ORIGINAL RESEARCH

Clinical Features and Vitreous Biomarkers of Early-Onset Type 2 Diabetes Mellitus Complicated with Proliferative Diabetic Retinopathy

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Purpose: To compare the clinical features and vitreous biomarkers of proliferative diabetic retinopathy (PDR) between patients with early-onset and late-onset type 2 diabetes mellitus (T2DM).

Materials and Methods: This case-control study analyzed the clinical data of 74 patients with PDR who underwent vitrectomy. The patients were divided into the early-onset (T2DM diagnosis age ≤ 40 years, n = 39) and late-onset (T2DM diagnosis age > 40 years, n = 35) groups. Thirty-six specimens were collected, and the liquid chip technology was used to detect the content of 27 types of cytokines in the vitreous. Differences in clinical features and cytokine levels between the two groups were evaluated. Bonferroni correction was applied for multiple comparisons.

Results: Compared with the late-onset group, the levels of hemoglobin A1c (HbA1c) and total cholesterol were significantly higher in the early-onset group ($P < 0.001$ and $P = 0.009$, respectively). Patients with early-onset T2DM PDR had worse visual prognoses and a higher rate of postoperative recurrent vitreous hemorrhage. The results of cytokine detection showed that the levels of interleukin-4 (IL-4), IL-6, IL-8, IL-9, granulocyte colony-stimulating factor, interferon-γ, interferon-inducible 10 kDa, monocyte chemotactic protein 1, macrophage inflammatory protein (MIP)-1α, and MIP-1β in the early-onset group were significantly higher than those in the late-onset group ($p < 0.0026$). Age at diabetes diagnosis and HbA1c, IL-4, and regulated upon activation, normal T cell expressed and secreted levels were independent risk factors for visual acuity after undergoing vitrectomy.

Conclusion: Early-onset T2DM PDR patients had poor blood glucose and lipid metabolism, higher levels of inflammatory factors, and worse visual prognosis. Stricter metabolic management and earlier anti-inflammatory interventions may be required for patients with early-onset T2DM.

Keywords: early-onset type 2 diabetes mellitus, proliferative diabetic retinopathy, vitrectomy, cytokines, hemoglobin A1c

Introduction

Proliferative diabetic retinopathy (PDR), a common complication of diabetes mellitus (DM), can lead to severe visual impairment and blindness. The age at onset of DM is considered a risk factor in the occurrence and development of diabetic retinopathy (DR).[1,2] With the changes in people's modern lifestyles and diet structure, the onset age of Type 2 DM (T2DM) is getting lower. Epidemiological investigations indicated that the incidence of early-onset T2DM (defined as a DM diagnosis age ≤ 40 years) in China increased nearly fourfold from 1997 to 2010.[3] Patients with early-onset T2DM have a higher risk of progressive chronic kidney disease compared with late-onset patients.[4,5] Similarly, the prevalence of DR and diabetic macular edema (DME) is significantly higher among patients with early-onset T2DM than among those with late-onset T2DM.[3] However, no study has reported the difference in clinical manifestation and prognosis between patients with early-onset and late-onset T2DM complicated with PDR.

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Accumulating evidence has shown that the inflammatory process is implicated in the pathogenesis of DR.[6,7] Multiple factors, such as vascular endothelial growth factor (VEGF) and inflammatory cytokines, have been identified in the intraocular fluid of patients with PDR.[8–12] Some investigators speculate that young people with diabetes may respond more strongly to hypoxia and hyperglycaemia, resulting in large amounts of VEGF in the eyes, consequently, they are more likely to develop retinopathy. This suggests that the identification of cytokines may provide an important clue for explaining the differences in clinical features and prognosis between early-onset and late-onset T2DM PDR. In addition, compared with the aqueous humor, vitreous specimens are closer to the retina and can truly reflect the cytokine levels, which are associated with the pathogenesis of PDR.[13]

The current study aimed to investigate the clinical features, prognosis, and risk factors for early-onset and late-onset T2DM PDR among patients who underwent vitrectomy, and to compare the differences in cytokines in the vitreous between the two groups. Awareness of these will help in early patient management, and the differences in cytokines may help in the prediction of disease outcomes and formulation of more targeted treatment.

Materials and Methods
Calculation of Sample Size
Since no previous literature was found, the sample size was estimated based on postoperative visual acuity in the pre-experiment. The postoperative visual acuity of early-onset group and late-onset group were 0.95 ±0.11 and 0.78 ±0.18 respectively. Using an alpha value of 0.05 and a power of 90%, the sample size was calculated by PASS15 software, we got the required sample size for early-onset group = 16 eyes and late-onset group = 16 eyes, and considering 10% of the lost follow-up, at least 36 cases should be included in the study. To achieve this sample size in the vitreous study, we recruited 48 eyes of early-onset patients and 38 eyes of late-onset patients.

Inclusion and Exclusion Criteria
This study was approved by the Ethics Committee of Tongji Hospital and conducted in accordance with the tenets of the Declaration of Helsinki. All participants signed informed consent forms after full explanation of the nature and potential risks of the study. From August 2019 to March 2021, consecutive patients who were diagnosed with PDR and underwent pars plana vitrectomy (PPV) were enrolled for the study. Patients with T1DM (7 eyes), inflammatory and ischemic intraocular diseases (3 eyes) or previous vitrectomy (6 eyes) were excluded. The final analysis included 86 eyes of T2DM patients (Figure 1). The patients were aged 18–80 years and were operated on by the same ophthalmologist. Surgical indications include uncleared vitreous hemorrhage (VH), extensive fibrovascular proliferation (FVP), tractional retinal detachment (TRD) and tractional rhegmatogenous retinal detachment (TRRD). The patients were divided into two groups according to the age of onset of diabetes: early-onset T2DM group (diagnosis age ≤ 40 years) and late-onset T2DM group (diagnosis age > 40 years).

The exclusion criteria were as follows: (I) Type 1 DM complicated with PDR, (II) the presence of other inflammatory and ischemic intraocular diseases, (III) previous vitrectomy, or other ocular surgery in the preceding three months.

Data and Sample Collection
Clinical characteristics of each patient were collected, including age, gender, duration of diabetes, age at diagnosis of diabetes, systemic conditions, body mass index (BMI), hypertension, and family history of DM. The hemoglobin A1c (HbA1c) levels, glomerular filtration rate (GFR), total cholesterol (TC), blood pressure, and blood glucose (FBG) were measured before operation. Preoperative ophthalmologic examinations included measurements of best-corrected visual acuity (BCVA) and intraocular pressure (IOP), slit lamp microscopy, ocular B-ultrasound, optical coherence tomography, and indirect ophthalmoscopic fundus examination. The abnormalities of eye examination before and during operation were recorded, including the existence of iris neovascularization, DME, VH, FVP, TRD and TRRD. If the FVP contained visible neovascularization tissue, it would be defined as active FVP. Combined intraoperative procedures, including cataract extraction, anti-VEGF surgery, and vitreous cavity packing, were also recorded. Regular follow-up was performed to record
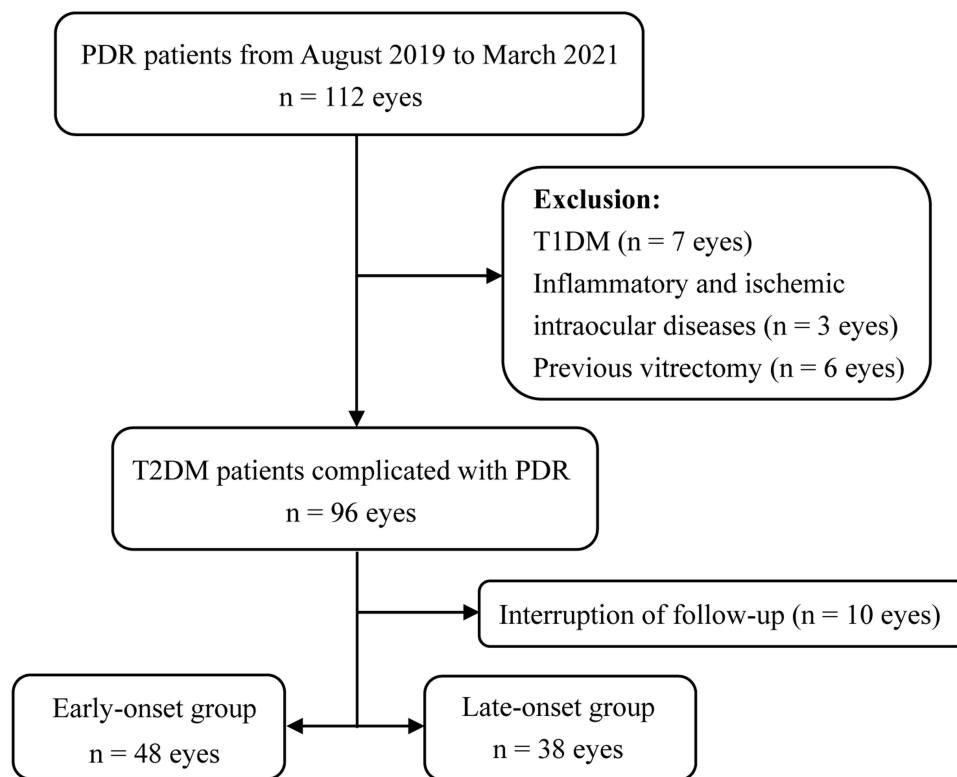
Figure 1 Flow chart of the study from recruiting participants to conducting analysis.

the BCVA, IOP, and fundus conditions at one, three, and six months after operation, as well as postoperative complications, such as recurrent VH, DME, neovascular glaucoma (NVG), cataract, and recurrent retinal detachment (RD).

Vitreous samples were collected from the eyes of the patients during the first vitrectomy. Before starting infusion, a 25G vitreous cutter was used to collect at least 1 mL undiluted vitreous samples. It is worth mentioning that we excluded patients who received anti-VEGF injection and photocoagulation within three months, while the rest of the patients who required anti-VEGF therapy underwent the operation after vitrectomy. No complication associated with sampling was observed. In total, 36 samples were collected and stored at −80 °C until analysis.

Cytokine Measurements

Before measuring 27 cytokines, vitreous samples were centrifuged 10,000g for 10 min to remove cellular components. Vitreous samples were measured using a Bio-Plex Pro Human Cytokine 27-plex (Bio-Rad Cat# M500KCAF0Y, RRID: AB_2893118, California, USA), which included the following 27 cytokines: interleukin (IL)-1β, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, basic fibroblast growth factor (b-FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), interferon-inducible 10 kDa protein (IP-10), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor (PDGF), regulated upon activation, normal T cell expressed and secreted (RANTES), tumor necrosis factor alpha (TNF-α), and VEGF. The experimental procedure for the cytokine assay was in accordance with the instructions. When the measured values fell below the limit of detection, we set the recorded concentration at the limit of detection. A cytokine was excluded if more than 10% of the samples were below the detection limit.

Statistical Analysis

Statistical analysis was performed using IBM SPSS statistics software, version 22.0. (IBM corporation, Armonk, N.Y., USA). The missing data in this study was less than 5%, which was excluded from the statistical analysis. Mahalanobis

distance method was used to identify multivariable outliers, the test standard was 0.005, and no outliers were found. A one-sample Shapiro–Wilk test was used to examine whether data were normally distributed. Normally distributed continuous variables are presented as mean ± standard deviation (SD) and were compared using the independent-samples t test. Categorical data are presented as percentages and were compared using the chi-square test. Non-normally distributed variables are presented as the median and interquartiles range (25th,75th percentile), and a non-parametric statistical analysis was employed. Intergroup comparisons were conducted using the Mann–Whitney U-test and Bonferroni correction, at a level of significance of $P < 0.0026$ (0.05/19). Spearman's rank-order correlation coefficients were calculated to test the relationships between cytokine concentrations and clinical characteristics. R values < 0.30 were considered low or weak correlations; 0.30–0.70, modest or moderate correlations; and > 0.70, strong or high correlations. Simultaneously, multivariate stepwise linear regression analysis was used to explore the risk factors for postoperative visual outcomes. The independent variables included in the linear analysis were based on the variables associated with postoperative visual outcomes in the correlation analysis (R > 0.30). $P < 0.05$ was considered statistically significant.

Results

In total, 48 eyes (39 patients) were assessed in the early-onset T2DM PDR group, with a mean patient age of 44.69 ± 8.51 years. Further, 38 eyes (35 patients) were assessed in the late-onset T2DM PDR group (control), with a mean patient age of 57.76 ± 7.53 years. The mean ages at DM diagnosis were 32.33 ± 5.18 and 47.84 ± 4.77 years in the early-onset and late-onset groups, respectively, ($P < 0.001$). Moreover, mean HbA1c and TC levels in the early-onset group were significantly higher than those in the late-onset group ($P < 0.001$ and $P = 0.009$, respectively). There were no significant differences in other basic characteristics, except for the high incidence of family history of diabetes in the early-onset group. The detailed general conditions of patients with PDR are summarized in Table 1.

Preoperative and Intraoperative Outcomes

The median preoperative BCVAs were 1.85 (1.6, 2.3) and 1.85 (1.48, 2.3) in the early-onset and late-onset groups, respectively, ($P = 0.620$). In the early-onset group, the surgical indications were mainly TRD/TRRD (64.6%) and FVP (33.3%), while VH (28.9%) and FVP (47.4%) were the main reasons for undergoing PPV among patients in late-onset group. The rates of preoperative panretinal laser photocoagulation (PRP) and perioperative anti-VEGF were also significantly higher in the early-onset group (45.8% vs 21.1% and 70.8% vs 28.9%, respectively). Moreover, the early-onset group had a higher proportion of active FVP (77.1% vs 47.4%, $P = 0.004$). The proportions of preoperative DME, cataract extraction and vitreous tamponade were similar for the two groups (Table 1).

Postoperative Visual Outcomes and Complications

There was no significant difference in the median of preoperative BCVA between the two groups ($P = 0.620$). Figure 2A shows the trend of BCVA changes at different times. Although the median postoperative BCVA was significantly increased in both groups ($P < 0.05$). The median BCVA at 1 month (1.0 vs 0.7 logMAR, $P < 0.05$), 3 months (0.9 vs 0.6 logMAR, $P < 0.05$) and 6 months (0.9 vs 0.5 logMAR, $P < 0.05$) after operation in the early-onset group was significantly higher than that in the late-onset group (Figure 2A).

Of the complications that occurred within 6 months after PPV, the incidence of recurrent VH in the early-onset group was significantly higher than that in the late-onset group (20.3% vs 5.8%, $P = 0.039$). The incidences of DME, NVG, cataract, and recurrent RD were also higher in the early-onset group than that in the late-onset group; however, there was no statistical difference between the two groups (Table 2, Figure 2B).

Cytokine Expression Profiles

The vitreous cytokine levels in 36 PDR patients in the two groups are summarized in Table 3. The detection rates of IL-5, IL-7, IL-12, IL-15, b-FGF, Eotaxin, PDGF-BB, and GM-CSF were less than 90%, and were not included in subsequent analyses.

Among the 19 cytokines tested in the vitreous fluid, after Bonferroni correction, the early-onset group showed significantly higher levels of IL-4, IL-6, IL-8, IL-9, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, and MIP-1β compared to those in the late-onset group ($P < 0.0026$ as statistically significant).

Table 1 Clinical Characteristics and Surgical Methods in the Early- and Late-Onset Groups

Clinical Characteristics	Early-Onset Group (n = 48)	Late-Onset Group (n = 38)	P-value
Age (years)	44.69 ± 8.51	57.76 ± 7.53	<0.001
Eyes (right, %)	23 (47.9%)	22 (57.9%)	0.358
Sex (male, %)	30 (62.5%)	17 (44.7%)	0.100
Age at DM diagnosis (years)	32.33 ± 5.18	47.84 ± 4.77	<0.001
Course of DM (years)	13.5 (7, 20)	10 (4.5, 15.75)	0.099
BMI (kg/m^2)	24.42 (22.73, 27.20)	23.45 (20.33, 23.45)	0.057
Family history of DM	23 (47.9%)	4 (10.5%)	<0.001
FBG (mmol/L)	7.76 (5.09, 10.61)	6.33 (5.05, 9.96)	0.256
HbA1c (%)	9.41 ± 2.28	7.61 ± 1.2	<0.001
TC (mmol/L)	5.12 ± 1.34	4.41 ± 1.03	0.009
e-GFR	74.90 (35.18, 103.93)	76.55 (55.75, 95.40)	0.774
Systolic blood pressure (mmHg)	143.92 ± 21.11	142.53 ± 20.13	0.758
Diastolic blood pressure (mmHg)	85.56 ± 11.30	82.71 ± 12.13	0.264
Pre-operative BCVA (LogMAR)	1.85 (1.6, 2.3)	1.85 (1.48, 2.3)	0.620
Pre-operative IOP (mmHg)	16.83 ± 3.84	15.95 ± 3.85	0.292
Preoperative DME	4 (8.3%)	2 (5.3%)	0.69
Iris neovascularization	2 (4.2%)	0 (0%)	0.501
Active FVP	37 (77.1%)	18 (47.4%)	0.004
Surgical indications			0.002
VH only	1 (2.1%)	11 (28.9%)	
FVP with or without VH	31 (64.6%)	18 (47.4%)	
TRD/TRRD with or without VH	16 (33.3%)	9 (23.7%)	
Preoperative PRP (n, %)	22 (45.8%)	8 (21.1%)	0.017
Perioperative anti-VEGF (n, %)	34 (70.8%)	11 (28.9%)	<0.001
Cataract extraction (n, %)	7 (14.6%)	11 (28.9%)	0.104
Vitreous tamponade			0.599
BSS	13 (27.1%)	14 (36.8%)	
Gas	22 (45.8%)	16 (42.1%)	
Silicone oil	13 (27.1%)	8 (21.1%)	

Abbreviations: BCVA, best corrected visual acuity; BMI, body mass index; DM, diabetes mellitus; FBG, fasting blood glucose; DME, diabetic macular oedema; FVP, fibrovascular proliferation; GFR, glomerular filtration rate; IOP, intraocular pressure; PRP, panretinal laser photocoagulation; TC, total cholesterol; TRD, tractional retinal detachment; TRRD, tractional rhegmatogenous retinal detachment; VH, vitreous haemorrhage; VEGF, vascular endothelial growth factor; BSS, balanced salt solution.

Correlation Between Cytokine Concentrations and Clinical Features

Correlation between cytokine concentrations and clinical features are summarized in Figure 3. Significant positive correlations were observed among most cytokines. Likewise, the BCVA at 6 months after operation had significant positive correlations with the following cytokines: IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-9, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, TNF-α, VEGF, and HbA1c. The level of HbA1c was positively correlated with some inflammatory factors and chemokines. However, the age at DM diagnosis showed significant negative correlations with many inflammatory factors and chemokines, as well as the BCVA at 6 months after operation.

Prognostic Factors for Visual Outcome

Stepwise multiple linear regression analysis was performed to identify potential risk factors for visual prognosis (Table 4, Supplementary data Tables S1–S3). Based on the correlation analysis between clinical characteristics and the cytokine concentration, variables significantly related to the BCVA at 6 months post-operation were included in the multiple linear regression analysis. After adjusting for preoperative BCVA, IL-1ra, IL-1β, IL-2, IL-6, IL-8, IL-9, G-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, TNF-α, and VEGF levels, the results revealed that the age at DM diagnosis (β =−0.028, P = 0.002), HbA1c (β = 0.110, P = 0.002), IL-4 (β = 0.355, P = 0.001), and RANTES level (β =0.004, P = 0.006) were independent predictors for visual outcomes at 6 months post-operation.
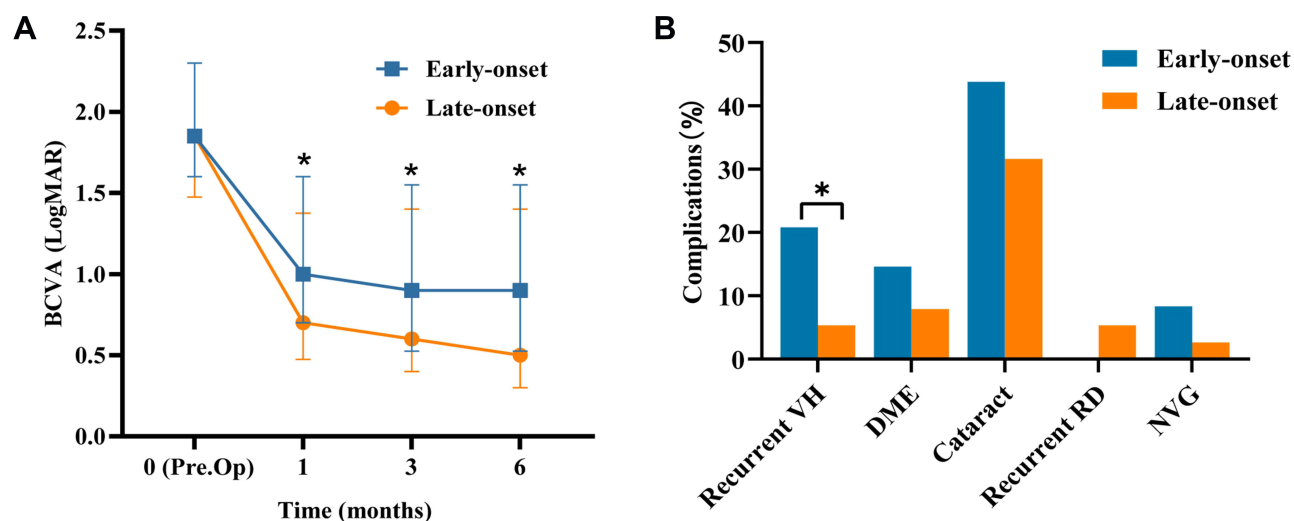
Figure 2 (**A**) Changes in BCVA in the early-onset and late-onset groups before operation and 1 months, 3 months, and 6 months after operation. (**B**) Postoperative complications in the two groups. *P < 0.05.

Discussion

In the present study, PDR patients with early-onset T2DM showed poor blood glucose control and lipid metabolism, and a great likelihood of severe retinopathy. These findings are consistent with the clinical characteristics of early-onset T2DM patients reported in previous studies.[14–16] Early-onset T2DM is often associated with genetic susceptibility and adolescent obesity.[15,17] Compared with patients with late-onset T2DM, patients with early-onset T2DM had higher levels of HbA1c and TC, and a higher prevalence of DR.[14] Moreover, the risk of DR and PDR increased significantly with the increase in HbA1c level.[18] This suggests that more attention should be paid to the screening of complications in patients with early-onset T2DM, and that a strict diet control should be carried out.

Vitreoretinopathy progresses rapidly in patients with early-onset T2DM. A previous longitudinal study showed that nearly 1/3 patients with early-onset T2DM progressed from non-proliferative DR to PDR during an average follow-up of 7.1 years.[19] In our study, we found that the incidence of simple VH was lower in the early-onset group, but the proportions of FVP and TRD/TRRD in the early-onset group were higher than those in the late-onset group. The proportions of gas and silicone oil tamponade were also higher in the early-onset group, this partly reflected the severity of retinopathy. However, the mechanism has not been fully clarified at present.

In this study, we proved that the levels of 10 cytokines including IL-4, IL-6, IL-8, IL-9, G-CSF, IFN- γ, IP-10, MCP-1, MIP-1α and MIP-1β in the early-onset group were significantly higher than those in the late-onset group. In previous studies, several cytokines have been identified in the eyes of patients with PDR; for example, MCP-1, IL-6, IL-8, and VEGF have been demonstrated to be the four main cytokines upregulated in eyes of patients with PDR.[8,9,20,21] MCP-1 recruits mononuclear macrophages and has been shown to play a central role in the development of chronic fibroproliferative

Table 2 Postoperative Complications in the Two Groups

Postoperative Complications	Early-Onset Group (n = 48)	Late-Onset Group (n = 38)	P-value
Recurrent VH	10 (20.8%)	2 (5.3%)	0.039
DME	7 (14.6%)	3 (7.9%)	0.534
Cataract	21 (43.8%)	12 (31.6%)	0.249
Recurrent RD	0 (0%)	2 (5.3%)	0.375
NVG	4 (8.3%)	1 (2.6%)	0.510

Abbreviations: DME, diabetic macular oedema; NVG, neovascular glaucoma; RD, retinal detachment; VH, vitreous haemorrhage.

Table 3 Vitreous Cytokine Levels in the Early- and Late-Onset Groups

Cytokine (pg/mL)	Early-Onset Group (n = 20)	Late-Onset Group (n = 16)	P-value
IL-1ra	373.97 (219.40, 692.88)	198.56 (103.26, 526.85)	0.053
IL-1β	0.50 (0.35, 0.68)	0.26 (0.19, 0.41)	0.011
IL-2	2.11 (1.53, 2.58)	1.07 (1.07, 1.53)	0.012
IL-4	0.70 (0.44, 1.02)	0.25 (0.21, 0.61)	0.002*
IL-6	142.21 (96.78, 353.30)	40.54 (20.33, 79.21)	<0.001*
IL-8	221.67 (132.33, 519.97)	58.59 (20.91, 116.83)	0.001*
IL-9	24.14 (9.23, 33.60)	3.27 (1.84, 8.02)	<0.001*
IL-10	1.90 (1.52, 3.53)	1.52 (1.17, 2.05)	0.386
IL-13	1.38 (0.78, 1.65)	1.2 (0.66, 1.74)	0.422
IL-17	3.01 (2.15, 4.06)	2.84 (1.80, 5.18)	0.838
G-CSF	113.71 (67.66, 178.64)	27.53 (20.42, 65.97)	<0.001*
IFN-γ	95.31 (53.47, 163.70)	34.37 (13.75, 51.60)	0.001*
IP-10	2583.5 (1870.25, 6421.75)	595.67 (361.26, 1163.75)	<0.001*
MCP-1	1268.0 (979.03, 1509.25)	613.89 (308.81, 962.31)	<0.001*
MIP-1α	2.33 (1.47, 3.75)	0.76 (0.49, 1.62)	<0.001*
MIP-1β	21.51 (14.81, 33.85)	5.87 (3.29, 12.05)	<0.001*
RANTES	7.66 (6.14, 11.38)	5.88 (2.92, 11.20)	0.211
TNF-α	6.43 (4.76, 10.36)	4.36 (2.44, 9.03)	0.178
VEGF	110.48 (37.16, 200.94)	33.94 (13.14, 117.68)	0.036

Note: *A Bonferroni correction was adopted with significance set for a P-value less than 0.0026.
Abbreviations: G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon-γ; IL, interleukin; IL-1ra, IL-1 receptor antagonist; IP-10, interferon-inducible 10 kDa protein; MCP-1, monocyte chemotactic protein 1; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

diseases.[22] IL-6 is secreted by T cells and macrophages and causes inflammation in damaged tissue. Cohen et al[23] confirmed that IL-6 may indirectly induce angiogenesis by inducing the expression of VEGF. IL-8 also plays a role in inducing inflammation and promoting angiogenesis in the eye.[24,25] Yoshida et al[26] believed that elevated levels of MCP-1, IL-6, and IL-8 were associated with postoperative fibrous proliferation in PDR. Our study shows that the expression of 10 cytokines, including IL-6, IL-8, MCP-1, were significantly increased in early-onset group. Moreover, there was a significant correlation between these cytokines. Interestingly, the level of VEGF was significantly higher in the early-onset group than that in the late-onset group before Bonferroni correction (P=0.036). These findings may partly explain why early-onset patients are more likely to develop postoperative recurrent VH. However, this statistical difference disappeared after Bonferroni correction, suggesting that the level of VEGF may not play a leading role in patients with early-onset T2DM.

IFN-γ is produced by T cells and is an immunomodulatory factor. IP-10 belongs to chemokine, which is mainly induced by INF-γ. Both of them cause inflammatory response. The concentrations of IFN-γ and IP-10 in intraocular fluid of patients with DR were increased.[27,28] MIP-1α, MIP-1β, IL-9 and G-CSF in vitreous of patients with DR are rarely studied. MIP-1α and MIP-1β belong to the chemokine family and are involved in the process of local inflammatory injury. And MIP-1α can induce corneal neovascularization in mice,[29] MIP-1β can also induce interstitial fibrosis.[30] IL-9 can be used as a proinflammatory cytokine in autoimmunity and allergic reactions. G-CSF is a colony stimulating factor involved in regulating the proliferation and differentiation of inflammatory cells. Various chemokines can act as leukocyte attractants and angiogenesis inducers to act on endothelial cells together with pro-inflammatory cytokines, resulting in the destruction of blood-retinal barrier and neovascularization.[31]

The age at DM diagnosis and level of HbA1c are associated with multiple inflammatory factors and chemokines. The correlation analysis showed that the age at DM diagnosis was significantly negatively correlated with the above 10 cytokines, while HbA1c was was significantly positively correlated with IL-6, G-CSF, IP-10, MCP-1 and MIP-1 α. In addition, patients with early-onset T2DM had higher HbA1c level and more severe retinopathy than patients with late-onset T2DM. Therefore, we speculate that in the process of long-term chronic retinal inflammation caused by hyperglycemia, the combined action of inflammatory factors and chemokines leads to the rapid progression of retinopathy in
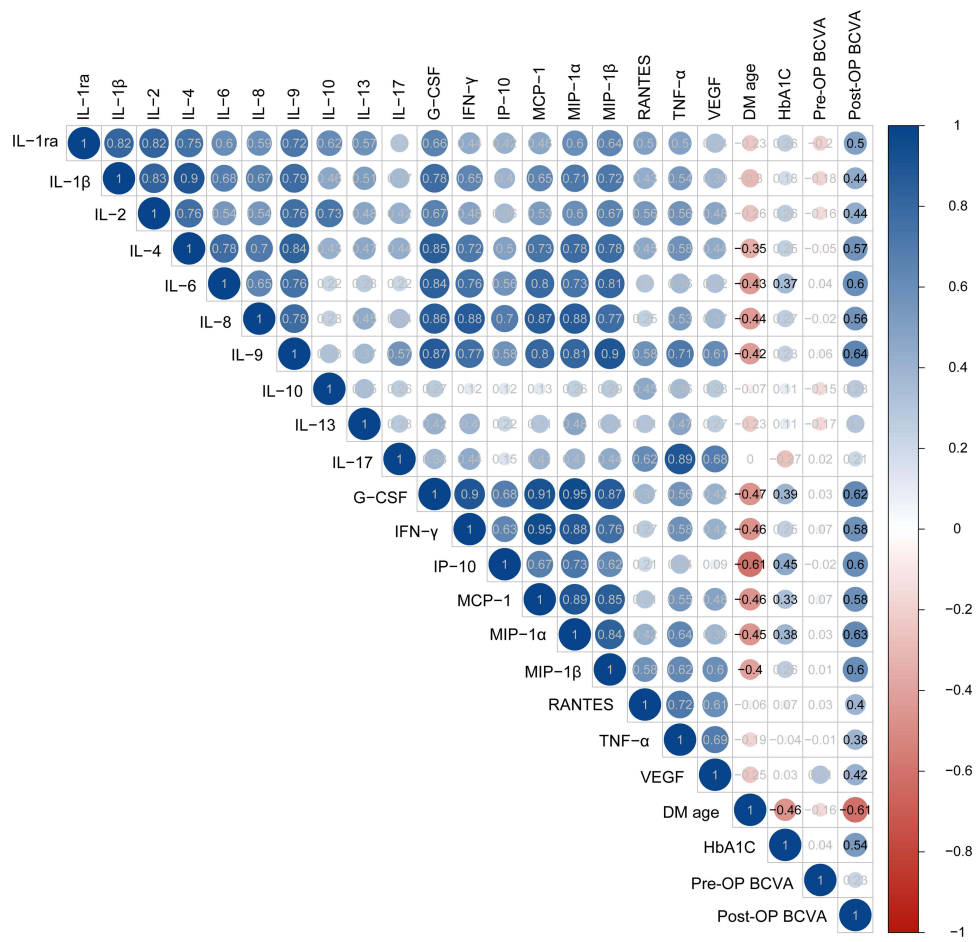
Figure 3 A heatmap of correlation coefficients between cytokine concentrations and clinical characteristics. Dark blue denotes high correlation (R→1); deep red, high negative correlation (R→−1); and white, a lack of correlation (R→0). An R value of 0.3 was set as the threshold and P < 0.05 was considered significant.

Abbreviations: DM, diabetes mellitus; Pre.OP, preoperative; DM age, age at diagnosis of DM; BCVA, best corrected visual acuity; Post.OP BCVA, postoperative BCVA at last follow-up; DME, diabetic macular oedema; NVG, neovascular glaucoma; RD, retinal detachment; VH, vitreous haemorrhage; G-CSF, granulocyte colony-stimulating factor; HbA1c, haemoglobin A1c; IFN-γ, interferon-γ; IL, interleukin; IL-1ra, IL-1 receptor antagonist; IP-10, interferon-inducible 10 kDa protein; MCP-1, monocyte chemotactic protein 1; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

patients with early-onset T2DM PDR. It also suggests that anti-inflammatory therapy, not just anti-VEGF therapy, can be performed in the early stage of the disease for patients with early-onset T2DM PDR. A recent clinical trial found that the combination of dexamethasone and silicone oil tamponade during PPV is a safe and effective treatment for PDR, and may reduce the incidence of DME and the need for anti-VEGF injections, which provides further support for anti-inflammatory therapies.[32]

Table 4 Stepwise Multivariate Linear Regression Analysis of Risk Factors for Visual Prognosis (the BCVA at 6 Months After Operation) of PDR

Variables	Unstandardized Coefficients		Standardized Coefficients	P-value
	β	Std. Error	β	
Age at DM diagnosis	−0.028	0.008	−0.385	0.002
HbA$_{1C}$	0.110	0.033	0.383	0.002
IL-4	0.355	0.094	0.387	0.001
RANTES	0.004	0.001	0.307	0.006

Abbreviations: HbA1c, haemoglobin A1c; IL, interleukin; RANTES, regulated upon activation, normal T cell expressed and secreted; BCVA, best corrected visual acuity; PDR, proliferative diabetic retinopathy.

The relationship between the age at DM diagnosis, cytokine levels, and visual prognosis after PPV in patients with PDR is poorly understood. Multiple linear regression analysis showed that the age at DM diagnosis was negatively correlated with postoperative LogMAR BCVA. However, HbA1c, IL-4, and RANTES were positively correlated with postoperative LogMAR BCVA. This suggests that the younger the age at T2DM onset, the worse the prognosis of visual acuity after vitrectomy. These results are consistent with the findings of Lv et al, who proved that early-onset diabetes is an independent risk factor for the development of PDR in T2DM patients with microalbuminuria.[33] Many studies have demonstrated the relationship between the level of glycaemic control and progression of DR.[34–37] Harris et al[34] found that for every one point increase in the HbA1c level, the risk of progression to PDR increased by 14%, after adjusting for confounding factors. This suggests stricter blood glucose management should be performed in patients with early-onset diabetes. Blood glucose remains the most important modifiable measure to reduce the risk of progression of DR and vision loss. RANTES is a member of the chemokine family. It causes leukocyte infiltration, vascular injury, and neovascularization locally in the retina by chemotaxis of other inflammatory factors. A significant increase in RANTES has been proved to be associated with DR severity.[38] IL-4 is considered an anti-inflammatory factor; however, its role remains controversial, with some studies finding elevated IL-4 levels in DR[39,40] and others finding the opposite.[41] In our study, the increased expression of the anti-inflammatory cytokine IL-4 could be interpreted as a compensatory response aimed at reducing PDR-related inflammation. The exact role of IL-4 in DR requires further investigation.

It is worth mentioning that our research has several advantages and limitations. One advantage is that, to our knowledge, this is the first study to evaluate the relationship between early-onset T2DM PDR and vitreous cytokines. Another advantage is that the effect of the age at onset of T2DM on the severity and prognosis of PDR has also been studied. The first limitation of this study is that the sample size was small and all patients were hospitalized in the same centre. This may have led to selection bias. The sample size of vitreous specimens was also small, which may lead to the omission of some predictive factors. Therefore, the results of this study should be verified with a larger sample size that includes a multicentre population. Second, since the onset of T2DM is often insidious, the exact onset age cannot be determined; therefore, the observed onset age may be higher than the actual onset age. In addition, the postoperative follow-up period was only six months; longer follow-ups and observations should be conducted in the future.

Conclusion

This study found that, compared with patients with late-onset T2DM PDR, patients with early-onset T2DM PDR had poorer blood glucose control and lipid metabolism, a more severe retinopathy, higher levels of inflammatory factors, and a poorer visual prognosis. Age at DM diagnosis, HbA1c, IL-4, and RANTES levels were independent predictors of visual acuity after PPV. These findings emphasize that attention should be paid to patients with early-onset Type 2 diabetes, with stricter metabolic management and earlier anti-inflammatory interventions.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

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Disclosure

The authors report no conflicts of interest, financial or otherwise for this work.

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