INVESTIGATING THE ASSOCIATION OF BIOCHEMICAL AND CLINICAL PARAMETERS WITH THE SEVERITY OF DIABETIC RETINOPATHY IN BANGLADESH

Afroz Jahan, Md. Ismail Hosen and Hossain Uddin Shekhar*

Clinical Biochemistry and Translational Medicine Laboratory, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh

Bioresearch Communications
Volume 12, Issue 1, January 2026

DOI:
doi.org/10.3329/brc.v12i1.86761

ABSTRACT

Diabetic retinopathy (DR) is a significant microvascular complication of diabetes leading to vision impairment and blindness. While various clinical and biochemical parameters are linked with the severity of DR, there is no such study to ascertain these association in Bangladeshi population. This cross-sectional study investigated the association of various biochemical and clinical parameters with the severity of non-proliferative diabetic retinopathy in Bangladeshi patients. A cohort of 90 Bangladeshi patients with non-proliferative DR was recruited and followed for 8 months for developing different stages of DR severity (mild, modern and severe). Visual acuity (VA), Non-Contact Tonometer (NCT) readings, and biochemical parameters including C-reactive protein (CRP), random blood sugar (RBS), lipid profile (triglyceride, total cholesterol), serum ferritin, and vitamin D levels were assessed using appropriate laboratory techniques. The average value for visual activities (V/A) in different stages of non-proliferative DR is significantly different ($p<0.001$). In the same way, the average for Non-Contact Tonometer (NCT), C-reactive protein (CRP), Random Blood Sugar (RBS), and Diastolic Blood Pressure is significantly different in different stages of non-proliferative DR. No significant associations were found with lipid profile, serum ferritin, or vitamin D. Correlation analysis revealed a moderate positive correlation between RBS and NCT ($r=0.225$, $p<0.05$), and significant negative correlations of V/A, NCT, and CRP, reinforcing the link between inflammation/ocular pressure and visual function. Linear regression models identified CRP, RBS, and Duration of Diabetes as positive predictors for NCT, and total cholesterol (positive), CRP (negative), and RBS (negative) as predictors for visual acuity. Our findings indicate that visual acuity, non-contact tonometer measurements, C-reactive protein, and random blood sugar levels are significantly associated with the severity of non-proliferative diabetic retinopathy. These parameters serve as valuable indicators for assessing DR progression and emphasize the critical roles of regular ophthalmic examinations and stringent glycemic control in managing the disease. Longitudinal studies are needed to further validate these associations and their predictive power.

KEYWORDS: Diabetic Retinopathy, Visual Acuity, Non-Contact Tonometer, C-reactive protein (CRP).

RECEIVED: 22 October 2025, ACCEPTED: 08 December 2025

TYPE: Original Article

***CORRESPONDING AUTHORS: Hossain Uddin Shekhar, PhD, Clinical Biochemistry and Translational Medicine Laboratory, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh**
Email: hossainshekhar@du.ac.bd

Introduction

Diabetes mellitus is a chronic condition characterized by an absolute or relative lack of insulin and causes organ mutilation, dysfunction, and failure, including the retina, heart, kidney, nervous system, and blood vessels (Singh et al. 2025). Anyone with a history of long-term uncontrolled diabetes is potentially at risk of developing diabetic retinopathy at some stages (Duh, Sun, and Stitt 2017). Diabetic retinopathy (DR) is an eye condition that can cause vision loss and blindness in people who have diabetes. It threatens the sight of about 10% of people with diabetes (Tan, Endocrinology, and 2023 2023; Lin et al. 2021). It affects blood vessels in the retina. Diabetic retinopathy can be proliferative (growing) or non-proliferative (not growing) (Z. Yang et al. 2022). Several biochemical parameters such as the serum levels of C-reactive protein (CRP), lipid profile, serum ferritin and Vitamin D levels have been shown to be associated with the severity of diabetic retinopathy (Valle, Russo, and Malaguarnera 2021; Stanimirovic et al. 2022; Lin et al. 2021; Tecilazich, Formenti, and Giustina 2021; Ouyang, Zhou, and Wang 2023; Li et al. 2023). Patients with diabetic retinopathy had higher serum levels of the CRP than those without diabetic retinopathy (Stanimirovic et al. 2022). The conclusion of a meta-analysis was that CRP level might be considered as a biomarker to determine the severity of diabetic retinopathy (Song et al. 2015b). A strong association were found between DR progression and triglyceride (TG), systolic blood pressure, and LDL-cholesterol in recent studies (Alattas et al. 2022; Ezhilvendhan et al. 2021). A number of clinical tests are available to assess the severity of diabetic retinopathy, including the visual acuity (VA), non-Contact Tonometer (NCT) and Fluorescein angiography (FA) tests (Glassman et al.

n.d.; Mukherjee et al. n.d.; Qureshi et al. n.d.). While the number of people diagnosed with diabetic retinopathy is increasing in Bangladesh, the combined correlation of theses clinical parameters and the above-mentioned biochemical parameters with the severity of diabetic retinopathy is yet to be investigated.

To our knowledge, there is no report on the severity of diabetic retinopathy and lipid profile, serum ferritin, vitamin D and CRP levels of the DR patients in Bangladesh. Thus, the aim of the study is to determine the association of lipid profile, serum ferritin, vitamin D and CRP levels in diabetic retinopathy and their association with different stages of the disease.

Materials and Methods

Study Population and related ethical approval

The study was approved by the ethical review committee of the department of Biochemistry and Molecular Biology, University of Dhaka. The study was designed as a cross-sectional study with purposive sampling of diabetic retinopathy (DR) patients at the Lion Eye Institute and Hospital, Dhaka, Bangladesh. A total of 90 patients with mild non-proliferative diabetic retinopathy were enrolled for the study. The inclusion criteria include diabetic retinopathy patients of both genders with no age limit. The mild non-proliferative DR patients were followed for 8 months and their data were collected for the progression into moderate and then severe non-proliferative DR. Prior to enrollment of study participants, a written informed consent along with the clinical information was collected from each of the patient.

Sample Collection and Processing

Approximately, 5.0 mL of blood specimens were collected from the DR patients at three time points (mild, moderate and severe stages of non-proliferative DR). Serum was separated after centrifugation of the whole blood at 2500g for 10 minutes. Then it was aliquoted in different tubes and stored at -20ºC for different types of analysis.

Visual acuity (VA)

The visual acuity test determines how small the letters on a standardized chart (Snellen chart) or a card held 20 feet (6 meters) away can be read. When testing at distances less than 20 feet, special charts are used (6 meters). All study participants underwent VA test. Additionally, Keeler Non-Contact Tonometer (NCT) (Desktop) was used for measuring NCT.

Estimation of Serum Vitamin D, CRP, Ferritin, and lipid

Serum levels of 25-hydroxyvitamin D was measured using the chemiluminescence immunoassay, following the method described by Han et al, 2020 (Han et al. 2020). Similarly, the serum levels of CRP, Ferritin, and lipid profiling was performed using standard protocols of the suppliers.

Statistical Analysis

All collected data were entered into a Microsoft Excel spreadsheet and subsequently analyzed using SPSS Statistics Version 26.0. Descriptive statistics were used to summarize the baseline characteristics of the study participants, presenting continuous variables as mean ± standard deviation (SD). To assess the association of biochemical and clinical parameters with the severity of non-proliferative diabetic retinopathy (mild, moderate, and severe stages), appropriate comparative analyses were performed. One-way Analysis of Variance (ANOVA) was utilized for comparing continuous variables across the three DR severity groups. Pearson's correlation coefficient (r) was employed to evaluate the strength and direction of linear relationships between continuous variables, such as RBS, VA, NCT, CRP, triglyceride, total cholesterol, ferritin, and vitamin D. Finally, linear regression analysis was conducted to identify significant predictors for key outcome variables, specifically visual acuity (VA) and Non-Contact Tonometer (NCT) readings. Independent variables included CRP, RBS, lipid profile components, serum ferritin, vitamin D, and duration of diabetes. The statistical significance level for all analyses was set at $p<0.05$.

Results

There are three common stages of non-proliferative diabetic retinopathy (DR): mild, moderate, and severe. One-way ANOVA of the mean values for all quantitative variables based on the different stages of non-proliferative DR shows that the average value for visual activities (V/A) in different stages of non-proliferative DR is significantly different with a p-value of less than 0.001. In the same way, the average for Non-Contact Tonometer (NCT), C-reactive protein (CRP), Random Blood Sugar (RBS), and Diastolic Blood Pressure is significantly different in different stages of non-proliferative DR (Table 1). The average visual activities (VA) are 19.36 ft. in mild stage, 17.54ft. in moderate stage and finally 17.33 ft. in severe stage of DR, indicating a significant association of the severity of DR with VA. We did not find any statistically significant difference in the levels of vitamin D, ferritin, lipid profile and RBS with the different stages of DR. An interesting observation is that there has been no significant change in the systolic blood pressure in different stages which is almost the same but the diastolic pressure is significantly decreased (**Table 1**).

Table 1. Average value of different variables for different stages of non-proliferative diabetic retinopathy with one-way ANOVA.

Variables	Mild (n=90)	Moderate (n=90)	Severe (n=90)	p-value (one-way ANOVA)
Visual acuity (V/A), ft	19.36	17.54	17.33	**<0.001**
Non-Contact Tonometer (NCT), mmHg	18.16	22.16	22.81	**<0.001**
Lipid profile: Triglyceride (TG), (mg/dL)	175.14	175.21	175.37	0.993
Lipid profile: Total Cholesterol (TC), (mg/dL)	254.53	254.44	254.73	0.997

Ferritin (ng/mL)	380.42	380.74	380.83	0.991
C-reactive Protein (CRP), (mg/dL)	1.56	1.57	1.68	**<0.001**
Vitamin D (ng/mL)	16.06	16.06	15.53	0.132
RBS	17.29	17.29	19.03	**<0.001**
BP systolic	134.11	134.0	132.33	0.403
BP diastolic	89.33	89.22	87.11	**0.005**
Duration of DM	15.39	15.39	15.37	0.998

Here, one-way ANOVA suggested that at least one pair has different means of the groups of each variable. To identify the pairwise difference of the parameters, paired t-test was performed and the results are presented in bar diagram (Figures 1-5).

Visual activities and Non-Contact Tonometer changes with the severity stages of DR

The visual activity (V/A) test result indicates a gradual decrease in the measurements of V/A (in feet) over time with the progression of DR into moderate and severe stages (Figure 1A). The results reveal that the mean value for the mild stage is significantly higher from both the moderate and severe stages of DR, with p-values less than 0.01 for both comparisons. This indicates that patients in the mild stage experience considerably higher mean values for visual activities than those in the other stages. However, the difference in mean values between the moderate and severe stages is not statistically significant, as indicated by a p-value of 0.27 (Figure 1A).

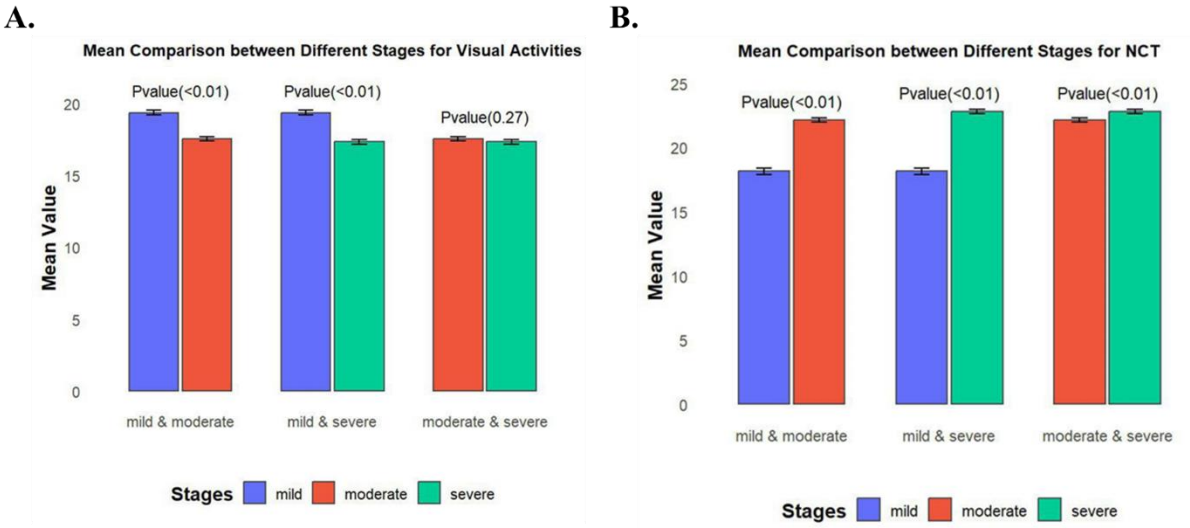

Figure 1. Cluster Bar diagram for comprising the means of V/A and NCT among different stages of DR. A. The bar chart compares the mean values of visual activities (V/A) at different stages of non-proliferative diabetic retinopathy: mild, moderate, and severe. The x-axis represents the three stages (mild, moderate, and severe), while the y-axis shows the mean values of visual activities in feet (ft). B. The presented bar chart provides a clear comparison of the mean values for the variable labeled "NCT" across the three stages. The x-axis illustrates these stages, while the y-axis displays the corresponding mean NCT values. Notably, the charts include statistical significance (p-values) for pairwise comparisons between the stages.

For NCT (Figure 1B), the findings reveal that all pairwise comparisons—mild & moderate, mild & severe, and moderate & severe show statistically significant differences in mean NCT values, as evidenced by p-values less than 0.01. This strongly indicates significant differences in mean NCT values across all stages. Specifically, we observe a progressive increase in mean values from mild to moderate and from moderate to severe.

Different stages of DR maintain similar lipid profile

Figure 2, illustrates the comparison of mean triglyceride and total cholesterol levels across the three stages of non-proliferative diabetic retinopathy: mild, moderate, and severe. In figure 2A, the x-axis represents the stages, and the y-axis shows the mean triglyceride values in mg/dL. Statistical significance (p-values) for pairwise comparisons between the stages is provided above the bars. The p-values for all comparisons (mild & moderate, mild & severe, and moderate & severe) are well above 0.05 (0.97, 0.91, and 0.94, respectively). This indicates that there are no statistically significant differences in triglyceride levels between any of the stages of diabetic retinopathy. Additionally, the chart in figure 2B shows that total cholesterol levels remain consistent, with no significant differences between the stages of DR.

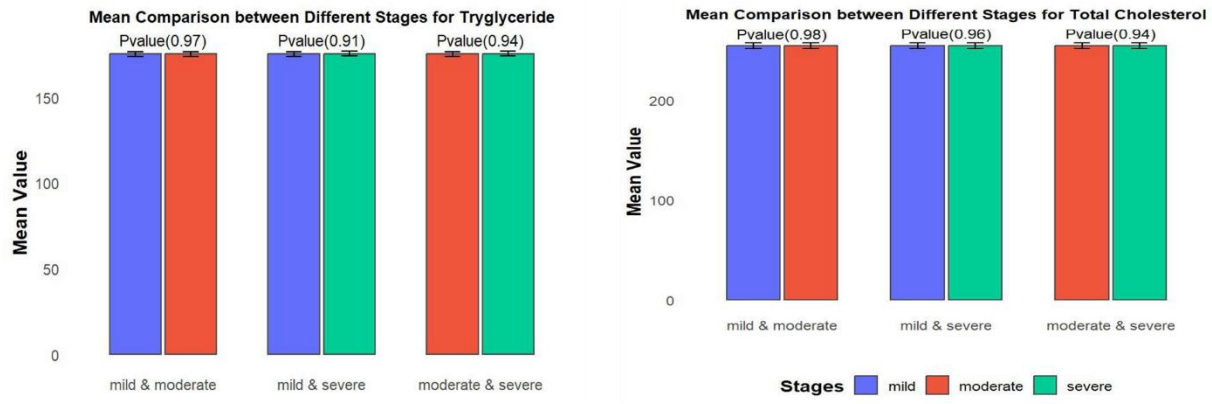

Figure 2. Cluster Bar diagram for comprising the levels of triglycerides and total cholesterol among different stages of DR. A. The bar chart compares average total cholesterol levels in mg/dL across three stages: mild, moderate, and severe. The x-axis represents pairwise comparisons, while the y-axis shows mean cholesterol values, with blue bars for mild, red for moderate, and green for severe. B. The bar chart shows the mean levels of total cholesterol in mg/dL in the different stages of DR. P-values indicate statistical significance.

No association of serum ferritin and vitamin D levels with the severity of DR

The bar chart in Figure 3A depicts the mean comparison of ferritin levels across different stages: mild, moderate, and severe. Additionally, the error bars at the top of each bar display minimal variability, reinforcing the consistency of ferritin levels across different stages. The chart suggests that ferritin levels remain relatively stable, with no meaningful differences between mild, moderate, and severe groups.

The comparison between mild and moderate stages has a p-value of 1, implying no difference in Vitamin D levels between these groups (Figure 3B). Similarly, the comparisons between mild & severe (p = 0.074) and moderate & severe (p = 0.07) suggest no statistically significant difference, although the p-values are relatively close to the conventional 0.05 threshold. This indicates that while there might be a slight decrease in Vitamin D levels as severity increases, the difference is not strong enough to be statistically significant.

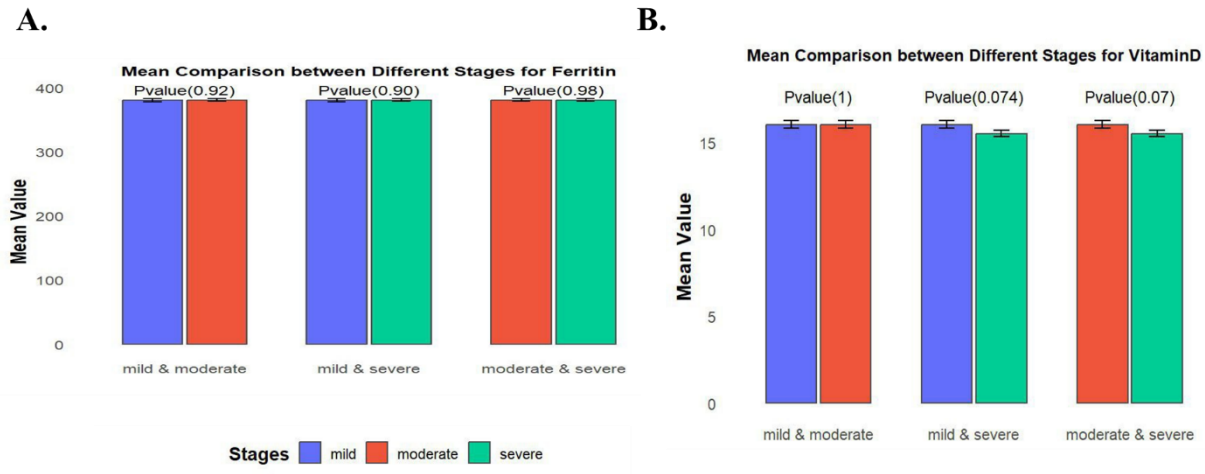

Figure 3. Cluster Bar diagram for comprising the levels of serum Ferritin and vitamin D among different stages of DR. A. For serum ferritin levels, the p-values are 0.92 for mild vs. moderate, 0.90 for mild vs. severe, and 0.98 for moderate vs. severe. These high p-values suggest that there are no significant differences in ferritin levels among the different stages. The x-axis represents pairwise comparisons between these stages, while the y-axis indicates the mean ferritin values. Each pair is represented by two colored bars: blue for mild, red for moderate, and green for severe. B. The bar chart compares the mean values of Vitamin D across different severity stages: mild, moderate, and severe. The bars are color-coded blue for mild, red for moderate, and green for severe. The y-axis represents the mean Vitamin D values (ng/mL), while the x-axis categorizes the pairwise comparisons of the severity stages. Error bars are included to show variability in the data, and p-values above each comparison indicate statistical significance.

CRP and random blood sugar levels are associated with the moderate and severe stages of DR

The comparison of serum CRP levels between the mild and moderate stages of DR shows a p-value of 0.77, suggesting no significant difference in CRP levels between these two groups (Figure 4). However, the second and third comparisons, between mild & severe and moderate & severe, both show p-values of less than 0.01, indicating a statistically significant difference in CRP levels. This suggests that CRP levels increase significantly when progressing from mild to severe and from moderate to severe stages.

The comparison of random blood sugar levels between the mild and moderate stages shows a p-value of 1, indicating no significant difference in blood sugar levels between these two groups (Figure 4B). However, the comparisons between mild & severe and moderate & severe both have p-values of less than 0.01, suggesting a statistically significant increase in blood sugar levels as severity progresses. This implies that severe cases exhibit significantly higher blood sugar levels than both mild and moderate cases.

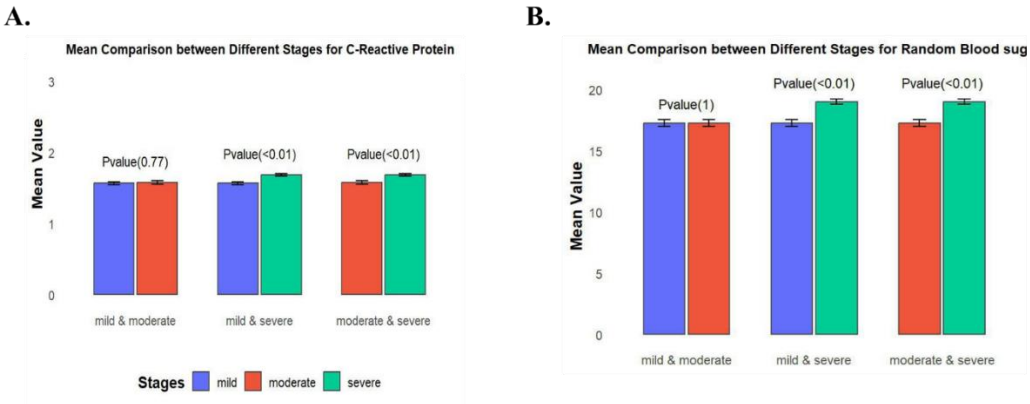

Figure 4. Cluster Bar diagram for comprising the means of CRP and RBS among different stages. A. The bar chart comparing the mean values of C-reactive protein (CRP) across different severity stages: mild, moderate, and severe. The bars are color-coded—blue for mild, red for moderate, and green for severe. The y-axis represents the mean CRP values, while the x-axis categorizes the different pairwise comparisons of the severity stages. Error bars indicate variability in the data, and statistical significance is provided through p-values above each comparison. B. The bar chart presents a comparison of the mean values of random blood sugar levels across different severity stages: mild, moderate, and severe. The bars are color-coded blue for mild, red for moderate, and green for severe. The y-axis represents the mean random blood sugar values, while the x-axis categorizes the different pairwise comparisons of the severity stages. Error bars are included to represent variability in the data, and statistical significance is provided through p-values above each comparison.

Association of blood pressure with different severity stages of DR

Data in Figure 5 shows the association of systolic and diastolic blood pressure with the stages of DR. For the systolic blood pressure plot in Figure 5A, the p-values for the comparisons between "mild & moderate" (0.95), "mild & severe" (0.21), and "moderate & severe" (0.23) indicate that there are no statistically significant differences between these stages. This suggests that the mean values for high blood pressure remain relatively similar across different severity levels.

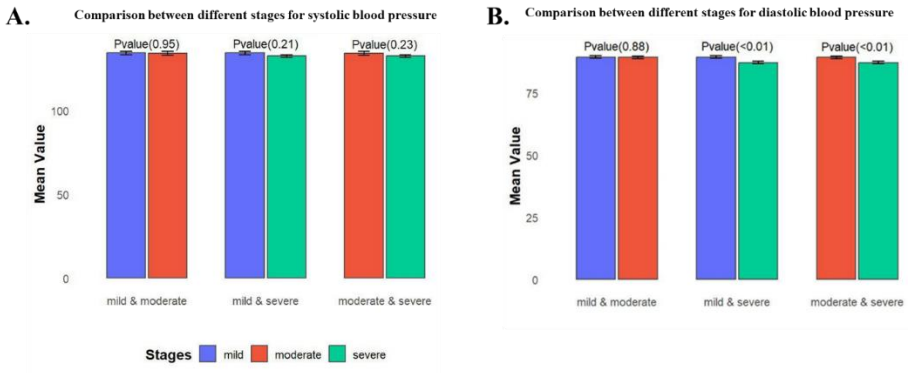

Figure 5. Cluster Bar diagram for comprising the means of systolic (A) and diastolic (B) blood pressure among different stages of DR. The bar charts present a comparison of the mean values of systolic (A) and diastolic (B) blood pressure levels across different severity stages: mild, moderate, and severe. The bars are color-coded blue for mild, red for moderate, and green for severe. The y-axis represents the mean systolic (A) and diastolic (B) blood pressure, while the x-axis categorizes the different pairwise comparisons of the severity stages. Error bars are included to describe variability in the data, and statistical significance is provided through p-values above each comparison.

For the diastolic blood pressure plot in Figure 5B, the p-values for "mild & moderate" (0.88) suggest no significant difference, but for "mild & severe" and "moderate & severe," the p-values are both less than 0.01, indicating statistically significant differences between these groups. This suggests that, unlike high blood pressure, there is a clear distinction in mean values between the severe stage and the other two stages in cases of low blood pressure.

Correlation among the Variables

The data presented in **Table 2** reveal several interesting correlations among the different variables. Firstly, a significant positive correlation (r=0.152, p<0.01) between RBS and visual activity suggests that as RBS levels increase, Visual activity may tend to increase. A moderate positive correlation (r=0.225, p< 0.05) was also observed between RBS and NCT (**Table 2**). These positive correlations indicate a potential association between sugar levels and NCT, consistent with the understanding that individuals with insulin resistance or type 2 diabetes often exhibit both hyperglycemia and dyslipidemia.

Furthermore, a positive correlation between RBS and triglyceride (r=0.153, p < .01) is observed in our dataset, but total cholesterol (r=0.078) has an insignificant correlation with RBS. Ferritin has a small negative correlation (r = -0.185, p < 0.01) with RBS indicating that the increase of RBS decreases the level of ferritin. While the CRP shows no significant correlation with RBS (**Table 2**).

Table 2. Correlation coefficient among all variables.

Variable	RBS	V/A	NCT	TG	TC	Ferritin	CRP	Vitamin D	Diastolic BP	Systolic BP	duration
RBS	1.00										
V/A	**0.152**	1.00									
NCT	**0.225**	**-0.494**	1.00								
TG	**0.153**	0.040	0.069	1.00							
TC	0.078	0.104	0.010	**0.752**	1.00						
Ferritin	**-0.185**	0.066	-0.022	**-0.128**	-0.018	1.00					
CRP	0.013	**-0.189**	**0.141**	0.063	0.041	-0.059	1.00				
Vitamin D	0.045	-0.033	-0.011	-0.069	0.073	0.017	0.111	1.00			
Diastolic BP	-0.022	0.090	-0.015	0.060	0.041	-0.053	-0.051	0.025	1.00		
Systolic BP	-0.064	**0.153**	-0.097	**0.210**	**0.244**	**-0.209**	**-0.136**	0.077	0.041	1.00	
Duration	-0.056	-0.056	0.106	**0.120**	0.120	0.084	-0.062	-0.009	0.000	0.111	1.00

*__Blue color__ significant at 5% level (moderate significant)
*__Green color__ significant at 1% level (Highly significant)

There is also some negative correlation between RBS and systolic and diastolic blood pressure but all the correlations are insignificant. There are notably negative correlations between V/A, NCT, and CRP, which are significant. Additionally, a significant but weak correlation is observed between V/A and systolic blood pressure (r=0.153). While there is a weak negative correlation between V/A, triglyceride, total cholesterol, ferritin, diastolic blood pressure, Vitamin D, and time duration, these are not statistically significant (**Table 2**).

Further, two linear models for dependent variables visual activities (VA) and NCT are prepared in **Table 3** and **Table 4** respectively, with the covariates of triglyceride, total cholesterol, ferritin, diastolic blood pressure, systolic blood pressure, vitamin D, and time duration. By The backward elimination method, we construct these models. This regression method is a stepwise technique used to build a simpler linear regression model by removing variables that are not significantly contributing to the model's predictive power. This method is especially useful when starting with many predictor variables and needing to identify the most relevant ones.

Table 3 presents the results of a multiple linear regression analysis predicting the dependent variable "V/A (Visual Activity)" from three independent variables: Lipid profile TC (total cholesterol), CRP (C-reactive protein), and RBS (random blood sugar). The intercept of the regression model is 20.261, and it is statistically significant with a p-value of less than 0.001. This value represents the expected value of Visual Activity when all independent variables are zero. The coefficient for total cholesterol is 0.008 9Table 3), indicating that for each unit increase in total cholesterol, the predicted V/A (Visual Activity) is increased by 0.008 units which is also statistically significant with a p-value of 0.037. The 95% Confidence Interval for this association of V/A with total cholesterol is 0.001 to 0.015, confirming the low positive association. Similarly, the coefficient for CRP is -1.481, indicating that for each unit increase in CRP, the predicted V/A (Visual Activity) decreases by 1.481 units (95% Confidence interval: -2.376 to -0.586) which is also statistically significant with a p-value of 0.001 (Table 3). The coefficient for RBS is -0.097, suggesting that for each unit increase in RBS, the predicted V/A (20 feet) decreases by 0.097 units (95% Confidence Interval: -0.168 to -0.026) with a p-value of 0.007 suggesting strong statistical significance (**Table 3**).

Table 3. Coefficient for linear regression model for visual activities with CRP, RBS, lower blood pressure, and their 95% confidence interval values.

Variables	Coefficient	95% confidence interval		p-value
		Lower value	Upper value	
intercept	20.261	17.733	22.790	<0.001
Lipid profile TC	0.008	0.001	0.015	0.037
CRP	-1.481	-2.376	-0.586	0.001
RBS	-0.097	-0.168	-0.026	0.007

Table 4 represents the results of a regression analysis constructed by the backward elimination method, with the dependent variable being NCT and the independent variables: being CRP (C-reactive protein), RBS (Random Blood Sugar), and Duration (year) within specific ranges.

Table 4. Coefficient for linear regression model for NCT with CRP, and RBS along with 95% confidence interval.

Variables	Coefficient	95% confidence interval		p-value
		Lower value	Upper value	
intercept	12.070	8.375	15.766	<0.001
CRP	1.833	0.382	3.283	0.013
RBS	0.228	0.114	0.343	<0.001
Duration	0.127	0.012	0.241	0.031

The intercept coefficient for NCT is 12.070 (**Table** 4), which is the predicted value of the dependent variable (NCT) when both CRP, RBS, and Duration are zero. The p-value is less than 0.001, indicating that the intercept is statistically significant. The coefficient for CRP is 1.833, meaning for every 1-unit increase in CRP, the dependent variable (NCT) is expected to increase on average by 1.833 units (95% Confidence Interval: 0.382, 3.283). The p-value (0.013) is less than 0.05, indicating that this predictor is statistically significant (Table 4). The coefficient for RBS (Random Blood Sugar) is 0.228. For every 1-unit increase in RBS, the dependent variable (NCT) is expected to increase on average by 0.228 units (95% confidence intervals: 0.114, 0.343**)**. The p-value is less than 0.001, indicating that this predictor is strongly statistically significant. Overall Interpretation is that both CRP, RBS, and Duration are statistically significant predictors of the dependent variable, NCT. CRP has a stronger effect (B = 1.735) compared to RBS (B = 0.221) and Duration (0.127) on NCT. The confidence intervals suggest that the coefficients for three variables are not to be zero, confirming their influence on the model. This model can predict NCT based on CRP, RBS and Duration values, with a reasonable level of confidence and statistical significance.

Discussion

This cross-sectional study aimed to investigate the association of various biochemical and clinical parameters with the severity of non-proliferative diabetic retinopathy (DR) in Bangladeshi patients. Our findings contribute to the understanding of DR progression and the potential utility of certain biomarkers in its assessment.

One of the most prominent findings of this study is the significant changes observed in both Visual Acuity (VA) and Non-Contact Tonometer (NCT) readings with the progression of DR severity. Visual acuity, as expected, showed a significant decline from the mild stage to the moderate and severe stages, reflecting the impact of the disease on vision. This is consistent with the well-established understanding that DR is a leading cause of vision impairment and blindness (Tan, Endocrinology, and 2023 2023; Z. Yang et al. 2022; Biswas et al. 2024). Our findings suggest that the progression from mild to moderate or severe stage is associated with a significant decline in visual activity performance. In contrast, the changes in visual activities between the moderate and severe stages may be less pronounced, indicating a possible plateau in the impact as the condition advances from moderate to severe stage. For the NCT, the result suggests that as the severity of diabetic retinopathy progresses, there is a consistent and significant change in the NCT variable. Such a pattern implies a direct correlation between the NCT measure and the severity of non-proliferative diabetic retinopathy. This progressive rise in NCT, an indicator of intraocular pressure, suggests a direct correlation between ocular tension and the worsening of non-proliferative DR. While the exact mechanistic link requires further investigation, this finding could imply that increased intraocular pressure plays a role in the pathophysiology or exacerbation of DR, or that the structural changes in the eye due to DR contribute to altered NCT readings.

In terms of biochemical parameters, our study found no statistically significant association between the severity of DR and lipid profile components (triglyceride and total cholesterol), serum ferritin levels, or vitamin D levels. This finding for lipid profile contrasts with some previous reports (Li et al. 2023; Jenkins, Grant, and Busik 2022; Cai et al. 123AD) that have shown correlations between elevated total cholesterol, triglyceride, and LDL levels with DR severity or progression. The discrepancy might be attributed to differences in study populations, methodologies, or the specific stage of DR studied. Similarly, while some literature suggests an association of

serum ferritin and vitamin D with diabetic complications (Ouyang, Zhou, and Wang 2023; Tecilazich, Formenti, and Giustina 2021; Valle, Russo, and Malaguarnera 2021), our study did not find these to be significantly different across the mild, moderate, and severe non-proliferative DR stages. This suggests that these specific biomarkers may not be reliable indicators for distinguishing the severity of non-proliferative DR in our study cohort.

Conversely, C-reactive protein (CRP) and Random Blood Sugar (RBS) levels emerged as significant indicators of advanced DR. While no significant difference was observed between mild and moderate stages for both CRP and RBS, both parameters were significantly elevated in severe DR compared to both mild and moderate stages. This aligns with existing literature indicating CRP as a potential biomarker for DR severity, reflecting systemic inflammation associated with the disease (X. Yang et al. n.d.; Qiu et al. 2020; Song et al. 2015a). The significant increase in RBS in severe DR also underscores the critical role of glycemic control in preventing DR progression. This suggests that while mild and moderate non-proliferative DR might not present with markedly different inflammatory or glycemic profiles, severe forms of the disease are characterized by a pronounced increase in these markers.

Regarding blood pressure, our study found no significant difference in systolic blood pressure across the DR stages. However, diastolic blood pressure showed statistically significant differences only between the severe stage and the other two stages, indicating a potential decrease in diastolic pressure in severe cases. This observation requires further exploration as the relationship between blood pressure components and DR severity can be complex and influenced by various factors.

The correlation analysis provided further insights. A significant positive correlation between RBS and visual activity was observed ($r=0.152$, $p<0.01$). While an increase in RBS is generally associated with worse outcomes in diabetes, the positive correlation with VA is counter-intuitive and warrants careful interpretation. It is possible that this reflects a specific sub-group dynamic or confounding factors within the dataset, rather than a direct beneficial effect of higher RBS on VA. This finding highlights the complexity of multi-parameter interactions and the need for more nuanced analyses. A moderate positive correlation was found between RBS and NCT ($r=0.225$, $p<0.05$), which is more consistent with the expectation that higher blood sugar levels can influence ocular health and intraocular pressure. The positive correlation between RBS and triglyceride ($r=0.153$, $p<0.01$) is also in line with the understanding of metabolic dysregulation in diabetes. The negative correlation between ferritin and RBS ($r=-0.185$, $p<0.01$) is an interesting finding that contradicts some studies suggesting elevated ferritin in diabetes; this could point to specific iron metabolism dynamics in this population. The significant negative correlations observed between V/A, NCT, and CRP are also noteworthy, further solidifying the inverse relationship between visual function/ocular pressure and inflammatory markers in DR.

The linear regression models provided predictive insights. For visual activity, total cholesterol showed a positive association, while CRP and RBS were negatively associated. The positive coefficient for total cholesterol is unexpected given the general understanding of lipid's role in DR and may require further investigation into potential confounding variables or specific mechanisms in this cohort. The negative association of CRP and RBS with VA is logical, indicating that higher inflammation and blood sugar levels are associated with poorer visual outcomes. For NCT, the model showed positive associations with CRP, RBS, and Duration of Diabetes. This strongly suggests that elevated inflammation (CRP), uncontrolled blood sugar (RBS), and longer disease duration are significant predictors of increased NCT, reinforcing their roles in DR progression and its impact on ocular health. The findings suggest that these clinical and biochemical parameters can serve as valuable predictors for assessing the severity of DR and its associated ocular changes.

A limitation of this study is its cross-sectional design, which precludes the establishment of cause-and-effect relationships. The purposive sampling method, while efficient for patient recruitment in a clinical setting, may limit the generalizability of the findings. Future longitudinal studies with larger and more diverse cohorts are needed to confirm these associations, elucidate the underlying mechanisms, and explore the predictive power of these biomarkers for DR progression over time. Further investigation into the unexpected positive correlation between RBS and VA, and the positive association of total cholesterol with VA in the regression model, would also be beneficial.

In conclusion, our study indicates that visual acuity and non-contact tonometer measurements, along with C-reactive protein and random blood sugar levels, are significantly associated with the severity of non-proliferative diabetic retinopathy in Bangladeshi patients. These findings highlight the importance of regular ophthalmic examinations and strict glycemic control in managing DR. While lipid profile, ferritin, and vitamin D did not show significant associations in this cohort, the predictive models for VA and NCT offer valuable insights for clinical assessment.

Acknowledgements and Funding statement

We would like to acknowledge the University of Dhaka (DU) for funding our study. The project was funded by the **UGC-DU research grant 2023-24**.

Competing interests

The authors declare no competing interests.

Availability of data and material

All the data are presented in this manuscript and the raw data are available on request to the corresponding author.

Declaration of AI assistance in manuscript preparation

During the preparation of this manuscript the author(s) used ChatGpt and Scispace in order to improve the readability and language of the manuscript. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

1. Alattas, Khadijah, Dania W Alsulami, Rahaf H Alem, Felwa S Alotaibi, Bayan A Alghamdi, and Layan S Baeesa. 2022. “Relation between Lipid Profile, Blood Pressure and Retinopathy in Diabetic Patients in King Abdulaziz University Hospital: A Retrospective Record Review

Study." *International Journal of Retina and Vitreous* 8: 20. https://doi.org/10.1186/s40942-022-00366-4.

2. Biswas, Arpon, Abhijit Deb Choudhury, Sristi Agrawal, Amol Chhatrapati Bisen, Sachin Nashik Sanap, Sarvesh Kumar Verma, Mukesh Kumar, et al. 2024. "Recent Insights into the Etiopathogenesis of Diabetic Retinopathy and Its Management." *Liebertpub.Com* 40 (1): 13–33. https://doi.org/10.1089/JOP.2023.0068.
3. Cai, Z, Y Yang, J Zhang - Scientific reports, and undefined 2021. 123AD. "A Systematic Review and Meta-Analysis of the Serum Lipid Profile in Prediction of Diabetic Neuropathy." *Nature.Com* 11: 499. https://doi.org/10.1038/s41598-020-79276-0.
4. Duh, Elia J., Jennifer K. Sun, and Alan W. Stitt. 2017. "Diabetic Retinopathy: Current Understanding, Mechanisms, and Treatment Strategies." *JCI Insight* 2 (14). https://doi.org/10.1172/JCI.INSIGHT.93751.
5. Ezhilvendhan, Kalaimamani, Anitha Sathiyamoorthy, B. Prakash, B. Bhava, and Arjun Shenoy. 2021. "Association of Dyslipidemia with Diabetic Retinopathy in Type 2 Diabetes Mellitus Patients: A Hospital-Based Study." *Journal of Pharmacy and Bioallied Sciences* 13 (6): S1062–67. https://doi.org/10.4103/JPBS.JPBS_164_21.
6. Glassman, AR, MA Elmasry, DE Baskin, … M Brigell - Ophthalmology, and undefined 2024. n.d. "Visual Function Measurements in Eyes with Diabetic Retinopathy: An Expert Opinion on Available Measures." *Elsevier*. Accessed June 18, 2025. https://www.sciencedirect.com/science/article/pii/S2666914524000551.
7. Han, Shuang, Wuxian Qiu, Junlan Zhang, Zhonghu Bai, and Xiao Tong. 2020. "Development of a Chemiluminescence Immunoassay for Quantification of 25-Hydroxyvitamin D in Human Serum." *Journal of Analytical Methods in Chemistry* 2020. https://doi.org/10.1155/2020/9039270.
8. Jenkins, Alicia J., Maria B. Grant, and Julia V. Busik. 2022. "Lipids, Hyperreflective Crystalline Deposits and Diabetic Retinopathy: Potential Systemic and Retinal-Specific Effect of Lipid-Lowering Therapies." *Springer* 65 (4): 587–603. https://doi.org/10.1007/S00125-022-05655-Z.
9. Li, Zhaoping, Yuan Yuan, Qianjin Qi, Qian Wang, and Li Feng. 2023. "Relationship between Dyslipidemia and Diabetic Retinopathy in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis." *Springer* 12 (1). https://doi.org/10.1186/S13643-023-02321-2.
10. Lin, Kuan Yu, Wen Hui Hsih, Yen Bo Lin, Chen Yu Wen, and Tien Jyun Chang. 2021. "Update in the Epidemiology, Risk Factors, Screening, and Treatment of Diabetic Retinopathy." *Wiley Online Library* 12 (8): 1322–25. https://doi.org/10.1111/JDI.13480.
11. Mukherjee, A, D Rathore, … S Shree - Int. Journal of, and undefined 2015. n.d. "Diagnosis of Diabetic Retinopathy." *Academia.Edu*. Accessed June 18, 2025. https://www.academia.edu/download/113008855/E502042124.pdf.
12. Ouyang, Junlin, Ling Zhou, and Qing Wang. 2023. "Spotlight on Iron and Ferroptosis: Research Progress in Diabetic Retinopathy." *Frontiersin.Org* 14. https://doi.org/10.3389/FENDO.2023.1234824/FULL.
13. Qiu, Fangfang, Xiang Ma, Young-Hwa Shin, Jianglei Chen, Qian Chen, Kelu Zhou, Wenjing Wu, et al. 2020. "Pathogenic Role of Human C-Reactive Protein in Diabetic Retinopathy." *Portlandpress.Com* 134: 1613–29. https://doi.org/10.1042/CS20200085.
14. Qureshi, I, J Ma, Q Abbas - Symmetry, and undefined 2019. n.d. "Recent Development on Detection Methods for the Diagnosis of Diabetic Retinopathy." *Mdpi.Com*. Accessed June 18, 2025. https://www.mdpi.com/2073-8994/11/6/749.
15. Singh, Aditi, Sucharita Shadangi, Pulkit Kr. Gupta, and Soumendra Rana. 2025. "Type 2 Diabetes Mellitus: A Comprehensive Review of Pathophysiology, Comorbidities, and Emerging Therapies." *Comprehensive Physiology* 15 (1). https://doi.org/10.1002/CPH4.70003')).
16. Song, Jian, Song Chen, Xiaoting Liu, Hongtao Duan, Jiahui Kong, and Zedong Li. 2015a. "Relationship between C-Reactive Protein Level and Diabetic Retinopathy: A Systematic Review and Meta-Analysis." *Journals.Plos.Org* 10 (12). https://doi.org/10.1371/JOURNAL.PONE.0144406.
17. ———. 2015b. "Relationship between C-Reactive Protein Level and Diabetic Retinopathy: A Systematic Review and Meta-Analysis." *PLoS ONE* 10 (12). https://doi.org/10.1371/JOURNAL.PONE.0144406.
18. Stanimirovic, Julijana, Jelena Radovanovic, Katarina Banjac, Milan Obradovic, Magbubah Essack, Sonja Zafirovic, Zoran Gluvic, Takashi Gojobori, and Esma R. Isenovic. 2022. "Role of C-reactive Protein in Diabetic Inflammation." *Wiley Online Library* 2022. https://doi.org/10.1155/2022/3706508.
19. Tan, TE, TY Wong - Frontiers in Endocrinology, and undefined 2023. 2023. "Diabetic Retinopathy: Looking Forward to 2030." *Frontiersin.Org* 13 (January). https://doi.org/10.3389/FENDO.2022.1077669/FULL.
20. Tecilazich, Francesco, Anna Maria Formenti, and Andrea Giustina. 2021. "Role of Vitamin D in Diabetic Retinopathy: Pathophysiological and Clinical Aspects." *Springer* 22 (4): 715–27. https://doi.org/10.1007/S11154-020-09575-4.
21. Valle, Maria Stella, Cristina Russo, and Lucia Malaguarnera. 2021. "Protective Role of Vitamin D against Oxidative Stress in Diabetic Retinopathy." *Wiley Online Library* 37 (8). https://doi.org/10.1002/DMRR.3447.
22. Yang, XF, Y Deng, H Gu, … A Lim - International, and undefined 2016. n.d. "C-Reactive Protein and Diabetic Retinopathy in Chinese Patients with Type 2 Diabetes Mellitus." *Pmc.Ncbi.Nlm.Nih.Gov*. Accessed June 19, 2025. https://pmc.ncbi.nlm.nih.gov/articles/PMC4768512/.
23. Yang, Zhengwei, Tien-En Tan, Yan Shao, Tien Yin Wong, Xiaorong Li, Xinyuan Zhang, Carmen Clapp, Mary Kinkel, and Tan T-e. 2022. "Classification of Diabetic Retinopathy: Past, Present and Future." *Frontiersin.Org* 13 (December): 1079217.https://doi.org/10.3389/FENDO.2022.1079217/FULL.