 1 mm region obtained from the software automatically. (B) Analysis outputs from the software showing parameters evaluated within the 1 mm area of the fovea.

Table 1. The BCVA and retinal microstructure before and after ERM surgery (N = 45).

	Baseline	1 month postoperatively	3 months postoperatively	6 months postoperatively
BCVA (logMAR)	0.82 ± 0.26	0.52 ± 0.23 (t = 5.37, $p < 0.0001$)	0.47 ± 0.15 (t = 7.29, $p < 0.0001$)	0.46 ± 0.25 (t = 6.44, $p < 0.0001$)
CRT (μm)	505.3 ± 83.3	433.8 ± 79.7 (t = 4.16, $p < 0.001$)	384.6 ± 74.8 (t = 7.24, $p < 0.0001$)	349.3 ± 64.5 (t = 9.94, $p < 0.0001$)
GCL (μm)	113.5 ± 28.1	108.9 ± 12.0 (t = 3.08, $p = 0.064$)	107.4 ± 12.8 (t = 6.24, $p = 0.13$)	98.1 ± 16.1 (t = 3.86, $p = 0.0002$)
INL (μm)	117.4 ± 29.5	99.7 ± 28.4 (t = 2.37, $p = 0.02$)	89.5 ± 25.7 (t = 3.44, $p < 0.001$)	85.3 ± 15.6 (t = 6.46, $p < 0.0001$)
ONL+OPL (μm)	166.4 ± 42.4	142.2 ± 25.4 (t = 2.51, $p = 0.01$)	138.0 ± 32.3 (t = 3.57, $p = 0.0006$)	130.7 ± 26.6 (t = 3.67, $p = 0.0005$)
M-Score	0.68 ± 0.24	0.65 ± 0.26 (t = 6.27, $p = 0.23$)	0.56 ± 0.19 (t = 2.36, $p = 0.02$)	0.44 ± 0.20 (t = 5.00, $p < 0.0001$)
IS/OS disruption, n (%)	12 (27)	7 (16) ($\chi^2 = 1.67$, $p = 0.19$)	6 (13) ($\chi^2 = 2.5$, $p = 0.11$)	4 (9) ($\chi^2 = 4.86$, $p = 0.027$)

Postoperative values at 1, 3 and 6 months were compared with baseline value respectively.

Data of all parameters, except for IS/OS disruption, are expressed as mean ± SD.

BCVA, best-corrected visual acuity; logMAR, logarithm of minimal angle of resolution; GCL, ganglion cell layer; ONL+OPL, outer nuclear layer + outer plexiform layer; IS/OS, inner/outer segment; SD, standard deviation.

Table 2. Multiple regression analysis of preoperative BCVA and M-score with other preoperative OCT parameters in iERM patients (N = 45).

	Preoperative BCVA				Preoperative M-score			
	t	SE	B (95% CI)	*p*	t	SE	B (95% CI)	*p*
CRT	78.17	0.006	0.469 (0.116, 1.125)	0.022*	136.33	0.003	0.409 (0.116, 0.624)	0.012*
GCL	2.08	0.013	0.027 (–0.056, 0.336)	0.834	101.12	0.002	0.202 (0.041, 0.613)	0.147
INL	–24.8	0.005	–0.124 (–0.306, –0.019)	0.460	49.25	0.008	0.394 (0.127, 0.571)	0.035*
ONL+OPL	19.44	0.009	0.175 (0.014, 0.307)	0.363	–25.47	0.015	–0.382 (–0.548, –0.106)	0.059
IS/OS disruption	16.09	0.021	0.338 (0.025, 0.512)	0.013*	15.52	0.012	0.186 (0.002, 0.341)	0.222

* $p < 0.05$.

Data such as age, gender, best-corrected visual acuity (BCVA), status of anterior segments especially lens, and medical history were collected from all subjects before surgery. Fundus ophthalmoscopy was employed to determine the condition of the fundus.

BCVA, data on deformation severity, and SD-OCT data were obtained preoperatively and 1, 3 and 6 months postoperatively. The BCVA measured by the international standard VA map was transformed into the logarithm of minimal angle of resolution (logMAR) BCVA for further investigation. The degree of metamorphopsia was quantified using M-charts [7]. For data analysis, the mean M-score was calculated from the M-score values derived from three replicate experiments. Retinal images were obtained after performing SD-OCT (Heidelberg Engineering, Heidelberg, Germany). With the obtained OCT images, the following parameters were recorded within the 1 mm area of the fovea: central retinal thickness (CRT), GCL thickness, INL and ONL+OPL, and continuity of IS/OS (IS/OS disruption; classified as intact, irregular/granular, or absent) (Fig. 1). These parameters correspond to the mean distance from the fovea center, that is, the central 1 mm region, obtained from the software automatically (Fig. 2). These parameters were measured three times for the purpose of computing the average values.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA). For continuous variables, the results were presented as mean ± standard deviation (SD). The results of categorical variables were presented as proportions (%). The BCVA and retinal microstructure before and after ERM surgery were compared using paired samples *t*-test (continuous variables) or Chi-square test (categorical variables). After controlling for some potential confounders (such as age, gender, and duration of disease) by univariate linear regression (data not shown), multiple linear regression was carried out to explore the association among BCVA, M-score and the parameters derived from OCT. The result is considered significant when $p < 0.05$.

Results

In this retrospective study, we analyzed 45 eyes from the recruited patients (15 males and 30 females), who had an average age of 65.7 ± 6.5 years old (ranging from 47 to 79 years old). We dissected the ERM and ILM successfully in all 45 cases, and cataract surgery combined with vitrectomy surgery was performed in 32 cases, and vitrectomy surgery was performed in the remaining 13 cases. The changes in SD-OCT parameters were observed preoperatively and 1, 3 and 6 months postoperatively.

As shown in Table 1, there were significant improvements in BCVA at 1, 3 and 6 months after operation in comparison with preoperative BCVA (t = 5.37, $p < 0.0001$; t = 7.29, $p < 0.0001$; t = 6.44, $p < 0.0001$ for 1, 3, and 6 months postoperatively, respectively) in all patients. Similarly, a

Table 3. Multiple regression analysis of postoperative 6-month BCVA and M-score with other preoperative OCT parameters in iERM patients (N = 45).

	Postoperative BCVA				Postoperative M-score			
	t	SE	B (95% CI)	*p*	t	SE	B (95% CI)	*p*
CRT	26.43	0.014	0.370 (0.112, 0.608)	0.012*	54.67	0.009	0.492 (0.024, 0.607)	0.001**
GCL	–43.21	0.002	–0.086 (–0.238, –0.007)	0.455	14.15	0.007	0.098 (0.001, 0.207)	0.523
INL	29.11	0.009	0.262 (0.024, 0.319)	0.098	39.75	0.012	0.477 (0.206, 0.628)	0.001**
ONL+OPL	17.57	0.021	0.369 (0.024, 0.548)	0.013*	22.75	0.008	0.182 (0.034, 0.341)	0.231
IS/OS disruption	15.38	0.013	0.200 (0.026, 0.345)	0.115	8.41	0.017	0.143 (0.012, 0.362)	0.264

* $p < 0.05$; ** $p < 0.001$.

Table 4. Multiple regression analysis of postoperative 6-month BCVA and M-score with other postoperative OCT parameters in iERM patients (N = 45).

	Postoperative BCVA				Postoperative M-score			
	t	SE	B (95% CI)	*p*	t	SE	B (95% CI)	*p*
CRT	52.67	0.006	0.316 (0.106, 0.547)	0.035*	112.33	0.003	0.337 (0.115, 0.492)	0.024*
GCL	–4.47	0.015	–0.067 (–0.119, –0.004)	0.536	4.87	0.008	0.039 (0.014, 0.165)	0.786
INL	7.26	0.007	0.049 (0.016, 0.108)	0.723	100.02	0.004	0.400 (0.224, 0.631)	0.006*
ONL+OPL	30.64	0.011	0.337 (0.204, 0.612)	0.023*	–9.38	0.008	–0.075 (–0.201, –0.027)	0.664
IS/OS disruption	47.52	0.014	0.665 (0.226, 0.812)	< 0.001**	–1.16	0.019	–0.022 (–0.161, 0.032)	0.903

* $p < 0.05$; ** $p < 0.001$.

significant reduction in CRT was found at each postoperative follow-up time (t = 4.16, $p < 0.001$; t = 7.24, $p < 0.0001$; t = 9.94, $p < 0.0001$ for 1, 3, and 6 months postoperatively, respectively) compared with the baseline data. Interestingly, only the GCL thickness at 6 months after operation has a considerable reduction than the value at baseline (t = 3.86, $p = 0.0002$). The values of INL thickness before operation and at 1, 3 and 6 months after operation were 117.4 ± 29.5, 99.7 ± 28.4, 89.5 ± 25.7, and 85.3 ± 15.6, respectively, showing a significant decrease (t = 2.37, $p = 0.02$; t = 3.44, $p < 0.001$; t = 6.46, $p < 0.0001$ at 1, 3, and 6 months postoperatively, respectively). The mean ONL+OPL thickness values at 1, 3, and 6 months after the operation were obviously lower than the preoperative value (t = 2.51, $p = 0.01$; t = 3.57, $p = 0.0006$; t = 3.67, $p = 0.0005$ at 1, 3, and 6 months postoperatively, respectively). Additionally, the M-scores at 3 and 6 months postoperatively were significantly lower than the baseline (t = 2.36, $p = 0.02$; t = 5.00, $p < 0.0001$, respectively). The proportion of IS/OS disruption was reduced from the preoperative value of 12 (27%) to 7 (16%), 6 (13%) and 4 (9%) at 1, 3 and 6 months after the operation ($\chi^2 = 1.67$, $p = 0.19$; $\chi^2 = 2.5$, $p = 0.11$; $\chi^2 = 4.86$, $p = 0.027$, respectively).

Multiple linear regression analysis revealed that the preoperative BCVA (logMAR) exhibited a significantly positive correlation with CRT ($p = 0.022$) and IS/OS disruption ($p = 0.013$), but not with the thickness of GCL, INL and ONL+OPL. M-score prior to surgery positively was correlated with CRT ($p = 0.012$) and INL thickness ($p = 0.035$) (Table 2).

Multiple linear regression analysis between postoperative BCVA and other preoperative OCT parameters revealed that the postoperative BCVA exhibited a significant association with CRT ($p = 0.012$) and ONL+OPL ($p = 0.013$). We also detected positive correlations of postoperative M-score with CRT ($p = 0.001$) and INL thickness ($p = 0.001$) (Table 3).

Multiple linear regression analysis between postoperative M-score and postoperative OCT findings indicated that the M-score exhibited a significant correlation with CRT ($p = 0.024$) and INL thickness ($p = 0.006$). Moreover, postoperative BCVA was significantly correlated with CRT ($p = 0.035$), ONL+OPL ($p = 0.023$), and IS/OS disruption ($p < 0.001$) (Table 4).

Discussion

iERM generates tractional stress across all layers of the retina, including retinal inner layers such as GCL and INL, and retinal outer layers such as OPL and ONL, possibly leading to metamorphopsia and decreased VA with macular distortion and/or edema [27]. However, the relationship of the intraretinal architectural morphology with the parameters of VA and metamorphopsia in iERM patients is still unclear. Thus, in this study, we determined the prognostic factors of metamorphopsia and VA by evaluating the SD-OCT parameters in iERM patients after vitrectomy.

Specifically, we investigated the relationship between BCVA, M-score, and foveal microstructure of iERM patients. We found that BCVA was significantly improved and CRT was clearly reduced after vitrectomy, suggesting that vitrectomy is a reliable method for improving visual function and alleviating retinal edema caused by contraction of iERM. M-score also reduced significantly after surgery, a finding consistent with a previous study [20]. This indicates that the morphologic characteristics of the retina can be analyzed based on the OCT findings, which can predict the changes in BCVA and metamorphopsia after vitrectomy.

Numerous studies have clarified the association between preoperative VA, M-score, and retinal microstructures in iERM patients, indicating that CRT, ONL+OPL thickness [18], INL thickness [17], and degree of IS/OS disruption [15,18] were associated with BCVA, whereas CRT [23] and INL thickness [24] were related to the degree of metamorphopsia. The results of this study showed that preoperative CRT and IS/OS disruption were significantly correlated with preoperative BCVA. In addition, an evident relationship of preoperative M-score with preoperative CRT and INL thickness was confirmed in this study. Our findings were congruent with those previously reported in most studies [15,21], that is, the retinal outer layer and inner layer characteristics were correlated with VA and metamorphopsia, respectively, with the exception of CRT, which was correlated with both VA and metamorphopsia.

The aim of the present study was to determine the factors influencing BCVA and deformation after vitrectomy. Hence, we focused on the association between postoperative BCVA, metamorphopsia and macular microstructure. Since the previous studies had inconsistent findings on the risk factors of postoperative visual function, in this study, we attempted to evaluate the correlation between postoperative 6-month BCVA and preoperative OCT parameters. The results suggested that preoperative CRT and ONL+OPL were significantly correlated with postoperative BCVA, consistent with several studies [12,13,16]. However, Lee *et al*. [19] verified a significant association between postoperative GCL thickness and postoperative BCVA. Besides, Bae *et al*. [28] and Takabatake *et al*. [29] demonstrated that no structural variables were significantly correlated with BCVA after surgery. Our study demonstrated that postoperative BCVA was significantly associated with postoperative thickness of CRT, ONL+OPL and IS/OS integrity, suggesting that the postoperative IS/OS disruption, but not the preoperative IS/OS disruption, contributed to better prediction of postoperative BCVA. In this study, the disrupted IS/OS in some cases was restored after vitrectomy combined with ERM/ILM peeling, supporting the hypothesis mentioned above.

Due to the clinical significance of metamorphopsia in determining the life quality of iERM patients, we attempted to determine distinct prognostic factors of metamorphopsia and VA. The evaluation of the association between postoperative M-score and retinal microstructure showed that both preoperative and postoperative CRT and INL thickness were obviously associated with postoperative M-score. Similar results that INL thickness was correlated with postoperative M-score have been reported by other researchers [23,24]. For instance, Okamoto *et al*. [20] found that the INL thickness was associated with the postoperative M-score after surveying the relationship between the thickness of INL and metamorphopsia determined by M-charts in ERM patients.

In this study, both metamorphopsia and VA were markedly correlated with pre- and post-operative CRT. This result was in line with previous studies, corroborating the notion that CRT is associated with not only VA but also metamorphopsia. Thus, CRT is the most distinctive prognostic factor for iERM. Moreover, our study showed that INL thickness had a significant influence on pre- and post-operative M-score, whereas ONL+OPL thickness and the integrity of IS/OS were associated with postoperative BCVA. Hibi *et al*. [30] assessed the retinal function of ERM patients and found that b-wave reduced more than a-wave did, and the ratio of the preoperative to postoperative b-wave was associated with the decrease of INL thickness. Accordingly, we inferred that the iERM contraction led to a thickness alteration in INL, which consists of horizontal, bipolar, amacrine, and Müller cell body structures. Moreover, INL thickening induces visual distortion and damages visual quality by restraining the general function of synaptic connections and decreasing the sensitivity of photoreceptors. Since the OPL covers the region where photoreceptors are in contact with the horizontal cells and bipolar cells while the ONL contains the photoreceptor cell bodies, the damage of ONL+OPL may result in decreased VA and metamorphopsia. Furthermore, we found that the significant change to all retinal layers only happened 6 months after vitrectomy, suggesting that the postoperative 6-month change of OCT parameters forms the baseline for predicting the improvement of iERM.

The present study has several limitations. First, most recruited patients had undergone cataract surgery combined with vitrectomy surgery, which could impact the results as the status of the lens could affect the evaluation of metamorphosis and VA (although the follow-up duration was limited to 6 months). Besides, the measurements of the thickness of different retinal layers in iERM eyes using manual segmentation could be biased, although two masked examiners were tasked with the measurement activity. Furthermore, this study employed a relatively small sample size and a follow-up period of only six months. Therefore, further studies employing larger sample sizes, longer follow-up periods, and advanced OCT technologies are warranted in the future.

Conclusions

In conclusion, compared with preoperative data, a significant improvement in BCVA was observed 1, 3, and 6 months postoperatively, whereas the M-score only decreased significantly 3 and 6 months after the surgery. In comparison with baseline, the CRT, INL and ONL+OPL thickness showed a significant decrease at each postoperative follow-up time, and GCL thickness and IS/OS disruption ratio were markedly reduced only 6 months postoperatively. We also found that pre- and post-operative CRT was significantly associated with both postoperative BCVA and M-score. Besides, pre- and post-operative INL thickness was correlated to postoperative metamorphopsia while postoperative BCVA was associated with postoperative ONL+OPL thickness and IS/OS integrity.

Abbreviations

BCVA, best-corrected visual acuity; CRT, central retinal thickness; GCL, ganglion cell layer; INL, inner nuclear layer; iERM, idiopathic epiretinal membrane; IS/OS, inner/outer segment; ONL+OPL, outer nuclear layer and outer plexiform layer; PPV, pars plana vitrectomy; SD-OCT, spectral-domain optical coherence tomography; VA, visual acuity; ILM, Internal limiting membrane; logMAR, logarithm of minimal angle of resolution; SD, standard deviation.

Availability of Data and Materials

All data are available from the corresponding author upon reasonable request.

Author Contributions

NM, LX, and ZZ designed the research; NM and LX conducted the data collection and contributed equally to this work; CS, YG and LX analyzed the data; NM and ZZ wrote the original draft. LX, YG and CS reviewed and edited the manuscript. The funding acquisition was from NM, ZZ and LX. All authors have read the final version and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All patients provided written informed consent to participate in this study and this study obtained ethics approval from the Ethics Committee of Affiliated People's Hospital, Jiangsu University (Approval number: SQK-20230057-W), which was conducted in accordance with the tenets of the 1964 Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

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