

EXTENDED REPORT

Risk evaluation of outcome of vitreous surgery for proliferative diabetic retinopathy based on vitreous level of vascular endothelial growth factor and angiotensin II

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Aims: To ascertain whether measurement of the vitreous fluid levels of vascular endothelial growth factor (VEGF) or angiotensin II (Ang II) could predict the outcome of vitreous surgery in patients with proliferative diabetic retinopathy (PDR).

Methods: A prospective observational case study was performed in 61 consecutive patients (61 eyes) with PDR who underwent vitreoretinal surgery. Vitreous fluid samples were obtained during surgery. The VEGF level in vitreous fluid and plasma was determined by enzyme linked immunosorbent assay, while the Ang II level was measured by radioimmunoassay. Patients were prospectively followed for 6 months and the postoperative outcome was analysed by logistic regression analysis.

Results: No improvement and/or progression of PDR was seen in 15 (25%) of the 61 eyes. Vitreous levels of VEGF and Ang II were significantly higher in eyes with progression of PDR than in eyes with regression of PDR ($p=0.0044$, and $p=0.0178$, respectively). Multivariate logistic regression analysis showed that the vitreous VEGF level increased along with the progression of PDR after vitreous surgery (odds ratio 2.48, $p=0.0008$).

Conclusion: A high vitreous fluid VEGF level is associated with a significant risk of postoperative progression of PDR. The vitreous level of VEGF at the time of surgery may be a useful predictor of the outcome.

The outcome of vitreous surgery for proliferative diabetic retinopathy (PDR) has improved during the past decade because the instruments and the surgical technique have been refined.¹ The initial indications and rationale for vitreous surgery were largely established on the basis of fundus findings such as new vessels elsewhere (NVE), new vessels on or within 1 disc diameter of the disc (NVD), fibrous proliferation elsewhere (FPE), vitreous haemorrhage, and tractional retinal detachment.¹ However, it now seems to be necessary to predict the outcome of vitreous surgery, because extensive neovascularisation can occur after surgery for PDR.

Recently, various cytokines have been suggested to play a part in the pathogenesis and progression of diabetic retinopathy. To assess the clinical significance of monitoring cytokines or growth factors, such as vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), and endostatin, we have been investigating the correlations between these peptides and the progression of PDR and/or diabetic macular oedema (DMO).^{2–7} We previously reported that both the level of angiotensin II (Ang II), a vasoactive factor, and that of VEGF, a stimulator of angiogenesis, were significantly higher in the vitreous fluid of patients with PDR than in that of non-diabetic patients or diabetic patients without retinopathy, and that the levels of both Ang II and VEGF were elevated in the active stage of PDR compared with in the quiescent stage.⁸ These findings suggest that Ang II may contribute to the development and progression of PDR together with VEGF.

In an attempt to predict the prognosis of PDR patients undergoing vitreous surgery, we investigated whether the vitreous levels of Ang II and VEGF were correlated with the outcome—that is, whether it was possible to predict the outcome of surgery by measuring Ang II and VEGF in

vitreous fluid obtained at operation, along with assessment of fundus findings.

PATIENTS AND METHODS

Patients

Undiluted vitreous fluid samples were harvested at the start of vitrectomy after informed consent was obtained from each subject following explanation of the purpose and potential adverse effects of the procedure. This study was performed in accordance with the Helsinki Declaration of 1975 (the 1983 revision), and the institutional review board of Tokyo Women's Medical University also approved the protocol for collection of vitreous fluid and blood samples. Vitreous fluid samples were obtained from 61 patients with PDR. Vitrectomy was performed to treat vitreous haemorrhage and/or preretinal haemorrhage in 33 patients, while it was done for tractional retinal detachment associated with fibrous proliferation in 28 patients.

Pars plana vitrectomy was done by a standard technique using three pars plana sclerotomy incisions. Undiluted samples of vitreous fluid (0.3–0.7 ml) were aspirated under standardised conditions from directly in the mid-vitreous at the beginning of surgery and were immediately transferred to sterile tubes. After collection of the vitreous fluid samples, the vitreous was removed as far as its base, followed by segmentation and delamination of proliferative membranes, removal of the posterior vitreous surface, and panretinal

Abbreviations: ACE, angiotensin converting enzyme; Ang II, angiotensin II; CV, coefficient of variation; ELISA, enzyme linked immunosorbent assay; FPE, fibrous proliferation elsewhere; NVD, new vessels on or within 1 disc diameter of the disc; NVE, new vessels elsewhere; PDR, proliferative diabetic retinopathy; RIA, radioimmunoassay; TRD, tractional retinal detachment; VEGF, vascular endothelial growth factor

Table 1 Clinical and laboratory characteristics of the patients

Characteristic	No* (n = 61)	Value
Age (years)	61	62.4 (9.6)†
Sex		
Male	35	57.4%
Female	26	42.6%
Duration of diabetes (years)	61	14.8 (7.3)†
Treatment:		
Diet	6	9.8%
Oral hypoglycaemic agents	33	54.1%
Insulin	22	36.1%
HbA _{1c} (%)	61	7.6 (2.2)†
Plasma VEGF concentration (pg/ml)	61	64.2 (30.7)†
Plasma Ang II concentration (pg/ml)	61	12.9 (7.5)†
Vitreous VEGF concentration (pg/ml)	61	1261.5 (1094.4)†
Vitreous Ang II concentration (pg/ml)	61	21.8 (16.2)†

*Numbers show the number of patients with available data. †Mean (SD).
 HbA_{1c} = haemoglobin A_{1c}; VEGF = vascular endothelial growth factor; Ang II = angiotensin II.

endolaser coagulation of the retina up to the ora serrata. Retinal detachment was treated with gas tamponade (25% SF₆) at the end of vitreous surgery. All operations were performed at Tokyo Women’s Medical University Hospital.

Exclusion criteria were: (1) treatment with an angiotensin converting enzyme (ACE) inhibitor or an Ang II type 1 (AT₁) receptor blocker (ARB), (2) previous ocular surgery, (3) a history of ocular inflammation, (4) retinal detachment associated with a retinal tear, and (5) rubeosis iridis.

Fundus findings

Preoperative, intraoperative, and postoperative fundus findings were recorded for each subject. The severity of diabetic retinopathy was assessed by standardised fundus colour photography and fluorescein angiography, and a preset lens with a slit lamp at 1 day before and 6 months after vitreous surgery.⁸⁻¹⁰ The severity of NVE, NVD, FPE, vitreous haemorrhage, retinal detachment, and diabetic retinopathy was graded according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS) system.^{8 11 12} The changes of fundus findings were assessed using the ETDRS severity score. The severity of fundus findings was graded in each photograph and the average severity was calculated. Fundus findings and the severity of retinopathy were compared between before and 6 months after vitreous surgery. The extent of retinal photocoagulation was classified using three grades: grade 0 was no photocoagulation, grade 1 was focal photocoagulation (defined as less than 1200 shots), and grade 2 was panretinal photocoagulation (defined as 1200 shots or more to the whole retina).

Sample collection

Samples of vitreous fluid were collected into sterile tubes at the time of vitreoretinal surgery and were rapidly frozen at -80°C. Blood samples were also collected from 61 patients. The blood was immediately placed on ice and centrifuged at 3000 × g for 5 minutes at 4°C, after which the separated plasma was rapidly frozen and stored at -80°C until assay.

Measurement of VEGF and Ang II

VEGF and Ang II were measured in the vitreous samples from all eyes as well as in the plasma samples. The VEGF concentration was measured by an enzyme linked immunosorbent assay (ELISA) for human VEGF (R&D System, Minneapolis, MN, USA) according to the manufacturer’s instructions, and the details have been reported previously.⁸⁻¹⁰ The VEGF levels in vitreous fluid and plasma were within the detection range of the assay, since the minimum detectable concentration was 15.6 pg/ml (intra-assay coefficient of variation (CV): 3.5% and interassay CV: 5.8%).

Vitreous and plasma Ang II levels were determined by radioimmunoassay (RIA) according to the manufacturers’ instructions and the details have been reported previously.⁸⁻¹⁰ The Ang II levels in vitreous fluid and plasma were within the detection range of these assays, since the minimum detectable concentration was 4.0 pg/ml for Ang II (intra-assay CV: 3.8% and interassay CV: 6.0%).

Statistical analysis

Analyses were performed with SAS System 8e software (SAS Institute Inc, Cary, NC, USA).¹³ Results are presented as the mean (SD) or as the geometric mean (SD) for logarithmic data. To identify independent predictors of the progression of PDR, univariate and multivariate logistic regression analyses were performed by the best subset variables selection method. To assess the relation between each factor tested and the severity of retinopathy, Spearman’s rank order correlation coefficients were calculated. Odds ratios were calculated using a logistic regression model with three dummy variables. A probability value (two tailed) of less than 0.05 was considered to indicate statistical significance.

RESULTS

Baseline patient profile

Complete data were available for 61 out of 67 patients, while six patients were lost to follow up because of transfer to another hospital or non-attendance at our outpatient clinic. Of the 61 remaining patients, 35 were men and 26 were women (table 1). Their mean age was 62.4 years (range 39–78 years), the mean duration of diabetes was 14.8 years (range 2–30 years), and the mean HbA_{1c} level was 7.6% (range 5.0–10.6%). These 61 patients were all followed for at least 6 months. The baseline PDR grade was level 71 in 20 eyes, level 75 in 13 eyes, level 81 in 17 eyes, and level 85 in 11 eyes (table 2). Panretinal photocoagulation had been performed before vitreous surgery in 37 patients and focal retinal photocoagulation had been done in 19 patients, while five patients had not undergone preoperative retinal photocoagulation.

Outcome of vitreous surgery

At the end of follow up, PDR showed no improvement and/or progression by one level or more in 15 patients (25%), while there was regression by one level or more in 46 patients (75%) (table 2). Forty two patients noted a significant improvement of visual acuity after surgery and 49 patients (80%) achieved a visual acuity of 20/200 or better. Among the remaining 12 patients (20%) with worsening of their visual acuity, four had diabetic macular oedema, four had vitreous haemorrhage, and four had macular atrophy. Intraoperative

Table 2 Severity of diabetic retinopathy at baseline and after 6 months

Severity at baseline*	Severity after 6 months*						Total
	47	53	61	71	81	85	
71	2	4	6	7	1	0	20
75	3	4	3	0	2	1	13
81	0	5	6	3	3	0	17
85	1	3	3	2	1	1	11
Total	6	16	18	12	7	2	61

*ETDRS retinopathy severity score.

retinal photocoagulation was done to a total of 1106.8 shots in patients who were photocoagulation grade 0 preoperatively, while patients who were grade 1 preoperatively received 721.6 shots and grade 2 patients received 236.5 shots. Three out of five patients (60.0%) who were grade 0 preoperatively showed progression versus 6 out of 19 (31.6%) and six out of 37 (16.2%) who were grade 1 and grade 2, respectively.

Vitreous and plasma levels of VEGF and Ang II

The vitreous fluid concentrations of VEGF and Ang II were significantly correlated with the baseline extent of retinal photocoagulation (VEGF; $\rho = -0.537$, $p = 0.0002$; Ang II; $\rho = -0.458$, $p = 0.0092$).

The vitreous fluid level of VEGF was significantly higher in patients who showed no improvement and/or progression of PDR (1845.6 pg/ml (224.3–3527.1)) when compared with the level in patients showing regression of PDR (791.2 pg/ml (75.6–2144.4)) ($p = 0.0044$). The vitreous fluid level of Ang II was also significantly higher in patients showing no improvement and/or progression of PDR (35.8 pg/ml (4.0–80.1)) than in patients with regression of PDR (9.7 pg/ml (4.0–22.1)) ($p = 0.0178$). On the other hand, there was no significant difference of plasma VEGF levels between no improvement and/or progression of PDR (65.8 pg/ml (15.6–218.5)) and regression of PDR (62.7 pg/ml (15.6–206.5)) ($p = 0.5972$). There was also no significant difference of plasma Ang II levels between no improvement and/or progression of PDR (13.8 pg/ml (4.0–23.4)) and regression of PDR (12.2 pg/ml (4.0–25.2)) ($p = 0.5329$).

The vitreous level of VEGF was significantly correlated with the severity of NVE, NVD, and FPE (NVE; $\rho = 0.48924$, $p = 0.0038$; NVE; $\rho = 0.35996$, $p = 0.0146$; FPE; $\rho = 0.29871$, $p = 0.0238$), but was not correlated with the severity of vitreous haemorrhage or retinal detachment (vitreous haemorrhage; $\rho = 0.18146$, $p = 0.3001$; retinal detachment; $\rho = 0.20165$, $p = 0.1832$). The vitreous level of Ang II was also significantly correlated with severity of NVE, NVD, and FPE (NVE; $\rho = 0.38926$, $p = 0.0138$; NVD; $\rho = 0.33667$, $p = 0.0264$; FPE; $\rho = 0.27643$, $p = 0.0486$), but not with

severity of vitreous haemorrhage or retinal detachment (vitreous haemorrhage; $\rho = 0.13246$, $p = 0.3782$; retinal detachment; $\rho = 0.19984$, $p = 0.1744$). On the other hand, plasma levels of VEGF were not significantly correlated with severity of NVE, NVD, vitreous haemorrhage, FPE, and retinal detachment (NVE; $\rho = 0.1441$, $p = 0.3027$; NVD; $\rho = 0.1274$, $p = 0.3356$; vitreous haemorrhage; $\rho = 0.0974$, $p = 0.4726$; FPE; $\rho = 0.1309$, $p = 0.3146$; retinal detachment; $\rho = 0.0883$, $p = 0.5124$). Plasma levels of Ang II were also not significantly correlated with those (NVE; $\rho = 0.1193$, $p = 0.3904$; NVD; $\rho = 0.1038$, $p = 0.4398$; vitreous haemorrhage; $\rho = 0.0902$, $p = 0.5516$; FPE; $\rho = 0.1298$, $p = 0.3217$; retinal detachment; $\rho = 0.0744$, $p = 0.5761$).

Outcome of vitreous surgery and risk factors

Univariate logistic regression analysis using the number of patients and the above mentioned factors showed that the vitreous levels of VEGF and Ang II, as well as the severity of NVE, NVD, and FPE, were associated with the progression of PDR (table 3).

Multivariate logistic regression analysis confirmed that VEGF was a significant predictor of the progression of PDR (table 4). Based on the estimated coefficients of PDR, a formula was devised to allow calculation of changes in the risk of PDR progression based on the quantitative changes of risk factors such as VEGF: estimated probability of progression of PDR = $1 / \{1 + \exp[+5.842 - 1.562 \times \log_{10}(\text{VEGF})]\}$.

Stratification of retinopathy progression by VEGF and Ang II level

The patients are stratified into four groups based on the median vitreous fluid levels of VEGF and Ang II (median VEGF level: 1120 pg/ml, median Ang II level: 25.0 pg/ml). In group 1 (low levels of both VEGF (<1120 pg/ml) and Ang II (<25.0 pg/ml)), 30 patients (94%) showed improvement of PDR and two patients (6%) showed progression. In group 2 (a high VEGF level (≥ 1120 pg/ml) and a low Ang II level (<25.0 pg/ml)), five out of eight patients (62%) showed improvement and three patients (38%) showed progression. In group 3 (a high Ang II level (≥ 25.0 pg/ml) and a low

Table 3 Univariate logistic regression analysis of factors related to the progression of proliferative diabetic retinopathy

Variable	Odds ratio (95% CI)	p Value
NVE	1.98 (1.11 to 3.24)	0.0098
NVD	1.42 (1.06 to 2.02)	0.0072
FPE	1.71 (1.01 to 2.52)	0.0219
Vitreous haemorrhage	1.32 (0.74 to 1.98)	0.1428
TRD	1.58 (1.02 to 2.12)	0.0892
Vitreous VEGF (log 10 pg/ml)	2.24 (1.08 to 3.58)	0.0012
Vitreous Ang II (log 10 pg/ml)	1.76 (1.18 to 2.43)	0.0136
Plasma VEGF (log 10 pg/ml)	1.14 (0.48 to 1.68)	0.1968
Plasma Ang II (log 10 pg/ml)	1.06 (0.42 to 1.63)	0.2014

CI = confidence interval; NVE = new vessels elsewhere; NVD = new vessels on or within 1 disc diameter of the disc; FPE = fibrous proliferation elsewhere; TRD = tractional retinal detachment; VEGF = vascular endothelial growth factor; Ang II = angiotensin II.

Table 4 Multivariate logistic regression analysis of factors related to the progression of proliferative diabetic retinopathy

Variable	Odds ratio (95% CI)	p Value
VEGF (log 10 pg/ml)	2.48 (1.02 to 4.98)	0.0008

CI = confidence interval; VEGF = vascular endothelial growth factor.

VEGF level (<1120 pg/ml)), two out of six patients (25%) showed progression of PDR, while eight out of 15 patients (53%) showed progression in group 4 (high levels of both VEGF (≥ 1120 pg/ml) and Ang II (≥ 25.0 pg/ml)). Groups 2, 3, and 4 had a significantly higher risk of PDR progression after vitreous surgery (group 2 odds ratio: 3.98, $p = 0.0316$, group 3 odds ratio: 2.46, $p = 0.0482$, group 4 odds ratio: 7.19, $p = 0.0019$) (table 5).

DISCUSSION

In this prospective study, we investigated whether the outcome of vitreous surgery for PDR was correlated with the vitreous fluid levels of VEGF and/or Ang II. We found that vitreous fluid levels of both VEGF and Ang II were significantly higher in patients who showed no improvement and/or progression of PDR when compared with the levels in patients who showed regression of PDR. The vitreous levels of VEGF and Ang II were also significantly correlated with the severity of NVE, NVD, and FPE, but not with the severity of vitreous haemorrhage or retinal detachment.

Various studies have shown that the vitreous levels of certain cytokines and growth factors are correlated with the severity of PDR.¹⁴⁻¹⁹ However, there have only been a few prospective studies on the prognosis of PDR and/or proliferative vitreoretinopathy (PVR),^{20, 21} and previous attempts at predicting the prognosis have not been very successful. Although vitreous surgery for PDR has been established for over 10 years, many patients still do not achieve a good outcome.¹ The main causes of a poor outcome after vitreous surgery are rubeotic glaucoma and tractional retinal detachment with fibrous vascular proliferation, so the prognosis seems to depend on the extent of neovascularisation. It is important to develop a method for predicting the outcome of vitreous surgery of PDR since this will help to improve the prognosis of surgical treatment. Measurement of VEGF and Ang II levels in the vitreous fluid may be useful in this respect, because both of these factors are correlated with neovascularisation. From our results, the plasma levels of VEGF or Ang II cannot be useful to predict the outcome of vitreous surgery, because there was no significant difference in plasma levels of VEGF or Ang II between no improvement and/or progression of PDR; regression of PDR and the plasma levels of VEGF and Ang II were not significantly correlated with severity of fundus parameters.

In this prospective study, univariate logistic regression analysis showed that several factors were associated with the progression of PDR. These factors included the vitreous levels of VEGF and Ang II, as well as the severity of NVE, NVD, and

FPE. All three fundus parameters reflect retinal neovascularisation, while VEGF and Ang II are involved in the onset and progression of new vessel formation.⁸ Therefore, a high level of neovascularisation is associated with the progression of PDR after vitreous surgery. Multivariate logistic regression analysis also showed that the vitreous level of VEGF was associated with the progression of PDR, while the vitreous level of Ang II and the fundus parameters no longer showed a significant association with PDR. The reason why the vitreous level of Ang II and the severity of NVE, NVD, or FPE were not selected as risk factors is that all these factors are significantly associated with the vitreous level of VEGF, which seems to be the most powerful risk factor. These results suggest that measurement of VEGF in the vitreous fluid may be useful for predicting the outcome of vitreous surgery for PDR. However, our sample size was relatively small, so a larger prospective study is required to confirm the predictive value of VEGF (or Ang II).

We have prospectively investigated the expression of VEGF and Ang II in the vitreous fluid of patients with PDR who underwent a primary vitrectomy. No improvement (containing the progression group) and regression of PDR was stratified into four groups according to the vitreous levels of VEGF and Ang II. The eyes with not only high levels of VEGF or Ang II but also both high levels of VEGF and Ang II in the vitreous fluid had a significantly high risk of progression of PDR in comparison with the eyes with low levels of both VEGF and Ang II. The advantage of this prospective study was the ability to perform a logistic regression model and estimated the odds ratio of postoperative progression according to the vitreous levels of VEGF and Ang II. Our results suggested that there is an interaction between VEGF and Ang II in the eye and the increase of both VEGF and Ang II production induces the activity of neovascularisation in patients with PDR.

Predicting the outcome of vitreous surgery for PDR is clinically important, but is not always easy. Stereoscopic fundus examination, fundus colour photography, FA, and examination using a preset lens with a slit lamp are all methods employed to diagnose the severity and activity of PDR. However, these clinical examinations are of limited value for predicting the outcome of vitreous surgery, probably because neovascularisation activity is not only dependent on fundus findings but also on the intraocular levels of mediators that promote new vessel formation and proliferation. We found that vitreous levels of VEGF and Ang II were higher in patients with active PDR than in patients with quiescent PDR.⁸ This suggests that measurement of the

Table 5 Odds ratios for groups based on the vitreous fluid levels of VEGF and Ang II

	Odds ratio (95% CI)	p Value
Group 1	1.00	
Group 2	3.98 (0.78 to 16.5)	0.0316
Group 3	2.46 (0.66 to 12.8)	0.0482
Group 4	7.19 (1.03 to 27.04)	0.0019

CI = confidence interval; group 1, VEGF < 1120 pg/ml and Ang II < 25.0 pg/ml; group 2, VEGF ≥ 1120 pg/ml and Ang II < 25.0 pg/ml; group 3, VEGF < 1120 pg/ml and Ang II ≥ 25.0 pg/ml; group 4, VEGF ≥ 1120 pg/ml and Ang II ≥ 25.0 pg/ml.

vitreous level of VEGF and/or Ang II may be useful to predict neovascularisation activity even when patients have already undergone retinal photocoagulation, and this test may possibly reduce the risk of visual disturbance caused by surgery for PDR.

In conclusion, this study revealed that the vitreous level of VEGF was correlated with the progression of PDR and was elevated when PDR showed progression. These findings suggest that VEGF (an angiogenesis stimulator) may determine whether progression or regression of PDR occurs after vitreous surgery. We also used these results to develop an equation for predicting the risk of postoperative progression of PDR. A high vitreous fluid VEGF level was a significant risk factor for and a potential predictor of the postoperative progression of PDR. Advances in diagnostic techniques may allow the intraoperative measurement of VEGF in the vitreous fluid and help to improve prediction of the outcome of vitreous surgery for PDR. However, a large scale clinical study is needed to determine whether the measurement of VEGF is actually useful or not.

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