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TRANSMISSION ELECTRON MICROSCOPY OF THE VITREOMACULAR BORDER IN CLINICALLY SIGNIFICANT DIABETIC MACULAR OEDEMA

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Abstract

Diabetic cystoid macular oedema (DME) is a common cause of visual acuity decrease. Good anatomical results and visual acuity (VA) of pars plana vitrectomy (PPV) in a case of a macular hole with internal limiting membrane peeling leads to the use of this technique in DME. A favourable result even in a case without vitreoretinal traction leads to the conclusion that pathogenesis of this disease is different.

We analysed retrospectively 20 eyes from 20 patients with DME that had undergone PPV and peeling ILM. Half of them were laser treated before surgery. All eyes had an attached posterior hyaloid membrane in the macular region, but without thickening and without traction. We examined parts of excised tissues by transmission electron microscopy (TEM). Median duration of DME at the time of PPV was approximately 18.0 months (range 12 – 24 months). The median preoperative best corrected VA of 0.4 (range 0, 01–1, 0), improved to a median postoperative VA of 0.55 (range 0, 01–1, 0). Ten eyes without preoperative laser coagulation had a median VA improvement of 112 %, while 10 eyes with preoperative focal macular laser treatment had a median VA improvement of 17 %. In all 20 eyes, DME was no longer visible on microscopic examination after a median period of 3.0 months after PPV. We classified TEM samples containing ILM, glial cells and connective tissue as monolayer membrane, multilayer membrane, and true epimacular fibrous membrane.

PPV and peeling ILM resulted in the resolution of oedema, with an improvement in visual acuity in the majority of cases. Eyes without preoperative macular photocoagulation had a significantly higher visual improvement than eyes with preoperative laser treatment, but PPV had an additive effect on final visual acuity in focal laser treated eyes. A randomised controlled prospective trial of PPV versus laser is needed to determine the role of PPV as a treatment modality for DME.

Key words

Clinically significant diabetic macular oedema, Transmission electron microscopy

Abbreviations used

DME, diabetic cystoid macular oedema; VA, visual acuity; PPV, pars plana vitrectomy; ILM. internal limiting membrane; TEM, transmission electron microscopy

INTRODUCTION

Macular oedema is present in various general and ophthalmological disorders and it is the most frequent cause of decreased vision acuity. It is also encountered as a complication of diabetic retinopathy. Its aetiology is complex. Impairment of the haematoretinal barrier and impaired blood supply to the retina are included among the causes. Another possible cause is also an early ageing of the vitreous body in diabetic patients, which is manifested by morphological and biochemical changes. Migration of glial and epithelial cells on the vitreoretinal border with the production of epiretinal membranes in the central area, which is morphologically manifested as a cystoid macular oedema, was described. It need not always be vitreomacular traction, however. Ischaemia of the central region of the retina (*1–2*) results in the production of humoral substances such as the vascular endothelial growth factor (VEGF) and interleukin (IL-6), (IL-8). Observations of some authors (*3*), who report occurrence of macular oedema in diabetic patients without obvious traction, as well as the possibility of spontaneous healing after detachment of the posterior vitreous body also support this version. Pars plana vitrectomy enabled a morphological analysis of the vitreomacular border and it is the subject of this study.

MATERIALS AND METHODS

In 2005 we performed posterior vitrectomy in 170 patients with the complication of diabetic retinopathy. Out of this number, the surgery was indicated 40 times for haemophthalmus, 100 times for proliferative diabetic retinopathy with traction detachment of the retina, which usually required a longer period of retinal tamponade with silicon oil as well, 20 times it was clinically significant chronic macular oedema (CSME), and 10 times a combination of the aforementioned pathologies. For the purpose of this paper, we dealt with the patients with macular oedema. We divided the patients into two groups: 10 patients were treated with focal laser coagulation at least 6 months and more prior to surgery and 10 patients with the same pathology, in whom laser coagulation of the central region or panretinal laser coagulation was not performed. Age distribution in both groups was similar. All patients signed the informed consent prior to the procedure. The diagnosis of CSME was confirmed by biomicroscopy and OCT examination. No patient had a vitreoretinal macular traction. All cases suffered from type 2 diabetes mellitus. The patients were treated with pars plana vitrectomy and, where possible, with peeling of the internal limiting membrane (ILM). A 100% removal of ILM was only possible in the group of patients who did not undergo focal laser coagulation; in the patients after laser coagulation ILM was removed in individual segments. Approval for clinical studies was obtained from the Faculty of Medicine Ethical Committee.

Preparations of TEM samples

The collected epiretinal tissue was processed for examination with transmission electron microscopy. The tissue specimens were fixed in 2.5% glutaraldehyde solution in phosphate buffer for 3 hours and post-fixated in 2% solution of osmium tetroxide. After dehydration in rising ethyl alcohol concentration, the tissue specimens were embedded into Durcupan ACM Fluca. Thin and ultrathin sections were cut with the ultramicrotome Reichert, Jung Ultacut E. After fixation with uranyl acetate and lead citrate, the ultrathin sections were examined and photographed with an FEI Morgagni transmission electron microscope.

RESULTS

The median of macular oedema at the time of PPV was 18 months (range 12–24). The patients' characteristics are shown in *Table 1*, the clinical data about pars plana vitrectomy group in *Table 2*, and the data about the focal laser treated group and pars plana vitrectomy in *Table 3*. After pars plana vitrectomy, there was a significant change in visual acuity in both groups (*Graph 1*). The thickness of the retina went below 200 μm in all patients. In patients with previous focal laser coagulation, pars plana vitrectomy had a positive additive effect.

In the collected material, we found an internal limiting membrane with fragments of collagen vitreous fibres (*Fig. 1*); in some samples there were glial cells and a fibrous membrane, which can be classified as a simple one-layer (*Fig. 2*), in more advanced stages of macular oedema we determined a genuine multilayer epimacular membrane (*Fig.3*). Microscopic examination of the samples showed no differences between both groups of the patients. Morphological pictures depend more on clinical progress. Throughout an 18-month period we observed flattening of the retina and disappearance of cystic changes in the macula in all patients. The ophthalmological finding was always stabilised.

DISCUSSION

Kishi and Shimizu (*4*) described the morphology of premacular vitreous body for the first time. *Gandorfer et al. (5)* properly analysed samples of the vitreoretinal border in patients with diabetic retinopathy and chronic macular oedema and found that in diabetic patients, even in case of vitreous body detachment, there are collagen fibres bound to ILM in the premacular region. They expressed a theory that ablation of the posterior vitreous body does not occur on the level of ILM. Our results support this theory. It seems that for a successful result of the operation it is not necessary to also remove ILM. This conclusion is supported by the regression of macular oedema also in those patients in whom it was not possible to remove ILM completely during the operation. The same opinion has also been held by many vitreoretinal surgeons. Focal laser coagulation in CSME is a proved standard according to an EDTRS multicentre study. Considering that the own collagen of the vitreous body has no contractile abilities, attention is paid mainly to cells in which contractile fibres were proved. Participation of the cell membrane together with pathological collagen fibres is necessary for the occurrence of tangential traction of the macular retina. In our material, we did not find any continuous cell membranes and a corresponding OCT finding. This would support the above-mentioned humoral and ischaemic theory. The same conclusion was expressed by *Gandorfer et al.(5)*. The effect of other factors besides the mechanical traction of the epiretinal tissue may be assumed in the aetiology of diabetic macular oedema. *Funatsu et al. (6, 7, 8*) and *Aiello et al. (9*) independently expressed a hypothesis that in the vitreous body of diabetic patients there are humoral substances such as the

Table 1
Group characteristics

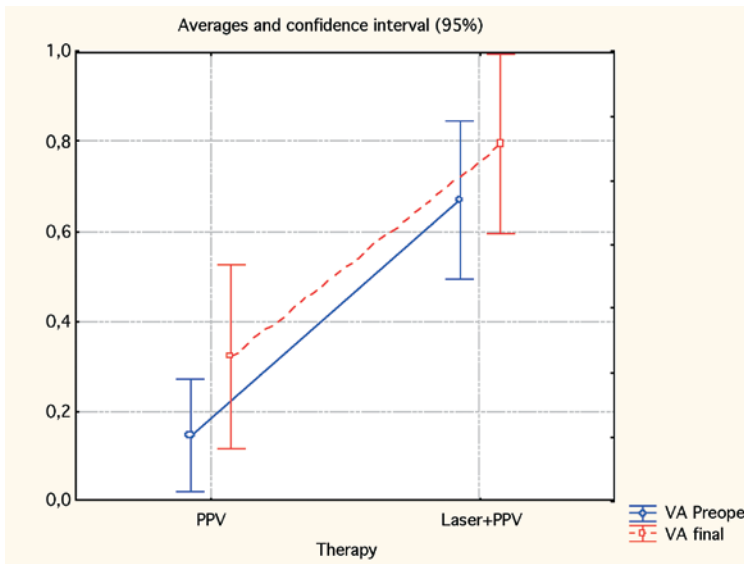
Characteristic	*Laser group*	*PPV group*
Male:female ratio	7:3	2:8
Age		
mean±SD	59.7±11.3	66.4±8.8
range	36–76	53–76

Table 2
Clinical data about PPV group only

Pacient	Gender	Age	V before	V after operaci	OCT	Folow up/years	Complication
1	M	58	5/15	1/50	650	2	Prolif.DR,
2	Ž	64	5/7,5	6/6	450	2	Cataract
3	M	61	6/36	6/9	480	2	Cataract
4	Ž	74	1/50	5/15	650	3	Prolif.DR
5	Ž	75	5/30	5/30	500	2	
6	Ž	72	5/30	5/15	480	2	
7	Ž	53	2,5m	2,5m	620	2	Cystoid macular edema
8	Ž	76	6/60	6/60	540		
9	Ž	55	5/50	5/15	580	5	
10	Ž	60	Pohyb	5/30	650	2	
11	M	74	1m	6/36	570	2	Prolif. DR
12	Ž	75	5/30	5/30	480	2	

Table 3
Clinical data about PPV and laser group

Pacient	Gender	Age	V before treatmett	V after treatment	OCT	Sledování r.	Komplikace
1	M	60	5/7,5	5/5	450	6	Cataract
2	M	50	5/5	5/5	500	2	
3	M	57	5/15	5/20	630	3	Cataract
4	M	60	6/9	6/12	600	2	
5	Ž	68	5/10;5/15	5/7,5;5/10	580	2	
6	Ž	76	5/10;5/10	5/15;5/15	580	2	
7	Ž	72	5/10;5/10	5/15;5/15	600	1	Glaucoma
8	M	36	5/5;5/7,5	5/7,5;5/15	580	2	Strabismus
9	M	57	5/7,5	5/5	450	2	
10	M	61	5/5;5/5	5/5;5/5	400	2	
11	M	42	5/5;5/5	5/5;5/5	420	5	
12	M	40	5/10;5/10	5/7,5;5/10	500	1	



Graph 1
Graph of visual acuity pre- and post-surgery

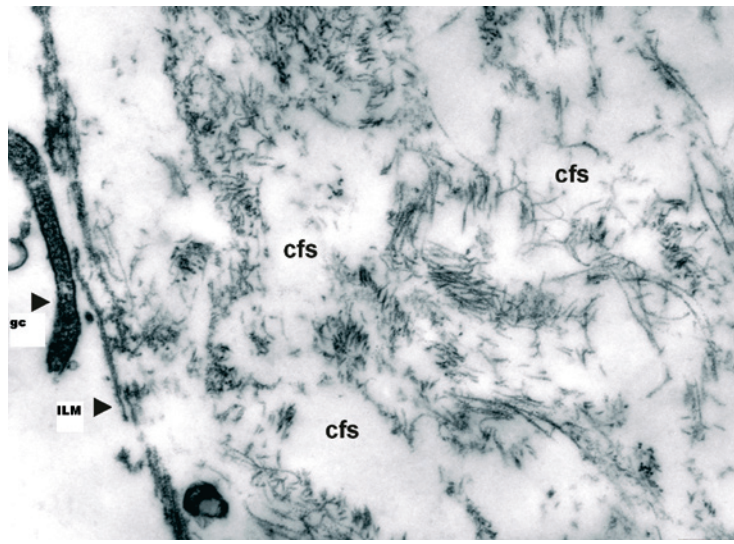


Fig. 1
Electronogram of the vitreoretinal border of patient No. 1 (PPV group).
cfs – collagenous microfibrils of corpus vitreum, gc ►(arrowhead) – glial cell,
ILM ►(arrowhead) – internal limiting membrane. Magnification approx. 24,000x

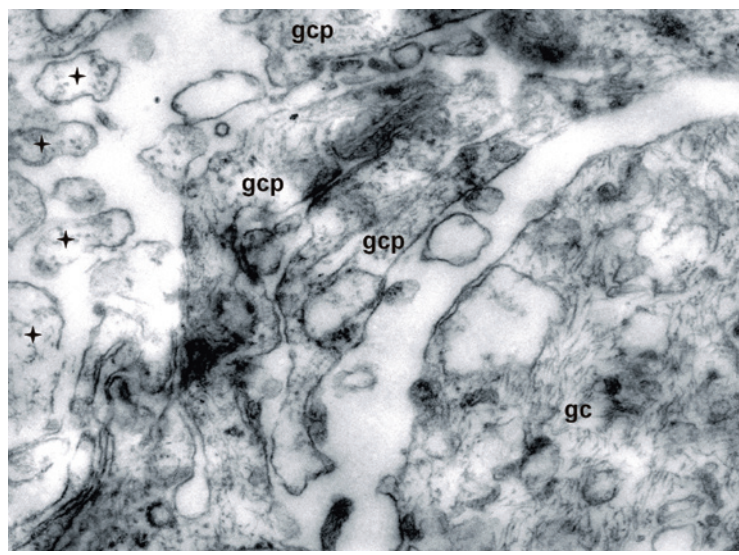


Fig. 2
Electronogram of the vitreoretinal border of patient No. 1 (Laser + PPV group).
Monolayer epimacular membrane. gc – glial cell, gcp – glial cell process.
Magnification approx. 33,000x

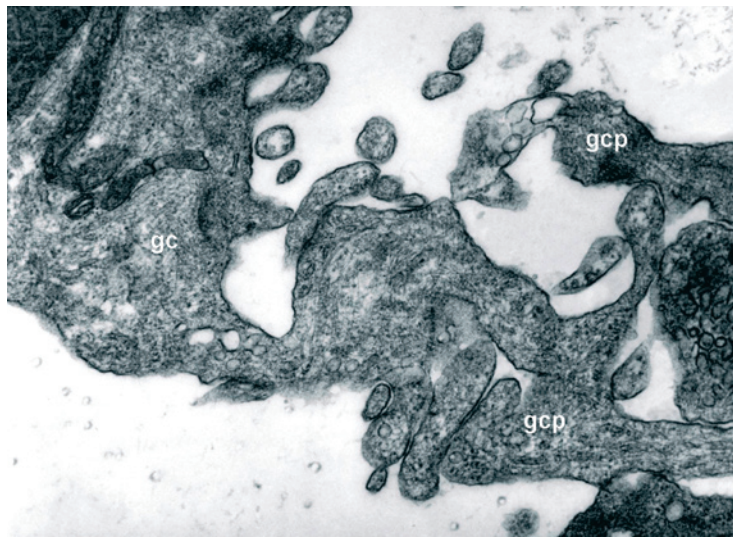


Fig. 3
Electronogram of the vitreoretinal border of No 3. (PPV group). Multilayer epimacular membrane. gcp – glial cell process, * - glial cell process, transversal section. Magnification approx. 60,000x

vascular endothelial growth factor (VEGF) and interleukin 6, produced in the cells of epiretinal membranes, which impair the haematoocular barrier and contribute to the development of macular oedema. Regression of macular oedema after pars plana vitrectomy may also be explained by a decrease or removal of the above factors by vitrectomy (*10*). This hypothesis is also supported by the gradual postoperation regression of CSME. The structure of the vitreomacular border in CSME may be characterised as:

ILM, which is covered with vitreous fibres;

cellular elements such as fibroblasts and fibrous astrocytes in the context of the vitreous body;

one or multi-layer cell membranes.

The efficiency of epimacular tissue removal and the pathogenesis of macular oedema is still a subject of discussions (*11*). Theoretically it can be assumed that PPV results in loosening of the traction forces and removal of factors increasing permeability. The benefit may also be an improved supply of the macula with oxygen and nutritive substances. Peeling ILM furthermore facilitates removal of the epimacular pathological tissue and the core of the vitreous body.

Pars plana vitrectomy and peeling of the posterior limiting membrane in diabetic retinopathy without obvious traction may result in regression of the clinically significant cystoid macular oedema and improvement of visual acuity. There will be

necessary follow-up randomised studies of the efficiency of simple PPV with peeling of the membrane compared with the therapy using focal macular laser coagulation (*12*).

CONCLUSIONS

PPV and peeling ILM resulted in the resolution of oedema, with an improvement in visual acuity in the majority of cases. Eyes without preoperative macular photocoagulation had a significantly higher visual improvement than eyes with preoperative laser treatment, but PPV had an additive effect on the final visual acuity of focal laser treated eyes. A randomised controlled prospective trial of PPV versus laser is needed to determine the role of PPV as a treatment modality for DME.

A c k n o w l e d g e m e n t

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