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Research Article

To Study the Role of Biochemical Biomarkers in Type 2 Diabetes Patients with Diabetic Retinopathy

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Abstract

Background: Diabetic retinopathy is the most common and severe microvascular complication of diabetes mellitus. It is the leading cause of irreversible blindness and the prevalence is expected to double by 2030. Comparing biochemical biomarkers in type2 diabetes patients with retinopathy (DR), patients without retinopathy (NDR) and controls.

Method: Study comprised of 3 groups DR (n = 66), NDR (n = 33) and controls (n = 33). Detailed work up included HbA1c levels, blood pressure, hs-CRP (high sensitivity C-reactive protein), white cell count, ESR, BMI, waist circumference.

Results: Mean age was 54yrs. Mean duration of DM was 10 and 4yrs in DR and NDR group. The mean hs-CRP values were significant between diabetics and controls irrespective of the status of retinopathy (p-0.00). For the diabetic population, BMI (body mass index) was positively correlated with CRP (p-0.001). Significance of this relationship was still maintained even after controlling effects of BMI. Amongst the study groups, mean ESR (erythrocyte sedimentation rate) values were higher significantly in diabetes with and without retinopathy than controls and also increased significantly with increasing severity of retinopathy (p-0.00). Patients with PDR (proliferative diabetic retinopathy) had the highest levels of ESR (38.35 ± 12.9).

Conclusion: Our study did not establish any significant relationship between diabetic retinopathy and hs-CRP. Thus, the role of hs-CRP remains controversial.

Keywords: *Diabetes; Diabetic Retinopathy; Non Proliferative Diabetic Retinopathy; Proliferative Diabetic Retinopathy; High Sensitivity C-Reactive Protein; ESR*

Introduction

Currently the world is facing a challenge of epidemic proportions with Diabetes Mellitus (DM). According to the estimates of the World Health Organization, the number of people worldwide with diabetes mellitus will rise to around 360 million by the year 2030 [1]. Diabetic retinopathy is one the most common and severe microvascular complication of DM and the leading cause of irreversible blindness [2]. Epidemiological, clinical, and laboratory studies have shown a number of interconnected mechanisms associated with hyperglycemia and increased our understanding of the pathophysiological changes of diabetic retinopathy (DR) culminating recently in screening programmes, early diagnosis and new treatment options for diabetic retinopathy but despite these advances, the prevalence of DR remains high around 40% [3] and not all patients are benefitted or prevented from developing the disease or its consequences, reinforcing the fact that DR is a multifactorial disease [3].

There are various established known risk factors of DR of which duration of diabetes is probably the strongest predictor of the disease for development and progression of retinopathy [4]. Others include degree of hyperglycemia, hypertension, deranged lipid profile etc [4].

Therapeutic approaches targeting inflammation such as intra-vitreal injection of anti-VEGF and corticosteroids have been shown to be effective in slowing down the development and progression of DR [3,5]. Inflammatory biomarkers such as White cell count, ESR, hs-CRP, prostaglandin E1 and E2, IL-6 have been studied in the pathogenesis of diabetic retinopathy [6].

Purpose of the Study

The purpose of this study is to evaluate the role and compare the various inflammatory biomarkers in diabetic patients with and without retinopathy and to associate these biomarkers with the stages of DR and macular edema. This may help in identifying diabetic patients at risk of developing DR, such patients can be screened and diagnosed at early stages.

Method

Patients which were included in the study had given a full, free and voluntary consent.

Study participants consisted of patients visiting the outpatient department of ophthalmology with Diabetes mellitus, referred from the department of endocrinology and metabolism and normal individuals at a tertiary eye hospital in north India over a period of 13 months from November 2016 to February 2018. This was an observational, cross sectional study.

Patients were divided into three groups:

- Group A comprising of diabetic patients with retinopathy,
- Group B diabetics without retinopathy and
- Group C non diabetic age and gender matched controls.

Group A was further divided into 4 groups according to severity of retinopathy:

- A1: Mild non proliferative diabetic retinopathy (NPDR),
- A2: Moderate non proliferative diabetic retinopathy,
- A3: Severe non proliferative diabetic retinopathy,
- A4: Proliferative diabetic retinopathy (PDR).

Maculopathy was not graded separately.

Patients were excluded if they were known to have (1) hemoglobinopathies, (2) acute and chronic inflammatory conditions, (3) with media opacities hindering diagnosis and classification of diabetic retinopathy, (4) on antiplatelet drugs for the past 2 weeks, (5) Unwilling and uncooperative patients.

Once the patient qualified for inclusion in the study (after screening), the detailed assessment of all study participants was done. It consisted of general physical examination, measurement of best corrected Snellen's distant visual acuity, slit-lamp evaluation of the anterior segment, followed by dilated fundus evaluation using 90 Dioptre lens and indirect ophthalmoscope after pupillary dilatation of at least 5 mm by 1% tropicamide eye drop.

On the basis of fundus examination, Diabetic Retinopathy was graded according to the 'International Clinical Classification System'.

This international clinical classification system is based on an evidence-based approach, particularly the findings of the ETDRS and the WESDR (Table 1 and 2).

Proposed Disease Severity Level	Findings Observable on Dilated ophthalmoscopy
No apparent retinopathy	No abnormalities
Mild nonproliferative DR	Microaneurysms only
Moderate nonproliferative DR	More than just microaneurysms, but less than severe non-proliferative diabetic retinopathy
Severe nonproliferative DR	Any of the following: • >20 intraretinal hemorrhages in each of 4 quadrants • Definite venous beading in 2+ quadrants • Prominent intraretinal microvascular abnormalities in 1+ quadrant and no signs of proliferative retinopathy
Proliferative diabetic retinopathy	One or more of the following: Neovascularisation, vitreal/preretinal hemorrhage

Table 1: *Diabetic retinopathy disease severity scale.*

Abbreviation: DR: Diabetic Retinopathy.

Proposed disease severity level	Findings Observable on Dilated Ophthalmoscopy
Diabetic macular edema apparently absent	No apparent retinal thickening or hard exudates in posterior pole
Diabetic macular edema apparently present	Some apparent retinal thickening or hard exudates in posterior pole
If diabetic macular edema is present, it can be categorized as follows:	**1. Mild diabetic macular edema**: Some retinal thickening or hard exudates in posterior pole but distant from the center of the macula **2. Moderate diabetic macular edema**: Retinal thickening or hard exudates approaching the center of the macula but not involving the center **3. Severe diabetic macular edema**: Retinal thickening or hard exudates involving the center of the macula

Table 2: *Diabetic macular edema disease severity scale.*

**Hard exudates are a sign of current or previous macular edema. Diabetic macular edema is defined as retinal thickening, and this requires a three-dimensional assessment that is best performed by a dilated examination using slit-lamp biomicroscopy and/or stereo fundus photography.*

Optical Coherence Tomography (OCT) scans were performed to measure the central macular thickness. {CIRRUS high definition optical coherence tomography (HD-OCT) 4000 (Carl Zeiss)}.

Fundus Fluorescein Angiography was done wherever it was possible and required.

Blood samples were collected and hs-CRP was done by the ELISA technique as per manufacturer's instructions. The samples were first diluted using dilution buffer 101 fold. Then 100 microlitre of EIA buffer was put into each well to which 25 microlitre of calibrators CAL 1-6 and samples were added. This was incubated for 30 minutes at 37°C. A minimum of 250 microlitre washing solution was put per well and the strips were washed 3 times. Now 100microlitre of HRP conjugate was dispensed into the wells and incubated at 37°C for 30 minutes. Strips were washed 5 times thereafter. Now 100 microlitre of TMB substrate was put into the wells and incubated for 15 minutes at 25°C. 100 microlitre of stop solution was dispensed into the wells thereafter. Optical density (OD) was then measured at 450 nm and point by point method was applied for data reduction.

Criteria for sample size selection and statistical analysis

Date was analysed using SPSS software version 20 of windows. Data was presented as mean ± SD or media ± range depending whether the data is normal or non-normal respectively. One way ANOVA/Kruskal Wallis test was used depending on normality conditions and post hoc Tukey test/Mann Whitney test was used to compare the values among the 3 groups.

Chi square test was used for comparing the proportion among the 3 groups.

Observations and Results

Descriptive analysis

Most patients belonged to the age group of 51-65 years (56.1%) of which 62.12% were diabetics with retinopathy, 54.5% were diabetics without retinopathy and 40% were healthy controls.

The mean age of patients was 53.24 ± 9.68 years in control group, 54.64 ± 10.79 years in diabetics without retinopathy and 54.59 ± 8.63 years in diabetics with retinopathy which was not significantly different from each other (p-value 0.774).

The male to female ratio was 1.59:1. The distribution of males and females was not significantly different between the groups (p-value 0.182).

The mean duration of diabetes in group A was 10.29 ± 7.2 years and group B was 3.83 ± 3.2 years. Duration of diabetes mellitus was significantly more in group A than B (p-value 0.00). 25% of PDR patients had diabetes of more than 15 years duration.

Inflammatory markers

Hs-CRP values

Hs-CRP values ranged from 0 to 366.82. The mean hs-CRP in the controls, diabetics without and with retinopathy was highly significant (p-value 0.00) (Table 3).

	Group A	Group B	Group C	Total
0-10.99	03	01	27	31
11-20.99	09	05	06	20
21-30.99	11	11	0	22
31-50.99	13	04	0	17
51-100.99	13	08	0	21
101-150.99	12	02	0	14
151-400	05	01	0	6
Total	66	32	33	131*

Table 3: *hs-CRP values in the three groups.*

**Hs-CRP value of one patient was not available due to machine error.*

Statistical analysis also showed that the hs-CRP values were significant between groups B and C (p-value 0.003) and also between groups A and C (p-value 0.000) but no significant difference was found between group A and B (p-value 0.358).

There was no statistically significant difference in the mean hs-CRP values in the subgroups of retinopathy group (p-value 0.836) showing that hs-CRP values are high in diabetics and still higher in diabetics with retinopathy than normal controls but do not correlate with the severity of diabetic retinopathy (Table 3).

When these hs-CRP values were compared between the three groups after controlling effects of BMI, the significance of relationship was still maintained (p-value 0.001).

ESR

ESR is a very non-specific marker of inflammation. The range was from 0 to 66 and the mean ESR being 23.74 ± 13.2. On comparing the mean ESR in the three groups the values came out to be statistically significant (p-value 0.00) (Table 4).

	Mean ESR	P value
Group A	30.74 ± 12	
Group B	23.39 ± 10.2	0.00
Group C	10.09 ± 4.6	
Total	23.74 ± 13.2	

Table 4: *Mean ESR values in the three groups.*

Mean ESR values of group A and B were compared separately also and showed a significant difference (p-value 0.003).

The mean ESR in the 4 subgroups was also statistically significant (p-value 0.04) (Table 5).

	Mean ESR	P value
Group A1	21.90 ± 2.6	0.04
Group A2	30.26 ± 11.7	
Group A3	28.88 ± 11.06	
Group A4	38.35 ± 12.9	

Table 5: *Mean ESR values in the subgroups of retinopathy group.*

Discussion

It is a multisystem disease affecting eyes, kidneys, peripheral nerves, and micro- and macro-vessels due to chronic hyperglycemia. Diabetic retinopathy (DR) is one of the most common and severe complication of DM and the leading cause of blindness in adults aged 20-75yrs [7].

The natural evolution of DR includes many mechanisms including abnormal metabolic pathways, oxidative stress and subclinical inflammation, however the specific mechanism is still not fully understood [8]. Some therapeutic approaches targeting inflammation such as intravitreal injection of anti-VEGF and corticosteroids have been shown to be effective in slowing down the progression of DR [3,5]. Therefore, inflammation has been postulated to be an important part in the development and progression of DR.

The role of inflammation in the pathogenesis of diabetic retinopathy was first observed by Powell and Field in 1960 where they found that diabetics treated with anti-inflammatory agents like 'salicylate' had a lower incidence rate of diabetic retinopathy [9].

Various inflammatory biomarkers such as White cell count, ESR, hs-CRP, prostaglandin E1 and E2, IL-6 have also been studied in the pathogenesis of diabetic retinopathy [6].

C-reactive protein (CRP), identified in the 1930s, is an acute-phase protein and is mainly synthesized by the liver and adipose tissue on microbial invasion or tissue injury [10], measurement of which is useful for diagnosis and treatment of acute or chronic inflammatory conditions [11].

Emerging laboratory and epidemiologic data have now associated hs-CRP levels with impaired insulin sensitivity and the development of dysglycemic conditions, including the cardiometabolic syndrome and incident of type 2 diabetes [12]. However, the role of CRP in the pathogenesis of DR is still unknown.

Various clinical studies have investigated the relationship between CRP level and DR, most of which have been inconclusive, like a study conducted by Gholamhossein., *et al*. [13], measured the mean plasma levels of erythropoietin in proliferative Diabetic Retinopathy group which showed a significant difference in comparison to other grades of retinopathy. The mean plasma levels of hs-CRP, cholesterol, triglyceride, apolipoproteins A and B, and fasting blood glucose were not significantly different in the three groups namely no retinopathy, non-proliferative retinopathy and proliferative retinopathy.

The absolute relative risk (ARR) also showed that erythropoietin was an increasing risk for proliferative DR (ARR, 1.17; 95% confidence interval, 1.060 to 1.420; odds ratio,1.060) [13].

The stepwise rise in mean plasma erythropoietin level demonstrated the significant correlation and role of erythropoietin with proliferative DR versus non proliferative retinopathy and no retinopathy in diabetes [13].

Similar to this, our study also did not find any conclusive association between hs-CRP levels and diabetic retinopathy. Although it was significantly higher in diabetics than controls, irrespective of the status of retinopathy (p-value 0.836). For the diabetic population, BMI was positively correlated with CRP (p-value 0.001). Therefore, BMI (p-value 0.001) was a significant predictor of CRP. When these hs-CRP values were compared between the three groups after controlling effects of BMI, the significance of relationship was still maintained.

As the CRP is produced and secreted from the liver in response to the pro-inflammation cytokines such as IL-6 and TNF-a, it is recognized as one of the significant markers for inflammation. But in the condition of obesity, the hs-CRP should, however, be elevated by the hypersecretion of adipocytokines such as IL-6 and TNF-a from the adipocytes without inflammation. In fact, the serum hs-CRP level is directly associated with body weight or BMI and is therefore elevated in obese participants [14].

In another study by Tsunoda., *et al*. [14], the clinical significance of serum high sensitivity C-reactive protein (hs-CRP) in relation to chronic diabetic complications using 114 Japanese patients with Type 2 diabetes mellitus was studied. Multiple regression analysis revealed that retinopathy was not associated with higher serum hs-CRP value in Type 2 diabetic patients, and became more prominent among diabetic patients with hypertension in which the serum hs-CRP level was elevated. This factor was not considered in our study [14].

Another study by Du J-H., *et al*. showed no significant relationship between sex, age, body mass index (BMI), blood pressure, CRP, hemoglobin A1c (HbA1c) in the three groups namely proliferative, non proliferative and no retinopathy [15].

The drawback of this study and the reason for this inconsistency may be due to the higher sensitivity of hs-CRP than CRP as in this study CRP was used as a marker of inflammation rather than high sensitivity CRP. High-sensitivity CRP (hs-CRP) is more precise than standard CRP when measuring baseline (i.e. normal) concentrations and enables a measure of chronic inflammation [16].

Also, C-reactive protein (CRP) is an acute-phase reactant and has high intra-individual variability. Therefore, a single test for high-sensitivity CRP (hs-CRP) may not reflect an individual patient's basal hs-CRP level. Repeat measurement may be required to firmly establish an individual's basal hs-CRP concentration. The lowest of the measurements should be used as the predictive value [16].

Another cause of inconsistency in various studies could be due to the difference in the pathogenesis in type 1 and type 2 diabetes if differentiation was not done in the study. But most of the studies cited here had taken only type 2 diabetic patients as subjects.

On the contrary, study conducted by Jia., *et al*. [17], determined serum ischemia modified albumin (IMA) and high sensitivity CRP in patients with diabetic retinopathy, diabetic patients with no retinopathy and controls and found out that serum IMA and hs-CRP concentrations were significantly higher in DR patients than those in controls and NDR patients, the serum IMA and hs-CRP concentration in NDR patients were significantly higher than those in controls ($F = 197.124, 34.561; q = 5.41\text{-}27.34; P < 0.01$); the serum IMA and hs-CRP concentration were significantly higher in PDR patients than those in NPDR patients ($t = 5.46, 4.89; P < 0.01$); there was significant positive correlation between serum IMA concentration and hs-CRP concentration in DR patients ($r = 0.617, P < 0.01$) [17].

This showed that the serum IMA and hs-CRP concentration were significantly high in DR patients, and were positively associated with the severity of DR, which may also contribute to the development of DR.

Another study by Izuora., *et al*. [6] was also done to see if there is any association between diabetic retinopathy and its severity with inflammatory markers and obtained similar results. Inflammation was measured as levels of CRP. Differences in the levels of the inflammatory markers were compared between the groups with different retinopathy grading. The comparisons were first made without any adjustment and were then repeated following adjustment for age, duration of diabetes, sex, and BMI [6]. PgE_2 levels were significantly higher in the diabetic population than in the control group ($P < 0.001$). This was true for all levels of retinopathy. This relationship remained significant after controlling for the effects of BMI [6].

A significant relationship was found (P-value 0.01) between grades of retinopathy and CRP. However, after controlling for age, duration of diabetes, sex, and BMI, the significance of the relationship was lost (P-value 0.42) [6].

None of the other markers of inflammation were significantly associated with retinopathy.

Another observation made in this study was that for the diabetic population, BMI was positively correlated with CRP and IL-6 before adjustment for age, sex, and duration of diabetes ($P < 0.001$ for both) and after adjustment ($P = 0.04$ and 0.02, respectively) similar to the present study. Age ($P = 0.002$), BMI ($P = 0.001$), and IL-6 ($P = 0.001$) were the only significant predictors of CRP [6].

Hence the role of hs-CRP is still controversial with some studies showing a relationship between retinopathy and hs-CRP [6,17] while some do not [13-15]. Our study also did not show any significant relation between hs-CRP and retinopathy.

Although not a specific marker of inflammation, ESR was compared in the three groups and the difference was significant with ESR increasing from healthy controls to diabetics without retinopathy to with retinopathy (p-value 0.00) (Table 4).

Mean ESR values compared between diabetics without retinopathy and with retinopathy also showed a significant difference (p-value 0.003).

The mean ESR in the 4 subgroups of the first group according to the severity of retinopathy were also statistically significant (p-value 0.04) (Table 5).

This is in contrast to the study by Izuora., *et al*. who found no significant association of retinopathy and ESR [6]. However, most of the studies had not compared ESR with retinopathy [13-15,17].

Conclusion

We found that hs-CRP values were significantly higher in diabetics irrespective of the status of retinopathy compared to the control group but there was no significant difference between the groups with and without retinopathy. Even after controlling the effect of BMI, this relationship was maintained. Therefore, we could not establish any significant relationship between hs-CRP and diabetic retinopathy or consider it as a biomarker for early detection of impending microvascular complications.

Bibliography

1. Gao L., *et al*. "High prevalence of diabetic retinopathy in diabetic patients concomitant with metabolic syndrome". *PLoS One* 11.1 (2016): e0145293.
2. Wild S., *et al*. "Global prevalence of diabetes". *Diabetes Care* 27.5 (2004): 1047-1053.
3. Heng LZ., *et al*. "Diabetic retinopathy: pathogenesis, clinical grading, management and future developments". *Diabetic Medicine* 30.6 (2013): 640-650.
4. Antonetti DA., *et al*. "Diabetic retinopathy". *New England Journal of Medicine* 366.13 (2012): 1227-1239.
5. Rangasamy S., *et al*. "Diabetic retinopathy and inflammation: novel therapeutic targets". *Middle East African Journal of Ophthalmology* 19.1 (2012): 52-59.
6. Colhoun HM., *et al*. "C-reactive protein in type 1 diabetes and its relationship to coronary artery calcification". *Diabetes Care* 25.10 (2002): 1813-1817.
7. Ayhan Tuzcu E., *et al*. "Relationship between mean platelet volume and retinopathy in patients with type 2 diabetes mellitus". *Graefe's Archive for Clinical and Experimental Ophthalmology* 252.2 (2014): 237-240.
8. Tang J and Kern TS. "Inflammation in diabetic retinopathy". *Progress in Retinal and Eye Research* 30.5 (2011): 343-358.
9. Powell EU and Field R. "Diabetic retinopathy and rheumatoid arthritis". *Lancet* 284.7349 (1964): 17-18.
10. Genest J. "C-reactive protein: risk factor, biomarker and/or therapeutic target?" *Canadian Journal of Cardiology* 26 (2010): 41A-44A.
11. Lim LS., *et al*. "C-reactive protein, body mass index, and diabetic retinopathy". *Investigative Ophthalmology and Visual Science* 51.9 (2010): 4458-4463.
12. Ndumele CE., *et al*. "Interrelationships between inflammation, C-reactive protein, and insulin resistance". *Journal of the CardioMetabolic Syndrome* 1.3 (2006): 190-196.
13. Gholamhossein Y., *et al*. "Diabetic retinopathy risk factors: plasma erythropoietin as a risk factor for proliferative diabetic retinopathy". *Korean Journal of Ophthalmology* 28.5 (2014): 373-378.

14. Tsunoda K., *et al*. "Retinopathy and hypertension affect serum high-sensitivity C-reactive protein levels in Type 2 diabetic patients". *Journal of Diabetes and its Complications* 19.3 (2005): 123-127.

15. Du J-H., *et al*. "Elevation of serum apelin-13 associated with proliferative diabetic retinopathy in type 2 diabetic patients". *International Journal of Ophthalmology* 7.6 (2014): 968-973.

16. HSCRP - Clinical: C-Reactive Protein, High Sensitivity, Serum.

17. Jia Z., *et al*. "[Changes of the concentration of serum ischemia modified albumin and high sensitivity C-reactive protein in type 2 diabetic patients with retinopathy]". *Zhonghua Yan Ke Za Zhi* 45.9 (2009): 805-808.

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