

Clinical Characteristics and Histopathology of Idiopathic Epiretinal Membrane in Vietnam

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Abstract

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BACKGROUND: Idiopathic epiretinal membrane (iERM) is an avascular proliferation of different types of cells between the posterior vitreous cortex and the internal limiting membrane. That causes visual impairment including blurry, distortion, scotoma. Many studies of iERM were done to describe the clinical characteristics and investigate the histopathology of this disease. Nonetheless, there has not been a study of iERM histopathology in Vietnam.

AIMS: To describe clinical characteristics and histopathological results of idiopathic retinal membrane and the association between them.

METHODS: A cross sectional descriptive study of 35 iERMs (33 patients) in Vietnam National Institute of Ophthalmology (VNIO).

RESULTS: High morbidity incidence was in group age >50 years (32/35), female gender (26/35), limited movement works (27/35), and high educational levels (28/35). Distortion was the highest (77.14%), scotoma and floater was less frequent (28.5%, 45.7%). Macular edema in all cases and PVD and exudate were high frequent (65.7%, 62.8%). Symptom duration was 8.2 ± 4.7 months, (1-21 months). Mean of central macular thickness was $468.51 \pm 97.24 \mu\text{m}$ (656-274 μm). Six types of cell were detected, including glial cell (35/35), fibroblast (23/35), myofibroblast (23/35), macrophage (13/35), lymphocyte (5/35) and neutrophil (2/35). The number of cell types in one sample ranged from 1-5 types (2.85 ± 1.28 cell types). Number of cell types were correlated to symptom duration ($r = 0.47$, $p = 0.004$, Pearson's test) and central macular thickness ($r = 0.72$, $p < 0.001$, Pearson's test).

CONCLUSION: There were 6 types of cells in iERM. Glial cell was the most frequent cell, inflammatory cells (macrophage, lymphocyte, neutrophil) was also detected. The number of cell types was statistically correlated to symptom duration and CMT.

Introduction

Epiretinal membrane (ERM) is described as cellophane maculopathy or macular pucker, which is also a proliferation of avascular cellular between the posterior vitreous membrane and internal limiting membrane (ILM), it causes visual impairments, primarily due to the mechanical distortion of the macular area [1], [2], [3]. Idiopathic epiretinal membrane (iERM) is an ERM in cases there is not any causative factors or ocular pathology was found

and its pathophysiology has remained unclear. That membrane contracts to the layers of retina, that due to changing of retinal surface to most of its layers, and causes macular edema [2].

There have been many studies about clinical characteristics and histopathology of the iERM to investigate the pathogenesis of this disease. Clinical characteristics include risk factors, symptom durations, visual impairment such as blurry, distortion, scotoma; and retinal structure change that was observed by OCT (Optical Coherence Tomography)

as central macular thickness (CMT), macular volume (MV) [1], [3], [4], [5]. In histopathology, two components of iERM were usually detected: the proliferative cells (origin or deprivation) and extracellular matrix (collagen, fibronectin) [2], [6], [7], [8], [9]. The proliferative cells were the most important component that formed extracellular matrix, thus always were the main investigated subjects in studies. Some types of cells were often found in iERM, such as glial cell, hyalocyte, retinal pigment epithelium (RPE) cell, macrophage, fibroblast, myofibroblast, and inflammatory cells (macrophage, lymphocyte, neutrophil). The glial cells were the most frequent cells in iERMs which caused a little traction while myofibroblasts were cell type caused significant traction to the retina. Glial cells were included three types of cells: muller cells, astroglia, microglia. These cells formed supported structures that stretched across full thickness of the retina, and were also at the limits of the retina at the OLM and ILM [2]. By using immunohistochemistry, glial cell was proven able to differentiate to other cells (macrophages, fibroblasts, myofibroblasts). On the other side, myofibroblasts (the major cell type in traction iERMs) did not appear in normal human retina. They could be differentiated from Muller cell, hyalocyte or RPE cell [2], [6], [8]. Others cells such as hyalocyte, RPE were only detected in some recent study using immunochemistry. Inflammatory cells were recently detected in iERM, that suggested the inflammatory mechanism in iERM formation [6].

These diversities of the cell types and the cell density in iERMs may be explained by different methods of histopathology (including chemical staining such as Hematoxylin Eosin (HE), Periodic Acid Schiff (PAS), immune marker staining such as immunocytochemistry, immunohistochemistry or detecting by transmission electron microscopy) and small sample sizes of researches (often less than 20 cases) [6], [8], [10]. The other difficult factor for histopathology of iERM was the small size of membrane sample after peeling that was not enough for analyzing. In Viet Nam, the number iERM cases has increased, but there were only few researches about clinical characteristics of iERM based on examinations and OCT imaging. Thus, we did this study to describe clinical characteristics and histopathological results of idiopathic retinal membrane and association between them.

Material and Methods

A cross sectional descriptive study was done in 35 iERM eyes (33 patients) whom were treated with vitrectomy and membrane peeling in Department of Vitreous and Retina, Vietnam National Institute of Ophthalmology (VNIO) from 7/2016-12/2016. The size

requirement of the iERM specimens was more than 2 x 2 mm. Then these specimens were histopathologically tested in pathology laboratory of VNIO and were rechecked in laboratory of 103 Military hospital.

Patients with iERM were diagnosed on clinical and OCT at stage 1 or 2 (Gass J.D 1993) [11]. Patients marked the checklist about history of diseases, then an across retinal section image was done by OCT (RTVue-100 Fourier-Domain Optical Coherence Tomography, Optovue, Inc.) to measure CMT, macular volume (MV). After that, 23 G vitrectomy with 3 ways through parsplana trocar with BIOM system was done to obtain the retinal membrane [12]. The epiretinal membrane was stained by Trypan blue (0.5 mL, 0.25%; Fluoron GmbH, Neu-Ulm, Germany) [13]. Then, the membrane was peeled by intraocular forceps, the epiretinal membrane was fixed immediately in 0.9% saline solution to transported to histopathological laboratory. These specimens were flattened on the antiseptic glass slide and dried in alcohol solution several times with decreasing alcohol concentration (100%, 90%, 80%, 70%). Afterwards, that were stained with Hematoxylin-Eosin Y 1% solution in 10 minutes and re-washed with distilled water within 5 minutes. The fixed specimens were checked by optical microscope with different magnifications (x 100, x 200, x 400). The cell density and cell type were identified based on the cell morphology by a histopathologist. A density of cells in specimen was classified to be dense and sparse. The dense density specimens were that there were 10 or more cells in a field with the magnification x 400. The other specimens were sparse density [2].

Statistical analysis

According to statistical methods Medicine by Excel and SPSS 16.0 program (SPSS, Inc., Chicago, IL, USA). In the analysis, the value of $p < 0.05$ as statistically significant.

Results

Clinical characteristics

Thirty-five eyes with iERM of 33 patients (9 men, 24 women) were collected in our study. The ages of our patients were from 38 to 76 years old and two third cases (22 patients) were more than 50 years old. Most of studied cases (25 patients) have limited movement works (who spends more time for sitting than moving) and 26 patients have high educational level (beyond high school).

Distortion was the highest ratio in each group of visual acuity and overall (77.1%). Scotoma and floater are less frequent (28.5%, 45.7%). In patients with high visual acuity ($> 20/100$), the symptoms were

easier to be realized (Table 1).

Table 1. Distribution of symptom by visual acuity

Visual acuity	Distortion	Scotoma	Floater
≤ 20/100 (n = 25)	17 (68.4%)	3 (8.6%)	7(28.1%)
20/100 – 20/40 (n = 9)	9 (100%)	7 (77.3%)	8 (88.4%)
≥ 20/40 (n = 1)	1 (100%)	0	1 (100%)
Total	27 (77.1%)	10 (28.5%)	16 (45.7%)

ME were in all of cases (100%), PVD and exudate were high frequent in iERM (65.7% and 62.8% respectively). There was only one case of RH in our study group (Table 2).

Table 2: Distribution of signs by iERM grade

Grade	ME	PVD	RH	Exudate
1 (n = 6)	6 (100%)	3 (50 %)	0 (0%)	4 (66.6%)
2 (n = 29)	29 (100%)	20 (68.9%)	1 (2.4%)	18 (62.1%)
Total	35 (100%)	23 (65.7%)	1 (2.8%)	22 (62.8%)

ME: macular edema; PVD: posterior vitreous detachment; RH: retinal hemorrhage.

Up to surgery time, symptom duration was from 1 to 21 months (8.2 ± 4.7 months). Mean of CMT was $468.51 \pm 97.24 \mu\text{m}$ (656-274 μm). The details of distribution of symptom duration and central macular thickness in study patients were shown in Figure 1, and Figure 2.

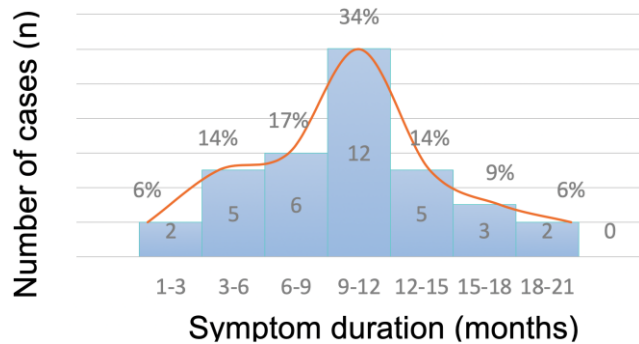


Figure 1: Distribution of symptom duration of iERM

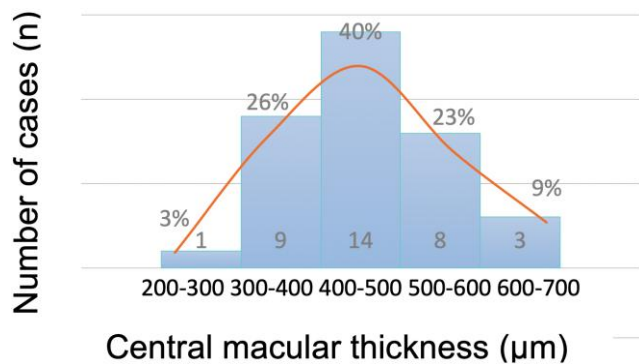


Figure 2: Distribution of central macular thickness in OCT

Histopathological results

There were 12 cases with sparse density A) and 23 cases with dense density B) (Figure 3).

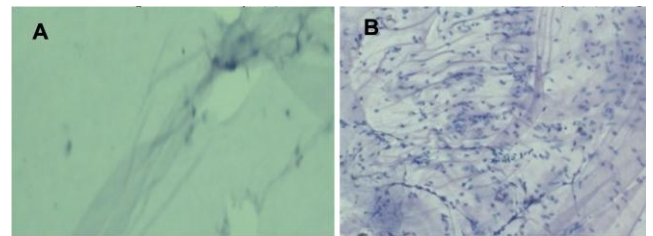


Figure 3: A) sparse density; B) dense density

The number of cell types ranged from 1 to 5 types of cells in one sample (average 2.8 ± 1.2 types of cells). There were statistically a greater number of cell types in dense density group (4.7 ± 2.1) than sparse density group (2.2 ± 1.1) ($p = 0.02$).

There were 6 types of cells in iERMs samples, including glial cell, contractile cells (myofibroblast, fibroblast), inflammatory cells (macrophage, lymphocyte, neutrophil) (Figure 4).

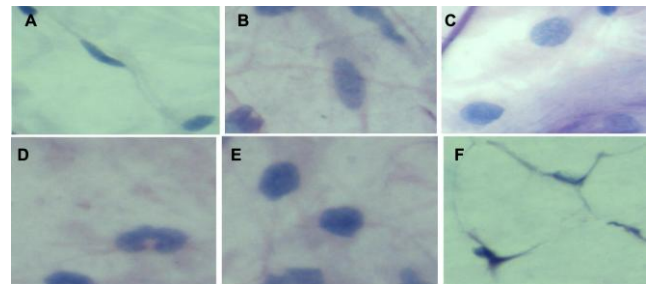


Figure 4: The cell types in our study sample; A) fibroblast; B) myofibroblast; C) macrophage; D) neutrophil; E) lymphocyte; F) glial cell

Glial cell was the most popular cell type (35/35 samples) that must have proved the important role of this cell in the mechanism of iERM. Fibroblast was the second frequent type of cell in 23 specimens (65.7%). Myofibroblasts was detected in 23 cases (65.7%). Macrophage was detected in 13 cases (37.2%). Lymphocyte was detected only in 5 cases (16.7%). Neutrophil was detected in only 2 cases in our study. The cells of connective tissue (glial cell, fibroblast, myofibroblast) were the main part of iERM which were detected in most samples. Otherwise, the inflammatory cells (macrophage, lymphocyte, neutrophil) just appeared in few samples with high number of cell types (from 3 cell types in a sample) (Table 3).

Table 3: Distribution of cell types in a sample

Cell type	Number of cell types in a sample				
	1 (n = 7)	2 (n = 7)	3 (n = 8)	4 (n = 10)	5 (n = 3)
Glial cell	7	7	8	10	3
Fibroblast	0	6	6	8	3
Myofibroblast	0	6	7	8	2
Macrophage	0	0	2	9	2
Lymphocyte	0	0	0	2	3
Neutrophil	0	0	0	0	2

Association between clinical characteristics and histopathology

We had looked for the association between histopathology and some of symptoms and sign of

iERMs in our patients. We found that number of cell types in a sample correlated to symptom duration ($r = 0.47$, $p = 0.004$, Pearson's test) and to central macular thickness was statistically significant ($r = 0.72$, $p < 0.001$, Pearson's test) (Figure 5).

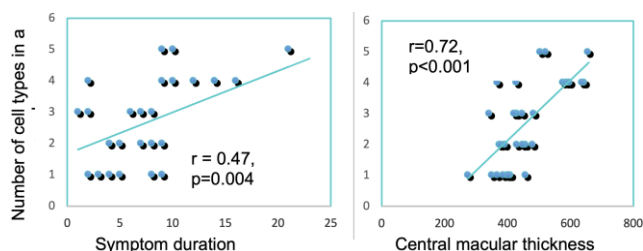


Figure 5: Association between number of cell types in a sample and symptom duration and central macular thickness

Discussion

The distortion was the most frequent iERM symptoms (77.1%) in our patients. This symptom is a manifestation of macular edema as a result of ERM. When the ERM contracted to the layers of macular, it changed the macular structure and made the fluid appear in the extra layers. Thus, after peeling this membrane, the distortion was standard criteria to assessment of surgery outcome [4]. There was macular edema in OCT images in all 35 eyes of our study, so the distortion was more specific symptom than others such as floater, scotoma or blurry and may be used for screening ERM. However, this symptom depends crucially on visual acuity, there were all ten eyes (100%) with visual acuity above 20/200 having the symptom of distortion, but there were only 17 eyes (68.4%) in poor vision group (visual acuity was lower than 20/200).

The manifestation of macular edema in higher level is scotoma. When the edema lasted long time and the function of retinal photoreceptor cells was damaged, the symptom of scotoma appeared. The other reason of scotoma in ERM is the thick membrane over the retina. If the scotoma was in the center or enlarged, visual acuity also decreased making difficult to clinically detect this symptom. This characteristic explained why there were only 10 eyes (28,5%) having scotoma in our study, and most of them (7 eyes) was in group with visual acuity from 20/200 to 20/40.

Central macular thickness (CMT) is quantitative measure of macular edema in OCT. It was an important factor in the pathophysiology of iERM and directly affected vision and other visual functions [5]. In our study, the average of CMT (468.51 μm) was higher than previous studies, which can be explained by the fact that all iERM eyes had

been prepared to operate and were in severe stage of disease [14].

In this study, 6 cell types were detected, including glial cell (35/35), fibroblast (23/35), myofibroblast (23/35), macrophage (13/35), lymphocyte (5/35) and neutrophil (2/35). Glial cells was most frequent detected, its collagen was also the important formation of iERM [15]. The importance of these cells in iERM formation had been proven by many researches [16], [17], [18],[15]. However, the subtype of glial cells (Müller cells, astrocytes...) that was the major cell type in iERMs have been controversial. A recent study that used immunohistochemical staining for glutamine synthetase (GS) (expressed specifically in Müller cells and did not appear in astrocytes) on surgically excised iERMs showed the characteristics of continuous, isodense pattern of immunoreactivity for GS. This result proved that Müller cell was the predominant cell type for iERM formation [15]. Using HE staining, this study did not find any other cells such as hyalocyte, astrocyte, retinal pigment epithelium. Fibroblast and myofibroblast which could produce fibril forming the membrane and cause significant traction to the retina were also major component cell in iERMs [6], [8]. These cells that normally did not appear in retina might be differentiated from hyalocytes, glial cell – normal cell in retina. In our study, there were 27 samples in 35 iERMs having this contractile cell (myofibroblast, fibroblast or both of them). This result explained why the CMT in OCT of the study eyes was higher than that in previous studies.

Inflammatory cells (macrophage, lymphocyte, neutrophil) were detected in 14 samples. When comparing the iERMs with inflammatory cell to the sign of retinal hard exudate, we found the significantly statistic correlation ($p < 0.05$, Chi-Square test). Hard exudative was lipoprotein structure that appeared intra retina as a consequence of increased retinal vascular permeability in inflammation. There were 22 eyes with retinal hard exudate with twelve iERMs having inflammatory cell, that may demonstrate the role of inflammation in iERMs mechanism. Some studies also proved the inflammation mechanism in pathophysiology of iERMs [6].

Number of cell types in a sample was different in studies, it ranged from 2 to 4 cell types in Bu S.C. research (2014), 2-3 cell types in Wang L.C. research (2015), 1-4 cell types in Marie Ueki [2], [19], [20]. This difference may be explained due to the method of histopathology. Most studies used HE staining to classified the type of cells and used immunohistochemistry to re-confirm. However, the identification of cells using immunohistopathology method depended on available specific marker and usually used to find out the cell origin. Some specific markers that were often used were glial fibrillary acidic protein (GFAP), vimentin, cellular retinaldehyde-binding protein (CRDLBP), Kir4.1, CD 45, CD 64, CD 168, pan cytokeratin, neurofilament [2], [6], [6], [17].

The cell types in iERM sample could be divided to 3 groups: the original glial cells, the secondary cells (fibroblast and myofibroblast) and others, and inflammatory cells. In our study, using HE staining images identification, there were 6 cell types and maximum 5 cell types in a sample. Looking for the association between histopathology results and some signs and symptoms, we only found the correlation between number cell type and symptom duration ($r = 0.47$, $p = 0.004$, Pearson's test), and CMT ($r = 0.72$, $p < 0.001$, Pearson's test).

The cell types in iERMs of the study were only identified by the cell morphology in HE staining, so it may not be very accurate in cell type classification and origin. The immunohistochemistry should be used to recognize exactly the origin and the type of cells.

In conclusion, our study found that the most frequent symptom of iERM was distortion and the CMT was $468.51 \pm 97.24 \mu\text{m}$ ($656\text{-}274 \mu\text{m}$). In histopathology result, the cell density was higher in dense group (> 10 cells/field $40 \times$). There were 6 different cell types, including glial cell (35/35), fibroblast (23/35), myofibroblast (23/35), macrophage (13/35), lymphocyte (5/35) and neutrophil (2/35). The number of cell types in a sample was from 1 to 5 and it correlated to CMT and symptom duration.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the institutional ethical review board of Vietnam National Institute of Ophthalmology.

Informed Consent

Informed consents were obtained from the patients included in the study.

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