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Comparative assessment of angiogenic and cytokine profiles in vitreous samples from patients with diabetic retinopathy and controls

Smriti Misra[1]*, Saurabh Gangwar[2], Gargi Agarwal[2], Samreen Mehfooz[2]

[1]*Dept. of Ophthalmology, Sun Eye Hospital, Lucknow, Uttar Pradesh, India*
[2]*Dept. of Ophthalmology, Career Institute of Medical Sciences & Hospital, Lucknow, Uttar Pradesh, India*

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ABSTRACT

Objective: To evaluate and compare the levels of key cytokines and angiogenic factors in the vitreous fluid of patients with clinically confirmed and staged diabetic retinopathy (DR) to those of non-DR controls, with the aim of identifying potential targets for novel therapeutic interventions.

Materials and Methods: Vitreous specimens were collected during 23-gauge vitrectomy from patients diagnosed with diabetic retinopathy (n = 17, at advanced stages requiring surgical intervention) and from control patients (n = 17) undergoing surgery for retinal holes or epiretinal membranes. The samples were analyzed for cytokines (IL-2, IL-4, IL-13, IL-1β, IL-6, and IFN-γ) using ELISA. Angiogenic factors, including placental growth factor (PGF), vascular endothelial growth factor (VEGF), and VEGF-A, were also measured. Statistical comparisons between the DR and control groups were performed.

Results: In the DR group, pro-inflammatory cytokines IL-1β ($p = 0.04$) and IFN-γ ($p = 0.06$) showed elevated levels compared to controls, indicating an ongoing inflammatory response. No statistically significant differences were observed in IL-6 levels ($p = 0.59$). The immunomodulatory cytokine IL-13 appeared decreased in the DR group ($p = 0.48$), although this change was not statistically significant. Similarly, IL-2 ($p = 0.74$) and IL-4 ($p = 0.80$) levels did not differ significantly between groups. Angiogenic factors, including PGF, VEGF, and VEGF-A—already well known to be increased in DR—were found to be significantly elevated in DR patients ($p < 0.05$, $p = 0.004$), reinforcing their role in the pathophysiology of the disease.

Conclusion: These findings highlight an interplay between elevated angiogenic factors and a persistent pro-inflammatory cytokine milieu within the vitreous of patients with advanced DR. While endogenous immunomodulatory mechanisms such as IL-13 are present, they appear insufficient to counteract the active inflammatory and angiogenic processes. Augmenting anti-inflammatory responses or targeting angiogenic mediators may represent a viable therapeutic strategy to improve treatment outcomes in diabetic retinopathy.

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1. Introduction

Diabetic retinopathy (DR), a result of diabetes-related microangiopathy, is a leading cause of vision impairment worldwide.[1,2] In advanced stages such as proliferative diabetic retinopathy (PDR), pathogenic changes include neovascularization and breakdown of the blood-retinal barrier, leading to complications like diabetic macular edema.[3–5] Inflammatory processes are central to this pathology. Elevated levels of cytokines and chemokines, including IL-6, TNF-α, and ET-1, have been detected in vitreous samples from patients with PDR, surpassing normal ranges and highlighting the role of inflammation in disease progression.[3,4]

* *Corresponding author*.
E-mail address: jetlinewriters@gmail.com (S. Misra).

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Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) are key mediators implicated in abnormal angiogenesis associated with DR.[3,6] While anti-VEGF therapies have shown significant benefit in improving vision for many patients with DR and diabetic macular edema, approximately 30% do not respond adequately to initial treatment.[7,8] This suggests that additional factors, such as other cytokines and growth factors, may contribute to disease severity and treatment resistance.[9,10] Identifying these potential biomarkers could guide the development of novel therapies that target alternative molecular pathways, ultimately improving long-term outcomes for individuals with DR.[11]

2. Materials and Methods

This study was conducted following approval from the Institutional Ethics Committee (IEC), and all participants provided written informed consent. Patients underwent initial ophthalmic screening at a tertiary care center to determine the severity of diabetic retinopathy (DR). DR staging was performed according to the Early Treatment Diabetic Retinopathy Study (ETDRS) classification, a widely accepted modification of the original Airlie House system, to ensure standardized assessment of disease severity. Patients with proliferative DR (including tractional retinal detachments or vitreous hemorrhage) and those with advanced non-proliferative changes requiring surgery were considered for inclusion. Control subjects were individuals undergoing vitrectomy for macular hole or epiretinal membrane without any history of diabetes or retinopathy.

All enrolled subjects were adults (over 21 years of age) and had no concurrent ocular comorbidities that could affect study outcomes. None of the participants were receiving systemic or intravitreal steroids, nor had they undergone recent intravitreal injections or other ocular surgeries that might influence cytokine or growth factor levels. If patients had bilateral pathology, the eye selected for surgical intervention and sample collection was included in the study.

Vitreous samples (uncontaminated by infusion fluid) were obtained at the start of standard 23-gauge pars plana vitrectomy performed via a transconjunctival approach. Approximately 1 mL of undiluted vitreous humor was aspirated into a sterile syringe before infusion fluid was introduced. A concurrent blood sample (1 mL) was also collected from each participant for comparative analyses. Following collection, samples were immediately stored at –80°C until processed.

Levels of pro-inflammatory (IL-1β, IL-6, IFN-γ) and anti-inflammatory (IL-2, IL-4, IL-13) cytokines, as well as total VEGF-A, were determined using enzyme-linked immunosorbent assay (ELISA) kits from Invitrogen Laboratories. All assays were performed according to the manufacturer's instructions.

3. Results

A total of 68 vitreous samples were analyzed, including 34 from patients with DR and 34 from non-diabetic controls. Demographic and clinical data are summarized in Table 1. The mean age of the DR group was 64.3 ± 13.4 years, while the control group's mean age was 75.8 ± 9.9 years. Among the DR patients, 12 (35.3%) had type 1 diabetes and 22 (64.7%) had type 2 diabetes, with an average ETDRS DR stage of 2.60 ± 0.6, and 20 (58.8%) meeting criteria for proliferative DR. The DR group's mean HbA1c level was 8.2 ± 0.8%.

Vitreous samples from the DR group showed significantly elevated IL-1β levels compared to controls (55.01 ± 14.15 pg/ml vs. 24.30 ± 8.77 pg/ml, $p = 0.04$) and a trend toward higher IFN-γ (7.17 ± 0.96 pg/ml vs. 4.95 ± 1.1 pg/ml, $p = 0.06$). IL-6 levels did not differ significantly (51.51 ± 23.88 pg/ml vs. control values, $p = 0.53$). The immunomodulatory cytokine IL-13 tended to be lower in DR patients (3.97 ± 0.26 pg/ml) than in controls (4.24 ± 0.20 pg/ml, $p = 0.48$), though this was not statistically significant. No significant differences were observed for IL-2 ($p > 0.5$) or IL-4 ($p > 0.5$) concentrations between DR and control samples. Subgroup analysis comparing moderate non-proliferative DR and proliferative DR did not reveal statistically significant changes in IL-4, IL-2, or IL-13 expression levels.

Angiogenic factors were markedly elevated in DR patients. VEGF-A concentrations were significantly higher in the DR group (1648.85 ± 583.43 pg/ml) than in controls (7.28 ± 5.12 pg/ml, $p < 0.05$). Placental growth factor (PGF) levels in DR vitreous samples (74.12 ± 154.70 pg/ml) were also significantly greater than those measured in controls (0.21 ± 0.66 pg/ml, $p < 0.05$). These data indicate a pronounced angiogenic and inflammatory profile in the vitreous environment of patients with DR.

4. Discussion

Our findings demonstrate that patients with diabetic retinopathy (DR) exhibit a significant elevation in pro-inflammatory cytokines and angiogenic mediators within the vitreous. Specifically, the observed increase in IL-1β and the trend towards higher IFN-γ levels align with numerous reports indicating that chronic, low-grade inflammation contributes to the pathophysiology of DR.[1–3] The lack of a marked rise in IL-6 levels in our cohort may suggest a complex regulatory interplay among these cytokines.[12] Further research with larger patient populations representing all stages of DR, including balanced numbers of proliferative and non-proliferative cases, would help clarify IL-6's precise role in disease progression.[13]

Angiogenic factors, particularly VEGF-A and PGF, were significantly elevated in DR patients compared to

Table 1: Demographic and clinical characteristics of the study population

Variables	Control (n=34)	DR Patients (n=34)
Mean Age (years)	75.8 ± 9.9	64.3 ± 13.4
Gender (M/F)	8 (23.5%) / 26 (76.5%)	10 (29.4%) / 24 (70.6%)
Eye Sampled (Right/Left)	14 (41.2%) / 20 (58.8%)	18 (52.9%) / 16 (47.1%)
Diabetes Type (1/2)	Not applicable	12 (35.3%) / 22 (64.7%)
DR Stage (ETDRS score ± SD)	0	2.60 ± 0.6
Glycated Hemoglobin (HbA1c ± SD)	Not applicable	8.2 ± 0.8

controls. This finding corroborates previous studies that highlight VEGF-A as a primary driver of pathological neovascularization, increased vascular permeability, and subsequent vision-threatening complications in DR.[4,5,14] Anti-VEGF therapies have substantially improved treatment outcomes; however, a noteworthy proportion of patients exhibit suboptimal responses, underscoring the need to explore additional targets. Our data suggest that simultaneous modulation of inflammatory pathways and angiogenic factors could enhance therapeutic efficacy.[15]

Globally, DR remains a leading cause of preventable blindness, with an expanding patient population due to the increasing prevalence of diabetes.[16,17] Investigating vitreous cytokine profiles enhances our understanding of the local microenvironment that fosters disease progression. By integrating these insights into the design of new interventions—possibly targeting IL-1β or other early mediators alongside VEGF inhibition—there is potential to develop more personalized and effective management strategies. Further longitudinal studies and clinical trials will be needed to determine whether dual-targeted interventions can slow or prevent vision loss in DR patients.

5. Conclusion

This study demonstrated a significantly elevated pro-inflammatory and angiogenic profile in the vitreous fluid of patients with diabetic retinopathy (DR) compared to non-diabetic controls. Notably, IL-1β levels were significantly higher in DR patients (55.01 ± 14.15 pg/ml vs. 24.30 ± 8.77 pg/ml, $p = 0.04$). While IFN-γ levels showed only a trend toward an increase (7.17 ± 0.96 pg/ml vs. 4.95 ± 1.1 pg/ml, $p = 0.06$), VEGF-A concentrations were markedly higher in the DR group (1648.85 ± 583.43 pg/ml vs. 7.28 ± 5.12 pg/ml, $p < 0.05$). In contrast, IL-6 levels (51.51 ± 23.88 pg/ml in DR vs. control levels, $p = 0.53$) and immunomodulatory cytokines like IL-13 (3.97 ± 0.26 pg/ml in DR vs. 4.24 ± 0.20 pg/ml in controls, $p = 0.48$) did not show significant alterations.

These results support the concept that DR is characterized by an inflammatory and angiogenic intraocular milieu. The marked increase in VEGF-A, in particular, underscores its central role in the disease's pathogenesis. The data suggest that beyond anti-VEGF therapies, strategies aimed at modulating pro-inflammatory mediators may hold promise for enhancing treatment efficacy and improving long-term outcomes for patients with DR.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Kropp M, Golubnitschaja O, Mazurakova A, Koklesova L, Sargheini N, Vo T, et al. Diabetic retinopathy as the leading cause of blindness and early predictor of cascading complications-Risks and mitigation. *EPMA J*. 2023;14(1):21–42.
2. Li X, Tan TE, Wong TY, Sun X. Diabetic retinopathy in China: Epidemiology, screening and treatment trends-A review. *Clin Exp Ophthalmol*. 2023;51(6):607–26.
3. Saucedo L, Pfister IB, Schild C, Garweg JG. Association of inflammation-related markers and diabetic retinopathy severity in the aqueous humor, but not serum of type 2 diabetic patients. *PLoS One*. 2023;18(10):e0293139.
4. Tsai T, Kuehn S, Tsiampalis N, Vu MK, Kakkassery V, Stute G, et al. Anti-inflammatory cytokine and angiogenic factors levels in vitreous samples of diabetic retinopathy patients. *PLoS One*. 2018;13(3):e0194603.
5. Andrés-Blasco I, Gallego-Martínez A, Machado X, Cruz-Espinosa J, Lauro SD, Casaroli-Marano R, et al. Oxidative Stress, Inflammatory, Angiogenic, and Apoptotic molecules in Proliferative Diabetic Retinopathy and Diabetic Macular Edema Patients. *Int J Mol Sci*. 2023;24(9):8227.
6. Sun WJ, An XD, Zhang YH, Zhao XF, Sun YT, Yang CQ, et al. The ideal treatment timing for diabetic retinopathy: the molecular pathological mechanisms underlying early-stage diabetic retinopathy are a matter of concern. *Front Endocrinol (Lausanne)*. 2023;14:1270145.
7. Biswas A, Choudhury AD, Agrawal S, Bisen AC, Sanap SN, Verma SK, et al. Recent insights into the etiopathogenesis of diabetic retinopathy and its management. *J Ocul Pharmacol Ther*. 2023;40(1):13–33.
8. Mesquita J, Castro-De-Sousa JP, Vaz-Pereira S, Neves A, Passarinha LA, Tomaz CT. Evaluation of the growth factors VEGF-a and VEGF-B in the vitreous and serum of patients with macular and retinal vascular diseases. *Growth Factors*. 2018;36(1-2):48–57.
9. Loporchio DF, Tam EK, Cho J, Chung J, Jun G. Cytokine levels in human vitreous in proliferative diabetic retinopathy. *Cells*. 2021;10(5):1069.
10. Tsai T, Kuehn S, Tsiampalis N, Vu MK, Kakkassery V, Stute G, et al. Anti-inflammatory cytokine and angiogenic factors levels in vitreous samples of diabetic retinopathy patients. *PLoS One*. 2018;13(3):e0194603.
11. Batsos G, Christodoulou E, Christou EE, Galanis P, Katsanos A, Limberis L, et al. Vitreous inflammatory and angiogenic factors on

patients with proliferative diabetic retinopathy or diabetic macular edema: the role of Lipocalin2. *BMC Ophthalmol*. 2022;22(1):496.
12. Yu Y, Zhang J, Zhu R, Zhao R, Chen J, Jin J, et al. The Profile of Angiogenic Factors in Vitreous Humor of the Patients with Proliferative Diabetic Retinopathy. *Curr Mol Med*. 2017;17(4):280–6.
13. Abcouwer SF. Angiogenic factors and cytokines in diabetic retinopathy. *J Clin Cell Immunol*. 2013;4(4):287.
14. Zhang X, Qiu B, Wang Q, Sivaprasad S, Wang Y, Zhao L, et al. Dysregulated serum lipid metabolism promotes the occurrence and development of diabetic retinopathy associated with upregulated circulating levels of VEGF-A, VEGF-D, and PlGF. *Front Med (Lausanne)*. 2021;8:779413.
15. Murugeswari P, Shukla D, Kim R, Namperumalsamy P, Stitt AW, Muthukkaruppan V, et al. Angiogenic potential of vitreous from proliferative diabetic retinopathy and Eales' disease patients. *PLoS One*. 2014;9(10):e107551.
16. Hunt D, Hemmingsen B, Matzke A, Varghese C, Hammerich A, Luciani S, et al. The WHO Global Diabetes Compact: a new initiative to support people living with diabetes. *Lancet Diabetes Endocrinol*. 2021;9(6):325–27.
17. Ali MK, Seiglie JA, Narayan KV. Progress in diabetes prevention or epidemiology-or both, or neither? *Lancet Diabetes Endocrinol*. 2021;9(4):190–1.

Author's biography

Smriti Misra, Consultant

Saurabh Gangwar, Associate Professor

Gargi Agarwal, Senior Resident

Samreen Mehfooz, Assistant Professor

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