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C-reactive protein as a biomarker for diabetic retinopathy: Insights from a tertiary referral hospital in India

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ABSTRACT

Background: High blood glucose levels in diabetes mellitus (DM) may damage the eyes and cause diabetic retinopathy leading to blindness. Diabetes retinopathy (DR) evolves from NPDR to PDR during the disease course. CRP and other inflammatory mediators can serve as markers for early DR identification.
Aims & Objective: To study C- reactive protein (CRP) as a potential biomarker for diabetic retinopathy and its severity in diabetic population.
Materials and Methods: An observational prospective study was performed from October 2020 to October 2022 at a tertiary care referral centre in western India. We enrolled 115 type 2 diabetics with any grade of diabetic retinopathy. Apart from thorough ophthalmological examination, CRP levels were measured using a particle-enhanced turbidimetric immunoassay. Data analysis was done using SPSS 26.0, with a $p<0.05$ considered as significant for this study. Results CRP was positive in 15.7% subjects. There were no significant gender or age differences between CRP positive and negative groups, with an average age of 56.82 ± 9.26 years. Most patients (69.6%) had uncontrolled diabetes and used medication. High CRP was strongly linked to high FBS, PP2BS, and HbA1c. Positive CRP was linked to increased PDR rates, showing a relationship between CRP and DR severity.
Conclusion: CRP levels are connected to high blood sugar and HbA1c, however few diabetic retinopathy (DR) patients have higher levels. DR screening cannot be done using CRP alone. Further study with bigger samples is required to validate these results and develop other inflammatory indicators for early DR identification and treatment.

1. Introduction

Diabetes mellitus (DM) is a life style disorder characterized by high blood glucose levels owing to inadequate insulin synthesis or action. Chronic hyperglycaemia linked with diabetes causes damage and malfunction in a variety of organs, including the retina, kidneys, neurological system, heart, and blood vessels.Among them, the eye is severely impacted, with microvascular consequences such as diabetic retinopathy (DR). Even though progression of diabetic retinopathy takes decades to develop blindness, DR already accounts for a large proportion of blindness cases in India. Not just in India, it is the leading cause of vision impairment and blindness worldwide. [1,2]

Pathogenesis and progression of DR can be divided in to two stages: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Microaneurysms, hemorrhages, cotton-wool patches, soft and hard exudates are seen in NPDR, while neovascularization of the retina (NVE), disc (NVD), iris (NVI) or angle (NVA), pre-retinal vitreous hemorrhages and resultant tractional retinal detachment comprises of

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PDR.[3,4] The pathophysiology of DR includes altered metabolic pathways, oxidative stress, and inflammation. Inflammatory mediators such as C-reactive protein (CRP), Tumor Necrosis Factor-a (TNF-a), and interleukin-6 (IL-6) play an important role in the pathophysiology of diabetic retinopathy, according to available literature. Some of the inflammatory mediators involved in both systemic and local inflammation have been investigated as potential biomarkers of DR.[5]

One of these markers is C-reactive protein (CRP). C-reactive protein (CRP) was discovered in 1930. The liver produces pentraxin-family calcium-dependent ligand-binding protein CRP in response to IL-6. It is an acute-phase protein that serves as a marker for inflammation and tissue damage. CRP has been linked with macrovascular disease.[6] Some studies have revealed a correlation between CRP levels and the occurrence of DR in both type 1 and type 2 diabetic individuals.[7]

The purpose of this research is to determine if CRP can be used as a marker to detect diabetic retinopathy in afflicted individuals. Given the absence of widespread DR screening in India owing to cost and logistical constraints, there is an urgent need for a simple, cost-effective, and reproducible screening method to aid in early identification and referral for subsequent therapy. To prevent permanent vision loss caused by DR, apart from broad public education on its incidence and risk factors, what we require is its timely diagnosis in this high-risk groups to treat it earlier and prevent burden of blindness in society where diabetic population is ever increasing. We believe that cost-effective screening tools will be extremely helpful in this battle especially in a growing economy like India.

The purpose of the study is to determine if CRP levels and the severity of diabetic retinopathy are related. Additionally, the study intends to evaluate changes in the association between the two variables at various phases of the disease.

2. Materials and Methods

Ours was a prospective observational study performed in a tertiary referral hospital in western India during october 2020 to october 2022. Approval was obtained from the Institutional Ethics Committee. We have strictly adhered to declaration of Helsinki of 1964 throughout the study.

We enrolled 115 patients of type 2 DM with diabetic retinopathy in our study with consecutive sampling method.We had included type 2 diabetic patients with age range of 18 to 70 years with fundus findings of NPDR or PDR who were willing to give consent. The exclusion criteria included any media opacity (such VH or SHH) unrelated to diabetic retinopathy or any past history of treatment of DR or having other systemic or local disorders that can affect CRP levels.

All the 115 patients were subjected to thourough ophthalmic examination including visual acuity by snellen's chart, slit lamp examination, IOP measurement by applanation tonometry or non-contact tonometer (NCT) and posterior segment evaluation by indirect ophthalmoscopy, slit lamp biomicroscopy and/or B-scan ultrasonography (USG) whenever required. Diabetic retinopathy severity was graded using the international diabetic retinopathy classification.[8]

CRP levels were assessed using a particle-enhanced turbidimetric immunoassay which is briefly described hereby.The principle of this method is using latex particles coated with anti-CRP antibodies, which, when combined with CRP in a sample, form insoluble antigen-antibody complexes. These complexes can increase turbidity, which is measured at 550 nm spectrophotometrically. The concentration of CRP in the sample is directly correlated with the extent of turbidity, which can be analysed quantitatively against a calibrator. The kit components include a buffer solution, latex reagent containing goat antibodies specific to CRP, and a calibrator comprising inactivated human serum with known CRP levels.

Mixing nine parts of the buffer with one part of the latex reagent makes the working reagent. Samples and the calibrator are pipetted into separate containers and then combined with the working reagent. The initial and final absorbance of each mixture is measured to calculate the concentration of CRP present in the sample. Control materials must consistently fall within defined ranges to ensure the accuracy and reliability of the CRP measurement. The reference range for CRP concentration provided by this method typically extends up to 6-8 mg/liter.

Data was gathered and processed in Excel. Numbers and percentages represented qualitative data. We used mean and standard deviation for quantitative data. SPSS 26.0 (IBM, SPSS, Inc.) performed statistical analysis. $P<0.05$ was considered as statistically significant for this study purpose.

3. Results

Out of 115 patients that we had enrolled in the study, 15.7% (18 patients) were CRP positive whereas 84.3% (97 patients) were CRP negative. The study population's average age was 56.82 ± 9.26 years. CRP positive patients had a mean age of 53.28±13.17 years, while the mean age of CRP negative patients was 57.48 ±8.27-years. Patients with CRP positive and negative did not vary in mean age in a way that was statistically significant ($p = 0.2070$).

In terms of gender distribution, 85 of the 115 patients were male (74%), while 30 patients were female (26%). Gender-specific analysis of the CRP status revealed that of the patients with a negative CRP, there were 25 females (83.3%) and 72 men (84.7%). There were 13 men (15.3%) and 5 women (16.7%) in the CRP positive group. Between

CRP positive and CRP negative individuals, there was no discernible variation in the gender distribution (p = 0.8588).

Present study had 50.4% (58 patients) individuals with only diabetes as a systemic illness, whereas 36.5% (42 patients) had hypertension with diabetes, and 13.0% (15 patients) had other illnesses such as breathlessness, heart disease, cerebrovascular events in past in addition to diabetes.

In terms of prior cataract surgery, 19 patients (16.52%) had its history in both the eyes. In 13% and 6.96% patients (15 and 8 patients respectively), only right and left eye was operated for cataract in the past. Majority 61.7% (73 patients) of the patients had no surgical history. There was no statistical correlation between CRP positivity cataract surgery. (p=0.304)

The study population's total mean duration of diabetes was 10.72 ±6.47 years. In particular, the mean duration of diabetes in CRP-negative individuals was 10.63±6.77 years, whereas the mean duration in CRP-positive patients was 11.25±4.75 years. No statistically significant association was found between duration of diabetes and CRP positivity/ negativity (p = 0.708).

The research population's diabetes control was adequate only in 35 patients (30.4%) of the total, whereas rest 80 patients (69.6%) of the group, had uncontrolled diabetes. Among 35 patients with well controlled diabetes, 31 patients (88.6%) were CRP negative, and rest 4 patients (11.4%) were CRP positive. Of the patients with uncontrolled diabetes, 14 patients (17.5%) had a positive CRP reading and 66 patients (82.5%) were CRP negative. Patients with CRP positive and negative did not significantly vary in their diabetes management (p = 0.4096).35 out of 115 (34%) of the patients in our study was not taking any treatment for their diabetes whereas the rest 69.6% (80 patients out of 115) were. When the CRP status of patients taking diabetic medication was examined, it was found that 4 patients (11.4%) without medication had a CRP positive state and 31 patients (88.6%) had a CRP negative condition. Of the individuals receiving medication, 66 (82.5%) were CRP negative and 14 (17.5%) cases were CRP positive. This difference was not statistically significant (p=0.4096).

Presenting best corrected visual acuity (BCVA) in our study group is summarised in the following table. (Table 1)

Table 1: Presenting best corrected visual acuity (BCVA)

Best corrected visual acuity (BCVA)	RE	LE
< 6/60	32	40
6-60 – 6/18	54	51
>6/18	29	24

Following is the distribution of grades of diabetic retinopathy as per international classification in both eyes of our study subjects. (Table 2) 15.65% (18 patients) of individuals with diabetic retinopathy tested positive for CRP.

Table 2: Distribution of grading of diabetic retinopathy in our study population

Grading of Diabetic retinopathy	RE (%)	LE (%)
Mild NPDR	4 (3.5)	7(6.1)
Moderate NPDR	31 (27)	27 (23.47)
Severe NPDR	25 (21.7)	21(18.3)
PDR	55(47.8)	60 (52.2)
Total	115 (100)	115 (100)

Proliferative diabetic retinopathy (PDR) instances were seen in all age groups among individuals with diabetic retinopathy. The age range of 61-70 years was the most frequent for severe NPDR, while the age group of 51-60 years was the most common for mild NPDR. The correlation of age with grade of diabetic retinopathy is as per Figure 1.

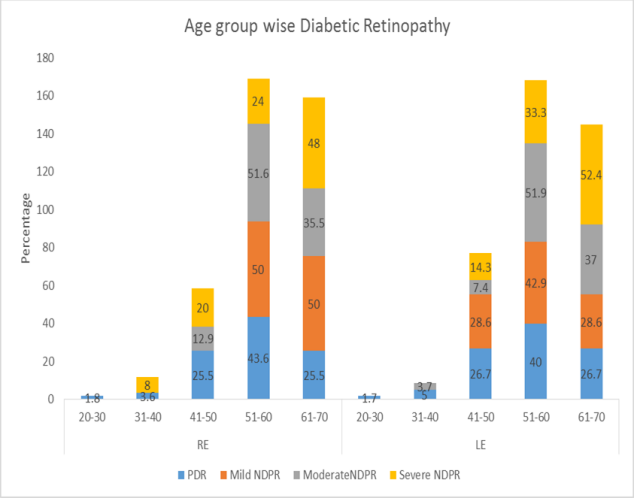

Figure 1: Correlation of age group with grades of diabetic retinopathy

Similarly, correlation between gender and different grades of diabetic retinopathy is summarised in Figure 2. Males were more likely than females to develop diabetic retinopathy, both NPDR and PDR, and this incidence was greater for all forms of retinopathy. 84.5% eyes (average of 88% right eye and 81% left eye) with severe NPDR belonged to males. And 72.55% eyes (average of 71% right eye and 74.1% left eye) with moderate NPDR belonged to males.

Patients with PDR had the highest fasting blood sugar (FBS) levels (mean: 171.17) among those with diabetic retinopathy. FBS levels gradually dropped in NPDR patients. Postprandial blood sugar (PP2BS) levels similarly peaked in PDR cases (mean: 224.15) and fell in NPDR cases. Echoing above 2 findings of FBS and PPBS, HbA1c values were greatest in PDR cases (mean: 7.39).

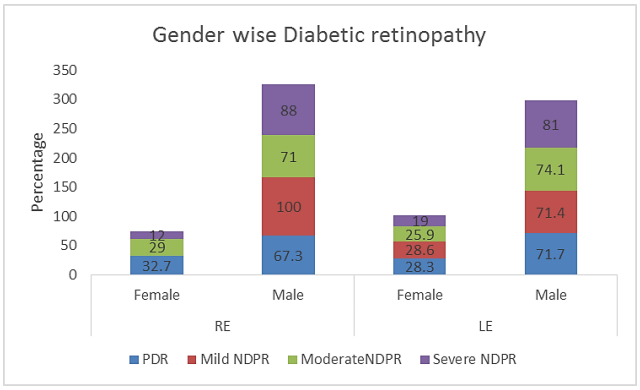

Figure 2: Correlation of gender with grades of diabetic retinopathy

Individuals with positive CRP were shown to have considerably higher blood sugar and HbA1c values than individuals with negative CRP. There was a significant difference (p = 0.0164) in the mean fasting blood sugar (FBS) for CRP negative patients and CRP positive patients, which was 145.11±60.97 and 184.95±77.48 respectively. A significant difference (p = 0.0175) was also seen in the mean postprandial blood sugar (PP2BS), which was 191.39± 70.93 for CRP negative patients and 239.34±107.19 for CRP positive individuals. There was a significant difference (p = 0.0354) in the mean HbA1c for CRP positive patients and CRP negative patients, which was 7.92±1.87 and 6.89±1.06, respectively.

4. Discussion

India is developing as a worldwide diabetes hotspot, with a continually growing diabetic population.[9] This rise brings a slew of problems, including diabetic retinopathy, a microvascular disease of the eyes. However, limited resources of our healthcare system and rapidly growing population of diabetic people in the country creates substantial hurdles for prompt diagnosis and treatment of diabetic retinopathy, which often results in permanent blindness. The time of appearance of diabetic retinopathy and its further course varies depending on diabetes duration and glycaemic control. Given the fluctuating nature of blood sugar levels, diabetic individuals need frequent medical checkups and eye exams to detect and manage diabetic retinopathy at the earliest for which routine eye examinations are crucial.

Many inflammatory mediators have been studied in past to find correlation between them and diabetes and diabetic retinopathy.[10–15] CRP is an easily measured inflammatory biomarker, and the relationship between CRP and systemic disease has been extensively studied. A large body of published data shows that higher CRP concentrations consistently correlate significantly with an increased risk of atherosclerosis, cardiovascular disease, and diabetes.[3,6,16–20] Prospective clinical investigations have shown a link between raised CRP levels and Type 1 and Type 2 diabetes, as well as an increased risk of diabetic complications such DR.

The purpose of this research was to determine the function of C- reactive protein in Diabetic Retinopathy and its relationship to various stages of retinopathy. The pathophysiology of diabetic retinopathy includes various inflammatory mediators; hence we have a variety of assays to identify these mediators. In this work, we looked at the function of one such mediator, the C-reactive protein. This might be used as an early screening tool for diabetic retinopathy when the patient has his normal blood test, and healthcare practitioners may be able to identify individuals who are at a higher risk of diabetic retinopathy and initiate treatment as soon as possible. This research attempted to identify a link between CRP and diabetic retinopathy and various degrees of retinopathy.

Present two-year analytical research included 115 diabetic retinopathy patients of various grades of the disease. Comprehensive eye exams, including visual acuity and posterior segment evaluation, were conducted, followed by CRP measurement utilizing an immunoturbidometric test.

In our study, the total mean age of CRP positive and negative patients did not vary significantly. (p=0.2070). Our research is analogous to the work of Sen D et al.[21] He also could not find a significant difference between CRP positive and negative patients. As a result, we may conclude that age was not connected with CRP levels. The present study found no significant difference between CRP positive men and females. (p=0.8588) in concordance with findings of Sen D et al.[21] Henceforth, gender and CRP levels does not seem to have statistically significant relationship.

We found that the level of CRP did not significantly correlate with number of diabetes patients on medication (p=0.4096), diabetic control (p=0.4096), or duration of diabetes (p=0.7080). In Yang XF. et al.'s research,[22] they also discovered that there was no significant difference in CRP levels among individuals with diabetic retinopathy.

We found that the mean HbA1C (p=0.0354), mean PP2BS (p=0.0175), and mean FBS (p=0.0164) of CRP positive patients were substantially greater than those of CRP negative patients. This statistically significant relationship was confirmed in the research of Yang XF. et al.[22]

We found that PDR cases had higher values for FBS, PP2BS, and HbA1c.

Total 18 patients were CRP positive in our study in which 72.22% had PDR, 11.11% had severe NPDR, 11.11% had moderate NPDR, and 5.55% had mild NDPR. Our research indicates that PDR was more common in individuals who tested positive for CRP and PDR patients had greater levels of these markers, indicating a relationship

between inflammation and disease severity. Our research was similar to that of Nada WM et al.,[23] Song et al.[3] and Gorska- Ciebiada et al.[24] Our study has 2 main limitations. One is small sample size which can be mitigated by further researches in future with larger sample sizes and population studies which can be helpful in reaffirming results of the present study. Also, diabetes affects numerous systems, including the renal system, and makes patients more susceptible to infections which might also increase the level of CRP, complicating patient selection for this research, particularly in those with severe retinopathy. Even when CRP is increased, it may not always indicate diabetic retinopathy. We have tried our best to avoid enrolling such patients to minimize effects of them on to CRP levels. More research is required to identify and eliminate all such confounding factors.

5. Conclusion

In the present study, CRP, an inflammatory mediator involved in the aetiology of diabetic retinopathy (DR), was examined for an association with DR severity. Ours was a mostly middle-aged male sample, with HbA1C levels associated with DR advancement. Most patients were recently diagnosed with DR and were unaware of the need of frequent fundus checks. While CRP levels were typically within normal limits and had no statistically consistent link with DR, they were significantly high in PDR. HbA1C, FBS, and PP2BS levels were significantly higher in C-reactive protein (CRP) positive individuals. Only 15.65% of diabetic retinopathy patients tested positive for CRP, but the majority of Proliferative DR (PDR) cases showed CRP positivity

However, the small number of CRP-positive DR patients implies that it is not a reliable indicator of DR, probably owing to its relationship with other systemic disorders. We found CRP's usefulness as a DR screening tool to be ambiguous. As a result, we conclude that CRP is not a reliable screening tool for diabetic retinopathy (DR). Further large sample size studies are essential not just to confirm our findings but also to find other inflammatory indicators that contribute to DR development, so that we can develop easy, rapid and cost-effective markers for early detection and management of DR and decrease the financial burden and burden of blindness on the suffering families and society at large.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Pandey SK, Sharma V. World diabetes day 2018: Battling the emerging epidemic of diabetic retinopathy. *Indian J Ophthalmol*. 2018;66(11):1652–3.
2. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. 2012;35(3):556–64.
3. Song J, Chen S, Liu X, Duan H, Kong J, Li Z. Relationship between C-Reactive Protein Level and Diabetic Retinopathy: A Systematic Review and Meta-Analysis. *PLoS One*. 2015;10(12):e0144406.
4. Chistiakov DA. Diabetic retinopathy: pathogenic mechanisms and current treatments. *Diabetes Metab Syndr*. 2011;5(3):165–72.
5. Kern TS. Contributions of inflammatory processes to the development of the early stages of diabetic retinopathy. *Exp Diabetes Res*. 2007;2007:95103.
6. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest*. 2003;111(12):1805–12.
7. Sasongko MB, Wong TY, Jenkins AJ, Nguyen TT, Shaw JE, Wang JJ. Circulating markers of inflammation and endothelial function, and their relationship to diabetic retinopathy. *Diabet Med*. 2015;32(5):689–91.
8. Wilkinson CP, Ferris FL, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology*. 2003;110(9):1677–82.
9. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK, et al. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR-INDIAB population-based cross-sectional study. *Lancet Diabetes Endocrinol*. 2017;5(8):585–96.
10. Spijkerman AM, Gall MA, Tarnow L, Twisk JW, Lauritzen E, Lund-Andersen H, et al. Endothelial dysfunction and low-grade inflammation and the progression of retinopathy in type 2 diabetes. *Diabet Med*. 2007;24(9):969–76.
11. Le D, Miles R, Savage PJ, Cornell E, Tracy RP, Knowler WC, et al. The association of plasma fibrinogen concentration with diabetic microvascular complications in young adults with early-onset of type 2 diabetes. *Diabetes Res Clin Pract*. 2008;82(3):317–23.
12. Klein BE, Knudtson MD, Tsai MY, Klein R. The Relation of Markers of Inflammation and Endothelial Dysfunction to the Prevalence and Progression of Diabetic Retinopathy. Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Arch Ophthalmol*. 2009;127(9):1175–82.
13. Nguyen TT, Alibrahim E, Islam FA, Klein R, Klein BE, Cotch MF, et al. Inflammatory, hemostatic, and other novel biomarkers for diabetic retinopathy: the multi-ethnic study of atherosclerosis. *Diabetes Care*. 2009;32(9):1704–9.
14. Koleva-Georgieva DN, Sivkova NP, Terzieva D. Serum inflammatory cytokines IL-1beta, IL-6, TNF-alpha and VEGF have influence on the development of diabetic retinopathy. *Folia Med (Plovdiv)*. 2011;53(2):44–50.
15. Rangasamy S, Srinivasan R, Maestas J, Mcguire PG, Das A. A potential role for angiopoietin 2 in the regulation of the blood-retinal barrier in diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2011;52(6):3784–91.
16. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 2003;107(3):363–9.
17. Kobayashi S, Inoue N, Ohashi Y, Terashima M, Matsui K, Mori T, et al. Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. *Arterioscler Thromb Vasc Biol*. 2003;23(8):1398–1404.
18. Liu F, Chen HY, Huang XR, Chung AC, Zhou L, Fu P, et al. C-reactive protein promotes diabetic kidney disease in a mouse model of type 1 diabetes. *Diabetologia*. 2011;54(10):2713–23.
19. Badimon L, Pena E, Arderiu G, Padro T, Slevin M, Vilahur G, et al. C-reactive protein in atherothrombosis and angiogenesis. *Front Immunol*. 2018;9:430.
20. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Front Immunol*. 2018;9:754.

21. Sen D, Ghosh S, Roy D. Correlation of C-reactive protein and body mass index with diabetic retinopathy in Indian population. *Diabetes Metab Syndr*. 2015;9(1):28–9.
22. Yang XF, Deng Y, Gu H, Lim A, Snellingen T, Liu XP, et al. C-reactive protein and diabetic retinopathy in Chinese patients with type 2 diabetes mellitus. *Int J Ophthalmol*. 2016;9(1):111–8.
23. Nada WM, Da AM. Serum C-reactive protein and diabetic retinopathy. *Open J Ophthalmol*. 2017;7(2):73–81.
24. Gorska-Ciebiada M, Saryusz-Wolska M, Borkowska A, Ciebiada M, Loba J. C-reactive protein, advanced glycation end products, and their receptor in type 2 diabetic, elderly patients with mild cognitive impairment. *Front Aging Neurosci*. 2015;7:209.

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