

# Association between Total Bilirubin Levels and Diabetic Retinopathy in Patients with Type 2 Diabetes Mellitus

Dr. Kushagra Sharma<sup>1</sup>, Dr. V K Malik<sup>2</sup>, Dr. Sanjay Sharma<sup>3</sup>

Department of Ophthalmology, Subharti Medical College, India

**Abstract:** ***Aims:** To investigate the association between Total Bilirubin levels and Diabetic Retinopathy in patients with Type 2 Diabetes Mellitus. **Material and Methods:** In this prospective, randomized study, a total of 60 diabetic patients presenting to Subharti Hospital, Meerut were divided in following categories. 1) Mild Non-Proliferative Diabetic Retinopathy (NPDR). 2) Moderate NPDR. 3) Severe NPDR. 4) Proliferative Diabetic Retinopathy (PDR) 5) No DR. The eye with worse retinopathy was chosen for inclusion in the study examinations. If both eyes had equal retinopathy, the right eye was assigned to the study. Cases were followed up at 1 week, 1 month and 6 months after first visit. Serum total bilirubin levels were measured at each follow-up visit. **Observation and Result:** On correlation analysis, the two variables Diabetic retinopathy Fundus grading and Total Serum bilirubin levels are inversely and significantly correlated at baseline, 1 week, 1 month and 6 months. **Conclusion:** Serum bilirubin concentration is inversely and independently associated and inversely correlated with the prevalence of DR.*

**Keywords:** Diabetic Retinopathy, Serum bilirubin levels

## 1. Introduction

Diabetes mellitus is a systemic disease in which blood glucose levels become chronically, and often severely elevated either because insulin is not secreted from the pancreatic islet cells (type 1 diabetes), or because the insulin that is secreted is, for a variety of reasons, less than normally efficacious (type 2 diabetes). Over years of chronic hyperglycemia, many individuals develop severe damage to different organs and tissues: Among others, the kidneys, the peripheral nervous system, the heart and great vessels, and the retina.<sup>1</sup>

Diabetic retinopathy (DR) is a common microvascular complication of diabetes mellitus in people aged 36-69 years, and can lead to acquired blindness.<sup>2</sup> The incidence of DR in population-based studies ranges from 10 to 55%, and in clinic-based studies ranges from 11 to 65%.<sup>3</sup> Approximately 28.5% of blindness is attributed to DR in subjects aged 40 years and older.<sup>4</sup>

More and more evidence has shown that oxidative stress is a critical risk factor in the pathogenesis of DR.<sup>5</sup> Bilirubin is mainly generated from heme degradation, and has strong antioxidant and anti-inflammatory effects on the microvasculature.<sup>6</sup> Bilirubin can covalently bind to albumin with a combination rate of more than 99%. It not only scavenges superoxide radical and peroxide radical, but also prevents oxidation modifications of low density lipoprotein and lipid oxidation. Some prospective studies have suggested that there is a negative relationship between total bilirubin level (TBL) and cardiovascular disease.<sup>7-8</sup>

A meta-analysis on the relationship between TBL and cardiovascular disease also confirmed this inverse relationship.<sup>9</sup> However, several studies have reported that there was no association between TBL and retinopathy of prematurity.<sup>10-11</sup> Therefore, we carried out a study to

analyze the effect of TBL on DR and evaluate the relationship between TBL and DR.

## 2. Aims and Objectives

To investigate the association between Total Bilirubin levels and Diabetic Retinopathy in patients with Type 2 Diabetes Mellitus.

## 3. Materials and Methods

This is a hospital based cross sectional study, conducted on 60 patients of Type 2 DM coming to Ophthalmology department of Subharti Hospital, Meerut, India.

Informed consent was obtained from all participants.

All participants had a test of visual acuity, intra ocular pressure and complete ocular examination including fundus examination with slit lamp biomicroscope using a 90 diopter lens after dilatation of pupils using 1% tropicamide eyedrops.

The diabetic patients were assigned to one of the following groups based on presence and severity of retinopathy with the help of 90D lens exam, Indirect retinoscopy, fundus photographs and/or fundus fluorescein angiography.

- 1) Mild Non-Proliferative Diabetic Retinopathy (NPDR).
- 2) Moderate NPDR.
- 3) Severe NPDR
- 4) Proliferative Diabetic Retinopathy (PDR)
- 5) No DR

The eye with worse retinopathy was chosen for inclusion in the study examinations. If both eyes had equal retinopathy, the right eye was assigned to the study.

**Follow Up**

All the patients were followed up on 1 week, 1 month and 6 months. Total Bilirubin levels were determined on each visit and they were correlated with fundus changes over the time.

**Inclusion Criteria**

Patients with Type 2 Diabetes Mellitus.

**Exclusion Criteria**

- 1) History of chronic alcohol intake,
- 2) Smoking,
- 3) Intake of hepatotoxic medications within past six months,
- 4) Pre existing hepatobiliary abnormalities or chronic liver disease.
- 5) Patients with pre existing ocular diseases like glaucoma, high myopia, previous ocular surgery or photocoagulation were excluded from the study.
- 6) Patients with hypertension (>160/90 mmHg), anaemia or other systemic diseases like nephropathy which can accelerate the progression of DR were also excluded from the study.

**Statistics**

Statistical analysis was performed using IBM, SPSS Statistics version 25 (IBM Corp., New York, NY). Descriptive data was expressed as mean  $\pm$  standard deviation unless otherwise stated. Chi-square tests were used for proportions. Continuous variables were compared using t-tests. A one-way analysis of variance (ANOVA) was used to compare differences between the means of independent groups like best corrected vision (BCVA), intraocular pressure (IOP) and total serum bilirubin over 6 months (within group comparisons). A P value less than 0.05 was considered statistically significant. A correlation analysis was performed to study the relationship between serum bilirubin and stage of diabetic retinopathy. Pearson correlation coefficient,  $r > 0.5$  was considered strong correlation.

**4. Observations****Age Distribution**

The mean age of patients was  $55.12 \pm 9.8$  (range, 36-84 years). Table 1 shows the age distribution of subjects. The mean age of males ( $55.51 \pm 10.2$  years) was significantly higher (t-test,  $P < 0.05$ ) as compared to females ( $51.13 \pm 7.8$  years).

**Gender Distribution**

There were 27 males and 23 females in the study.

**Serum Total Bilirubin Levels**

Table 1 shows Serum Total bilirubin levels over the times

**Table 1:** Mean serum total bilirubin levels of the patients

Total Bilirubin	Mean	Standard Deviation
DAY 1	0.51	0.14
1 WEEK	0.57	0.14
1 MONTH	0.54	0.12
6 MONTHS	0.6	0.18

**Fundus Grading**

Table 2 shows fundus gradings at various times of follow ups

**Table 2:** Fundus gradings at various times of follow UPS

Time	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR
Baseline	10%	34%	20%	8%	28%
1 Week	10%	34%	20%	8%	28%
1 Month	10%	38%	16%	8%	28%
6 Months	10%	60%	-	-	30%

**Correlational Analysis**

Table 3 shows correlational analysis between Serum Total Bilirubin levels and Diabetic Retinopathy Fundus gradings.

**Table 3:** Correlation analysis between diabetic retinopathy fundus gradings and serum total bilirubin levels

Bilirubin- Diabetic Retinopathy Fundus Grading Correlation	Pearson's Correlation Coefficient (r)	Significance (P-value)
BASELINE	-0.471	0.001
1 WEEK	-0.451	0.001
1 MONTH	-0.431	0.001
6 MONTHS	-0.408	0.003

**5. Results**

On correlation analysis, the two variables Diabetic retinopathy Fundus grading and Total Serum bilirubin levels are inversely and significantly correlated at baseline, 1 week, 1 month and 6 months.

**6. Discussion**

In this study we observed that people with no retinopathy or mild diabetic retinopathy have higher serum total bilirubin levels compared to people who have severe retinopathy. Dave et al<sup>12</sup> conducted a cross section case control study at a tertiary care centre to study the correlation between serum bilirubin and severity of diabetic retinopathy in patients with type 2 diabetes. A total of 120 subjects were enrolled in the study. Out of these 40 patients without diabetes served as controls. Amongst 80 patients with diabetes, 36 patients had diabetic retinopathy and 44 did not. The authors found that total bilirubin was significantly higher in controls ( $P=0.01$ ) as compared to cases. The total bilirubin, direct bilirubin and indirect bilirubin was significantly ( $P < 0.001$ ) lower in patients with retinopathy in comparison to those without retinopathy. The prevalence of retinopathy was significantly lower among patients with the higher bilirubin quartile. In the present study, majority of the patients had diabetic retinopathy. On Pearson's correlation analysis, a significant ( $P=0.03$ ) and inverse correlation (Pearson's correlation coefficient,  $r=-0.417$ ) was found between total bilirubin and severity of diabetic retinopathy.

Zhang et al<sup>13</sup> conducted a cross sectional study to evaluate the association between total serum bilirubin and diabetic retinopathy in northeast China. They recruited 742 patients (419 men and 323 women) with type 2 diabetes in the study. The mean age of patients was  $59.55 \pm 10.6$  years, and the mean duration of diabetes was  $11.01 \pm 7.35$  years. The authors found that total serum bilirubin significantly

negatively correlated with duration of diabetes and microalbuminuria (Pearson's correlation coefficient,  $r=-0.213$ ). Moreover, total serum bilirubin inversely correlated with the risk of retinopathy (OR: 0.95, 95% CI: 0.93–0.99), after adjusting for confounding variables. The authors concluded that the negative correlation between these variables may be an early clinical marker for predicting the occurrence of retinopathy. The mean age of patients in the present study was  $55.12 \pm 9.8$  (range, 36–84 years). The correlation between total serum bilirubin and severity of retinopathy was much stronger in the present study (correlation coefficient,  $r=-0.417$ ). A small sample size in the present study could account for the difference in observations, despite significant results in both studies.

Shreelaxmi et al<sup>14</sup> conducted a case control study to find the association between total serum bilirubin and diabetic retinopathy in patients with type 2 diabetes. Duration of diabetes was more than 6 months. Diabetics without retinopathy served as controls. The study included 136 patients. Out of these, 68 were cases and 68 controls. The authors found that serum bilirubin inversely and independently associated and inversely correlated with prevalence of retinopathy ( $P<0.001$ ). The inference of the study was that an inverse correlation exists between serum bilirubin and the prevalence of retinopathy and may predict its progression of over time. A similar observation was seen in our study as well.

## 7. Limitations

The shortcomings of the present study were that there was no control group to compare the results. Second, the duration of follow up was small (6 months). Lastly, the sample size in the present study was small and patients were mainly selected from ophthalmic out-patient department. So, there may be a selection bias and cannot be considered representative of the entire population in this area. Further, prospective studies on large samples are required to better assess the effects of bilirubin on DR.

## 8. Conclusion

In conclusion, the exact pathological mechanisms underlying the association between serum bilirubin and diabetic retinopathy is not known, although there may be several possible explanations. Oxidative stress has been implicated in the pathogenesis of microvascular and macrovascular complications of diabetes and the antioxidant properties of bilirubin may play a protective role.

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