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Intraoperative extraocular Indocyanine Green (IE-ICG) dye test: a new method of detecting a peeled internal limiting membrane

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ABSTRACT

Aims: To develop an intraoperative, extraocular Indocyanine Green dye staining test (IE-ICG) for the differentiation of a peeled ILM from a thin epiretinal membrane, and to evaluate its efficacy.

Methods: This was a consecutive observational case and laboratory observational series. We performed ILM peeling in patients with an idiopathic macular hole (MH, n = 10) and diabetic macular oedema (DME, n = 10) without vital dye staining such as ICG or Trypan Blue. We also performed membrane peeling in patients with an idiopathic epiretinal membrane (ERM, n = 10). Then, the peeled membranes were stained with ICG (1.25 mg/ml) beyond the operation field and examined under a light microscope. After this examination, membranes were fixed with glutaraldehyde, and an electron microscope was used to confirm whether they were ILMs or thin ERM. The concordance rates between surgeon's intraoperative impression of membranes (SI), IE-ICG results (IT) and histological findings (HF) of peeled membranes were evaluated to reveal the efficacy of IE-ICG.

Results: The ILMs were homogeneously stained with ICG dye (positive IE-ICG), and the ERMs were not stained at all by ICG dye (negative IE-ICG). The concordance rate between IT and HF was 100% in all three groups of patients. However, concordance rates between SI and IT were 100% in MH, 80% in DME and 50% in ERM, respectively. The surgeon's impression of the membrane is inaccurate, especially in patients with idiopathic epiretinal membrane.

Conclusion: Considering the cost, difficulties of tissue preparation, and the time-consuming process of histological confirmation of an ILM, IE-ICG may be a useful alternative for the differentiation of a peeled ILM and a thin ERM.

Though definite effects remain controversial, many surgeons are performing internal limiting membrane (ILM) peeling to improve surgical outcome in patients with an idiopathic macular hole, diabetic macular oedema or idiopathic epiretinal membrane.^{1–6}

ILM peeling is a challenging procedure due to its inborn thinness and transparency. To improve its visibility, a vital dye such as Indocyanine Green, or Trypan Blue has been used for facilitating ILM peeling.^{7–11} However, many reports suggest that vital dyes are possibly toxic when applied during macular surgeries.^{12–23} Recently, intravitreal triamcinolone acetonide (IVTA) was used as an alternative option of vital dye for ILM peeling,²⁴ while some other expert surgeons have performed ILM peeling without any enhancing materials such as ICG, Trypan Blue, or IVTA.^{18–25} In cases when ILM peeling is conducted with triamcinolone or without a vital dye, it is difficult to know whether the peeled membrane is the ILM or the thin epiretinal membrane. Surgeons

usually depend on a personal feeling or impression during the peeling procedure, or use some other supportive sign such as petechial haemorrhage on the macular surface, disappearance of light reflex on the macular area or the scrolling nature of the peeled membrane. However, the only way to confirm that a peeled membrane as the ILM is to perform a histological examination, but such a confirmation involves high costs and is a difficult, time-consuming procedure. Further, the histological examination requires fixation and embedding, and would not be available until after completion of the case. Thus, we have developed a new method, which we refer to as the intraoperative extraocular ICG dye test (IE-ICG), which provides a feasible alternative for differentiating the ILM from the thin epiretinal membrane. Moreover, the devised method is non-toxic, rapid and cheap. We also evaluated the efficacy of the IE-ICG in patients with an idiopathic macular hole, diabetic macular oedema or an idiopathic epiretinal membrane.

MATERIALS AND METHODS

Patients

Patients with an idiopathic macular hole (n = 10), diabetic macular oedema (n = 10) and idiopathic epiretinal membrane (n = 10) were enrolled in this study. All operations were performed by one experienced vitreoretinal surgeon (KHP). The surgical procedure was briefly as follows. In macular hole patients, standard three-port pars planar vitrectomy was performed. Posterior vitreous detachment (PVD) was induced with active suction of ocutome over the optic disc if the PVD is not already present. ILM peeling was performed using end-gripping Eckardt forceps using an intravitreal injection of triamcinolone to enhance ILM visibility in five patients. The other five patients received ILM peeling without any enhancing material—for example, ICG, Trypan Blue or triamcinolone acetonide. The presumed ILM was removed from the eye and the IE-ICG performed off the surgical field.

In diabetic macular oedema patients, vitrectomy was performed, and PVD was induced if not present. ILM peeling was performed as described above. In five patients, triamcinolone was used, and in the other five no enhancing material was used.

In patients with idiopathic ERM, a vitrectomy was performed using the procedure described above, and ERM peeling was performed using end-gripping Eckardt forceps. ILM peeling was not attempted. An IE-ICG dye test was performed to determine whether the membrane was the ERM or ILM.

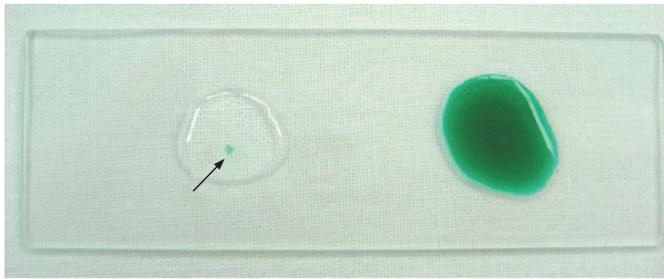


Figure 1 Intraoperative extraocular ICG (IE-ICG) dye test. The peeled membrane was dipped into a drop of ICG dye (1.25 mg/ml) on a slide glass for 30 s (right side of slide glass). The membrane was then suspended in a drop of BSS for examination under a light microscope (left side of the slide glass). A tiny ICG stained membrane was visible in a drop of BSS (arrow). If the peeled membrane is an ILM, the membrane is homogeneously stained by the green colour of the ICG dye (IE-ICG positive, fig 2A).

Intraoperative extraocular ICG dye test (IE-ICG)

The presumed ILM or ERM was removed as one piece from the eye and suspended in balanced salt solution (BSS). It was then dipped into a drop of ICG dye (1.25 mg/ml) on a slide glass for 30 s. Care must be taken to unfold the membrane with 30-gauge needle, if it is folded. This might improve exposure of membrane to the ICG dye and reduce the false negative results. The membrane was then resuspended in BSS and gently washed for 30 s. It was then suspended in a drop of BSS on a glass slide for examination under a light microscope (fig 1). When the peeled membrane was an ILM, the membrane was homogeneously stained with a green colour due to ICG dye (IE-ICG

positive; fig 2A). On the other hand, if it was an ERM, the membrane was not stained at all (IE-ICG negative, fig 2C).

Two experienced readers (PKH and KJH), masked to the subject information, interpreted the results of IE-ICG. Inter- and intraobserver variability was analysed by the κ statistic.²⁶ There was no intra-observer variability in the interpretation of IE-ICG. Inter-observer variability was observed only in the interpretation of membrane from ERM patients, and the κ value was 0.80. We also calculated the concordance rate between the surgeon's impression and IE-ICG results, and between the IE-ICG and histological findings in each group of patients.

Histological examination

After the IE-ICG, the peeled membranes were processed for histological examination to confirm their natures. Specimens were fixed in 4% phosphate-buffered glutaraldehyde solution and postfixed in osmium tetroxide. After dehydration in graded concentration of ethanol, membranes were embedded in Epon 812. For light-microscopic examination, a semithin section of 400 nm was stained with Toluidine Blue. An ultrathin section of 60 nm was contrasted with uranyl acetate and lead citrate for electron microscopy. Analysis and imaging were performed with an Olympus BX 51 light microscope (Olympus, Tokyo) and a Hitachi 7100 electron microscope (Hitachi, Tokyo).

RESULTS

All 30 patients were tested using the IE-ICG successfully without tissue loss. ICG dye mixed with distilled water had not shown any storage-related problems in performing the IE-ICG test until 1 month after make-up. We did not have any further

Figure 2 (A) Light-microscopic findings demonstrating positive IE-ICG dye test results ($\times 40$). If the peeled membrane is the ILM, the membrane is homogeneously stained with a green colour due to the ICG dye ("+" by the IE-ICG dye test). (B) Electron-microscopic findings of the same membrane showing a homogenous structure without a cellular component, proving that the membrane is in fact an ILM ($\times 4000$, bar = 8.75 μm). (C) Light microscopic finding demonstrates negative results by the IE-ICG dye test ($\times 40$). If the membrane is an ERM, it is not stained by ICG dye ("–" IE-ICG dye test). (D) Electron-microscopic finding of the same membrane showing irregularly intermingled collagen and attached cells adjacent to the membrane proving that the membrane is an ERM ($\times 7000$, bar = 5.0 μm). (E) Light-microscopic finding of an ERM obtained by epiretinal membrane peeling ($\times 40$). Removed membranes were observed to have two components; one was not stained with ICG dye (indicating that it was an ERM), while the other was stained by ICG dye (arrow) indicating that it was ILM. (F) Electron-microscopic findings of the same membrane. The two components of the adherent membrane were resolved. The upper part was an ERM (arrowhead) and the lower more homogenous membrane an ILM (arrow) ($\times 9000$, bar = 3.89 μm).

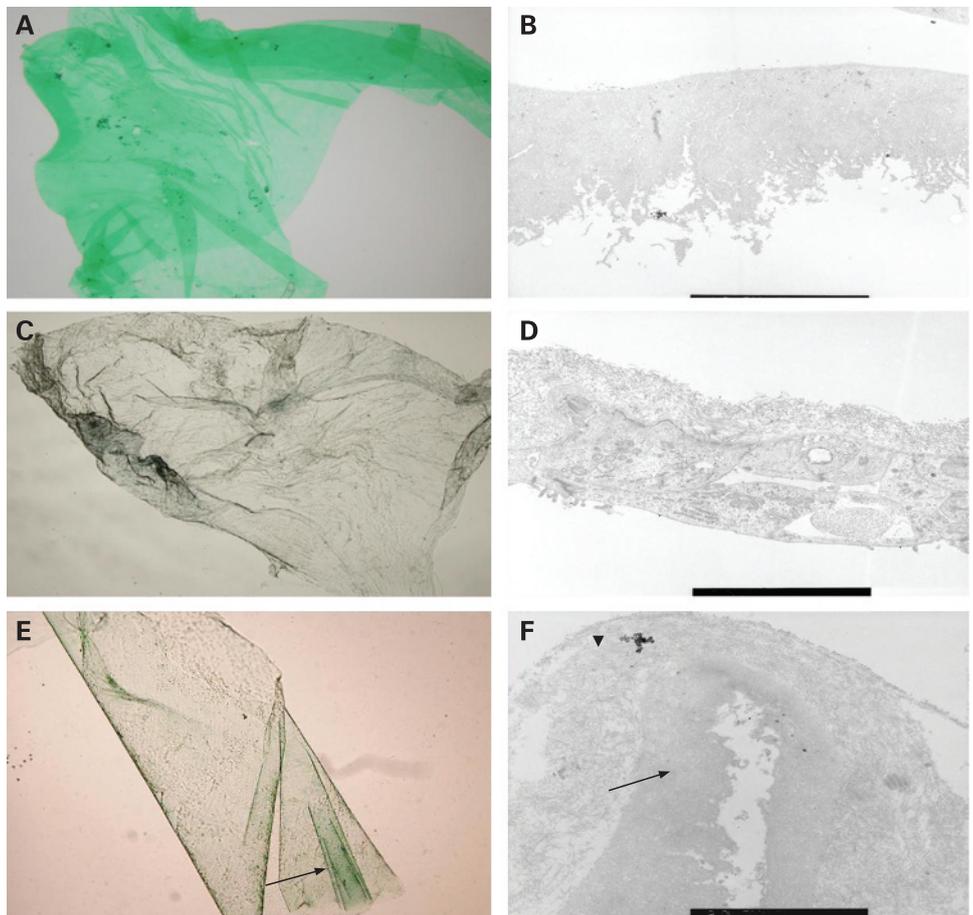


Table 1 Demographics, visual outcome and test results of the patients suffering from an idiopathic macular hole, diabetic macular oedema or idiopathic epiretinal membrane

Patient no.	Sex/age	Lat.	Diagnosis	Preop. VA*	Postop. VA*	Staining for membrane	Surgeon's impression	IE-ICG	Histology
1	M/73	L	MH	20/200	20/25	TA	ILM	ILM	ILM
2	F/53	R	MH	20/400	20/50	TA	ILM	ILM	ILM
3	M/60	R	MH	20/100	20/25	TA	ILM	ILM	ILM
4	M/62	L	MH	20/200	20/40	TA	ILM	ILM	ILM
5	F/58	R	MH	20/200	20/100	TA	ILM	ILM	ILM
6	F/74	R	MH	20/100	20/50	No	ILM	ILM	ILM
7	F/60	R	MH	20/400	20/50	No	ILM	ILM	ILM
8	F/69	R	MH	20/70	20/25	No	ILM	ILM	ILM
9	M/61	R	MH	20/100	20/40	No	ILM	ILM	ILM
10	F/59	L	MH	20/200	20/25	No	ILM	ILM	ILM
11	F/68	L	DME	20/70	20/50	TA	ILM	ILM	ILM
12	M/44	L	DME	20/200	20/40	TA	ILM	ILM	ILM
13	M/59	L	DME	20/400	20/200	TA	ILM	ILM	ILM
14	M/61	R	DME	20/100	20/40	TA	ILM	ILM	ILM
15	F/64	L	DME	20/200	20/50	TA	ILM	ERM	ERM
16	F/77	L	DME	20/200	20/100	No	ILM	ILM	ILM
17	F/50	R	DME	20/100	20/25	No	ILM	ILM	ILM
18	F/73	R	DME	20/100	20/70	No	ILM	ILM	ILM
19	F/66	R	DME	20/200	20/200	No	ILM	ILM	ILM
20	M/65	R	DME	20/200	20/200	No	ILM	ERM	ERM
21	F/68	R	ERM	20/70	20/40	No	ERM	ERM	ERM
22	F/68	L	ERM	20/100	20/40	No	ERM	ERM	ERM
23	F/73	L	ERM	20/100	20/30	No	ERM	ERM	ERM
24	F/66	L	ERM	20/70	20/40	No	ERM	ERM	ERM
25	F/60	R	ERM	20/70	20/25	No	ERM	ERM	ERM
26	M/74	R	ERM	20/40	20/30	No	ERM	ERM/ILM	ERM/ILM
27	M/53	L	ERM	20/50	20/40	No	ERM	ERM/ILM	ERM/ILM
28	F/61	R	ERM	20/40	20/40	No	ERM	ERM/ILM	ERM/ILM
29	F/60	L	ERM	20/50	20/25	No	ERM	ERM/ILM	ERM/ILM
30	F/76	R	ERM	20/200	20/70	No	ERM	ERM/ILM	ERM/ILM

*Visual acuity was measured using a Snellen chart.

DME, diabetic macular oedema; ERM, epiretinal membrane; F, female; ILM, internal limiting membrane; L, left eye; M, male; MH, idiopathic macular hole; No, no enhancing material for ILM peeling; R, right eye; TA, intravitreal triamcinolone acetonide for ILM peeling.

information concerning the longevity of ICG dye in storage, because that was not the aim of this study.

Ten of the 10 patients (100%) who underwent macular hole surgery with ILM peeling had a positive IE-ICG. It showed homogeneous greenish staining of membrane with ICG dye and was confirmed from the EM study as ILM (fig 2A, B). Eight of the 10 patients with diabetic macular oedema (80%) who underwent vitrectomy with ILM peeling had a positive IE-ICG. The other two presumed ILMs were negative by the IE-ICG, indicating that they were not ILMs but rather thin posterior hyaloid membranes (fig 2C) and confirmed as ERM by histological study (fig 2D). We carried out an additional ILM peeling procedure with the triamcinolone acetonide. Five of the 10 patients with idiopathic ERM who underwent ERM peeling also had a negative IE-ICG, and the other five had a mixed positive and negative, indicating that the ILM was inadvertently peeled during the membrane peeling procedure (fig 2E, F). In ERM patients, we did not perform a further procedure because we did not intend to peel the ILM. The concordance rates between the surgeon's impression and the IE-ICG results were 100% for macular hole surgery, 80% for diabetic macular oedema surgery and 50% for ERM surgery. However, the concordance rate between the IE-ICG and histological examination was 100% in all three groups (table 1).

These results showed that a surgeon's impressions related to ILM and ERM peeling are inaccurate in diabetic macular oedema and ERM patients. They also suggest that IE-ICG is as accurate

as histological examination in terms of discriminating ILM from ERM.

DISCUSSION

Considerations of the possible toxicity of vital dye during the ILM peeling procedure have encouraged some surgeons to perform ILM peeling without vital dye staining or with the use of intravitreal triamcinolone.^{18 25} In these situations, surgeons depend on their own feelings and impressions to determine the nature of a peeled membrane. However, this method is quite subjective and always has the possibility of introducing substantial errors. Actually, our findings revealed that a surgeon's impressions during the surgical procedure are likely to be accurate in patients with macular hole, which means IE-ICG may be unnecessary in patients with an idiopathic macular hole. However, more complicated cases such as a macular hole with retinal detachment in patients with high myopia showed a multiplicity of epiretinal components of peeled membrane.²⁷ The IE-ICG may be helpful in revealing the nature of a peeled membrane in these more complicated cases of macular hole patients. The surgeon's impression is somewhat inaccurate in patients with diabetic macular oedema, whereas in patients with an epiretinal membrane, the present study shows that there is a 50% chance of misdiagnosing the peeled membrane, which demonstrates that a surgeon's impression of the nature of a peeled membrane is inaccurate in patients with diabetic

macular oedema and an epiretinal membrane. Thus, if surgeons want to guarantee an ILM peeling in these patients, they should use vital dye or some other method utilised to determine whether a peeled membrane is in fact the ILM.

Histological confirmation is currently the only method capable of confirming the nature of a peeled membrane, but it is not a straightforward method and almost impossible for every peeled membrane. Therefore, a more feasible, less expensive method that can reveal the nature of a peeled membrane and be performed during the surgical procedure is required. It was already known that ICG selectively stains the internal limiting membrane.²⁸ However, the usage of ICG as adjunctive during surgical procedures is limited to intraocular injections intended to enhance ILM visibility. As yet, no one has attempted ICG staining of a peeled membrane outside the eyeball as a ready means of identifying the membrane type.

Therefore, we developed a new feasible method for differentiating the ILM from thin epiretinal membrane by dyeing removed membrane outside the operation field with ICG during operations. The described IE-ICG needs only a few minutes to produce a definitive result and can be performed in parallel with the surgical procedure, and so it may provide information as to whether an additional procedure is necessary or not. The devised test is cheap—it requires only a drop of ICG dye—and it does not require processes like fixation, embedding or tissue sectioning. Moreover, it is free of toxicity concerns because all procedures are performed outside the operation fields. In addition to these advantages, its sensitivity and specificity were found to be 100% versus histological findings irrespective of the disease groups. This means that the devised test is both feasible and highly accurate.

However, several concerns remain. First is the need for IE-ICG. The precise nature of the peeled membrane may not be crucial for the results of macular surgeries. One important consideration may not be whether the peeled membrane is ILM or not, but how to peel it to the intended amount while minimising trauma to the macula. Therefore, some may underestimate the necessity of this test. However, in the absence of a test like the IE-ICG, it is difficult to address questions such as, “How do you know the membrane you peeled without vital dye is the ILM?” without resorting to histological results. So, even though the clinical usefulness of IE-ICG may be limited in retinal surgeons, its academic value is still sufficient to sustain this test as a feasible alternative for histological examination. Second, the concordance rate between surgeons’ impressions of membrane peeling without any vital dyes and IE-ICG results may depend on surgeons’ experience. As a beginner, the concordance rate may be low, and it may be high in experts. It is possible that the IE-ICG could be used by surgeons to hone their intraoperative impression concerning the natures of the peeled membrane through trial-and-error basis learning. Third, inter-observer variability was observed in interpretation of membrane from ERM patients. However, this discrepancy was eliminated with further education and self-learning gained from the results of histological examination.

In conclusion, we describe a new test, which we refer to as the IE-ICG. Because of its negligible cost, ease of tissue preparation and rapidity versus histological confirmation, we believe that the IE-ICG will be found to be a useful ready alternative for the differentiation of peeled ILMs and thin epiretinal membranes.

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Competing interests: None declared.

Ethics approval: Institutional Ethics Committee approval was granted for the study.

Patient consent: Informed patient consent was obtained before surgery.

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