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Conjunctival vessel morphology in patients with type 2 diabetes mellitus: findings of a case-control study

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Abstract. – OBJECTIVE: Diabetic retinopathy is a serious complication of diabetes mellitus that may lead to vision loss. Retinal problems are more likely to occur as the illness advances. Micro- and macro-vascular angiopathy is both linked to diabetes mellitus. Examining the impact of diabetes on blood vessels is one approach to understanding the disease's outward symptoms. The purpose of this research is to evaluate the morphology and breadth of conjunctival vessels in people with type 2 diabetes mellitus and to establish a correlation between these alterations and clinical retinal changes.

PATIENTS AND METHODS: A case-control study was conducted in the Department of Ophthalmology, KIMS, Koppal, Karnataka. The study included diabetic patients who voluntarily participated and were assessed at the Ophthalmology Outpatient Department (OPD). The general and ophthalmic history was taken for both cases and control. The best corrected visual acuity was estimated. Each subject, including cases and controls, had a general and ophthalmic history recorded. The anterior segment was evaluated with a slit lamp. After completing the clinical examination, the subjects underwent a conjunctival vessel imaging study using the slit lamp. The imaging included the study of conjunctival vessel morphology using ImageNet.

RESULTS: Both the test and control groups are comparable in terms of age and gender. The severity of diabetic retinopathy was shown to correlate with the conjunctival width range. Mild diabetic retinopathy is characterized by a conjunctival width of 30-34 microns (mean: 34.9), moderate diabetic retinopathy by 35-39 microns (mean: 37.3), severe diabetic retinopathy by 40-44 microns (mean: 42.4), and proliferative diabetic retinopathy by 50-54 microns (mean: 45.6).

CONCLUSIONS: Diabetes mellitus patients have larger conjunctival arteries as the disease becomes more severe. Dilated and tortuous conjunctival arteries are visible indicators that correlate with worsening diabetic retinopathy. This could be used for screening to ensure timely referral.

Key Words:
Conjunctival vessels, Diabetes, Diabetic Retinopathy.

Introduction

The eye is only one of several organs that diabetes may damage. All parts of the eye, from the conjunctiva to the optic nerve, may be damaged by diabetes mellitus. Diabetic retinopathy is a serious complication of diabetes mellitus that may lead to vision loss. It is the leading cause of blindness among adults in developed nations[1]. Vision loss is caused by diabetes complications, including neovascular glaucoma, tractional retinal detachment, and vitreous hemorrhage, as well as maculopathy and proliferative diabetic retinopathy (PDR). The World Health Organization[2] predicts a 69% increase in diabetes cases in developing nations between 2010 and 2030, compared to a 20% increase in the developed world.

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Retinal issues are more likely to occur as the disease progresses. Up to 50% of type 1 diabetes patients and 30% of type 2 diabetes patients have potentially vision-threatening retinal abnormalities; however, patients are often unaware of early retinal changes[3].

Examining the impact of diabetes on blood vessels is one method for gaining insight into the condition's manifestations. Microvascular and macrovascular angiopathy are both linked to diabetes mellitus. Retinal blood vessels are the typical site of manifestation. Similar appearances may be seen in the renal, cardiac, and cerebral blood vessels as well as the retina. Studies[4,5] done on diabetic patients show potentially fatal visual impairments induced by the retinal manifestation. Studies[6,7] have been conducted on visual deficits and life-threatening complications and these studies have guided the treatment protocols. Recent research by Kramer and Retnakaran[6] suggests that the presence of nephropathy in people with type 1 DM increases the likelihood of developing diabetic retinopathy. Patients with type 1 diabetes who develop proliferative diabetic retinopathy have a higher risk of developing chronic nephropathy[7]. Diabetic retinopathy and nephropathy are established independent predictors of diabetes mellitus. The presence of pericytes and the need for equipment and trained personnel render visualization of retinal vessels difficult. However, visualization of the readily visible conjunctival vessels is comparatively easier. The problem is the need for expensive tools and skilled workers. The conjunctival vessels are the most easily seen and recorded vessels in the eye. The next inquiry is whether or not the conjunctival vessels are affected by diabetes. Similar to the well-known vascular abnormalities in the retina, diabetes is also associated with capillary loss and macro-vessel dilatation in the conjunctiva[8]. Vascular engorgement and straightening have been linked to a prolonged history of diabetes and macro-vessel dilatation[9]. The vascular changes in the retina mirror those seen in the conjunctiva, which become more tortuous as a result of diabetes[10]. Evaluation of diabetic conjunctival angiopathy may facilitate recognition of retinal abnormalities, even by non-clinical personnel. Can eye problems manifest in the conjunctiva before they show up in the retina? However, there is a dearth of Indian research on how diabetes affects the conjunctival blood vessels. Therefore, the purpose of this research was to examine the conjunctival symptoms of type 2 DM and establish a correlation with the retinal manifestations of the disease.

With this context in mind, this study was carried out to evaluate the morphology and breadth of conjunctival vessels in people with type 2 diabetes mellitus and to establish a correlation between these alterations and clinical retinal changes.

Patients and Methods

Study Design, Participants and Setting

A case-control study was conducted in the Department of Ophthalmology, KIMS, Koppal, Karnataka. Patients diagnosed with type 2 diabetes enrolled in the Ophthalmology Outpatient Department (OPD) for ocular assessment and willing to participate in the study were included in the study. The study was conducted over a period of 18 months. Those subjected to any ocular surgery, on topical anti-glaucoma medications and with media opacities severe enough to prevent visualization of the fundus were excluded from the study.

Study Procedure and Sampling

The individuals included in the research were recruited from the Ophthalmology Outpatient Department (OPD) of the institution after receiving approval from the Institute's Ethical Committee. The research was conducted between February 2021 and August 2022. Seventy people with type 2 diabetes were recruited, along with seventy healthy controls of similar age and gender, to take part in the research. All participants gave their written informed permission before being included in the research. Eye and overall health histories were recorded based on the study conducted by Sharma et al[11]. The anterior portion was examined using a slit light. Direct and indirect ophthalmoscopy, as well as a 90D slit lamp bio-microscope (Shreeji Microsystems, Ahmedabad, India), were used to examine the mydriatic fundus in great detail. Clinical evaluation of diabetic retinopathy severity was performed using the Early Treatment of Diabetic Retinopathy Study (ETDRS) classification[12]. Intraocular pressure was measured using Goldmann's applanation tonometry, and lacrimal drainage was evaluated by syringing. Following the clinical evaluation, participants underwent slit lamp imaging of conjunctival vascular morphology as part of a study of conjunctival vessels.

Study Technique

Slit Lamp Imaging System

The imaging system consists of:

Slit lamp Module

1) Code: TT

Part name: Table Top

• 36 inches × 16 inches

• Varying height of 7 inches controlled by leveling switch

2) Code: SL

Part name: Slit lamp

Eyepiece: 12.5X Eyepiece with +6 to –6-diopter adjustment

Interpupillary Distance: Adjustable from 52 mm-75 mm

Working distance: 100 mm (Table I).

Imaging Module

The Slit Lamp is employed with the components, namely,

1) 100% beam splitter

2) C-Mount

3) Digital color CCD Camera

4) Camera Power Adapter

5) External Light source

System Module

- Motherboard – Intel D845WN
- Processor – i3 2.10 GHz
- RAM – 4GB
- Display Card – AMD 512 MB
- Combo Drive – Samsung
- Hard Disk – 500 GB Seagate
- USB with video input.
- Keyboard – Dell
- Mouse – Dell
- Monitor – DELL 24 "monitor
- Appasamy Imaging Software version 3.4

Vessels on the temporal bulbar conjunctiva were photographed using the image-net. After that, a similar photograph was taken, this time without the red tint. Using the system's measuring software, we determined the breadth of the largest vessel in the red-free image. Then, the vascular findings in the conjunctiva were connected to those in the retina.

Statistical Analysis

MS Excel was used for data entry, and SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used for all analyses. The independent *t*-test and Chi-square test were used to verify that the ages and sexes of the cases and controls were comparable. Proportions and percentages were used to summarize the categorical variables, such as diabetes duration and diabetic retinopathy severity. The Chi-square test of association was used to examine the correlation between the severity of diabetic retinopathy and the breadth of the conjunctival sac. In this case, the Q-Q plot was used to visually evaluate the normality of the continuous data. One-way ANOVA was used to analyze the correlation between the average width of the conjunctiva and the severity of diabetic retinopathy. A *p*-value lower than 0.05 was considered statistically significant.

Results

The largest age group was between 56 and 65 years, comprising 50% of the sample. Equal numbers (30/70, or 42.8%, and 40/70, or 57.1%) apply to the comparison group. The onset of DM did not vary between the sexes. This shows that participants in the study and controls were of similar ages and genders.

The average length of time a patient was unwell was between 15 and 19 years. 48/70 individuals (68.57%) have been classified as having mild to moderate symptoms. Seven out of seventy patients (10%) had no retinopathy (Table II).

The average width of the conjunctival vessels in healthy people was 27.3 microns, with a median of 28 microns. In contrast, the study population had an average width of 36.6 microns and a median width of 37 microns. The significance level for this difference is 0.001 [Chi-Square (df): 131 (6)]. Figure 1 shows that people with diabetes mellitus have larger-than-average conjunctival arteries.

The difference between the conjunctival widths of both eyes in the control group and the study population was between 0.1 and 0.3 microns. The conjunctival width varied by less than 1 micron

Table I. Details of magnification.

Magnification setting	0.4 X	0.6 X	1.0 X	1.6 X	2.5 X
Field of view in mm	35	23.4	14	8.8	5.7
Total magnification	5.5 X	8.5 X	14 X	23 X	35 X
Image magnification in continuous grabbing window (17" Monitor)	9 X	13 X	22 X	35 X	55 X

Table II. Characteristics of diabetics mellitus patients [N=161].

Features	N (%)	
Duration of diabetes (years)		
05-09	11	15.71%
10-14	15	21.43%
15-19	30	42.86%
20-24	13	18.57%
25-29	1	1.43%
Severity of Diabetic Retinopathy		
No DR	27	39%
Mild PDR	7	10%
Moderate NPDR	21	30%
Severe NPDR	11	16%
PDR	4	6%

DR-Diabetic Retinopathy, PDR-Proliferative Diabetic Retinopathy, NPDR-Non-Proliferative Diabetic Retinopathy

in 33.1 microns (47.14%) of the eyes in the control group. Twenty-three (32.86%) patients had a difference in eye distance of more than 0.1 microns. Twenty-six (34.3%) controls and thirty-five (50%) patients of the study group had a difference of two microns or less. Thirteen controls (18.6%) and twelve research participants (17.1%) had a difference of 0.3 microns. All this data proves is that there is a little but insignificant change (p-value=0.2095) in conjunctival width between the two eyes.

Only 14 of the evaluated eyes out of a total of 140 with diabetic retinopathy were completely healthy. A total of 54, 42, 22, and 8 eyes, respectively, showed signs of mild, moderate, severe, or PD retinopathy. The conjunctival width was between 25 and 29 microns in the majority of patients (n=07, 50%) without diabetic retinopa-

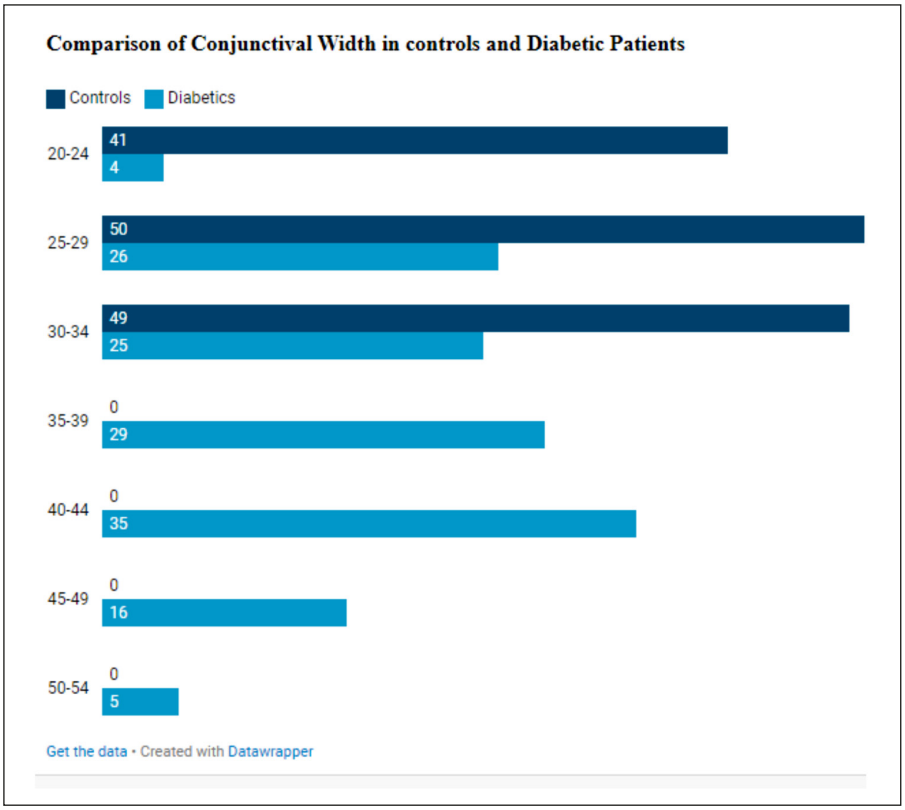

Figure 1. Comparison of conjunctival width in controls and diabetic patients.

thy, but between 30 and 34 microns in 30% of those with mild diabetic retinopathy. In 24% of moderate non-proliferative diabetic retinopathy (NPDR) and 41% of severe NPDR patients, the conjunctival width rose to 45-49 microns and 40-44 microns, respectively. Sixty-three percent of people had a conjunctival width of 50-54 microns. The results of the research show that as the severity of diabetic retinopathy worsens, so does the conjunctival width range. Figure 2 shows that this correlation is very significant (p-value <0.001).

Also, the width ranges from 30 to 34 microns (mean: 34.9) in patients with mild diabetic retinopathy, 35 to 39 microns (mean: 37.3) in those with moderate diabetic retinopathy, 40 to 44 microns (mean: 42.4) in those with severe diabetic retinopathy, and 50 to 54 microns (mean: 45.6) in those with proliferative diabetic retinopathy. With a p-value <0.0001 (Figure 3), the Chi-Square test deems this difference to be statistically significant.

Discussion

Diabetic patients visiting an eye OPD often vary in age from 56 to 65 and older. This agrees with the worldwide prevalence found in a study by Wild et al[13]. Diabetic retinopathy often develops in people with type 2 diabetes after 15 years[14]. The death rate was lower among people whose condition had been ongoing for more than 20 years (18.57%). Despite the small sample size, these findings may suggest that people with type 2 DM have a shorter life expectancy than normal after their condition has been established for at least 25 years.

In the present study, almost two-thirds of the diabetic participants had mild to severe NPDR. In addition, this figure is higher than the global prevalence[2]. Severe non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) were observed in 22% of patients, which is higher than the global prevalence of PDR [6.96 PDR + 10.2 visually threatening DR (VTDR)][2].

Microvascular abnormalities in the bulbar conjunctiva were studied by Cheung et al[15] in patients with type 2 diabetes mellitus. Using computer-assisted intravital microscopy (CAIM), the researchers looked at the microcirculation in the conjunctiva of 14 type 2 diabetes patients and 14 healthy control volunteers of the same age. T2DM patients' (n=14) conjunctival artery diameters were found to be significantly (p-value <0.01) larger than those of healthy, non-diabetic control participants (54.0±4.4 mm). The study cases had significantly (p-value <0.01) aberrant microvascular distribution, with uneven vessel distribution on the surface of the bulbar conjunc-

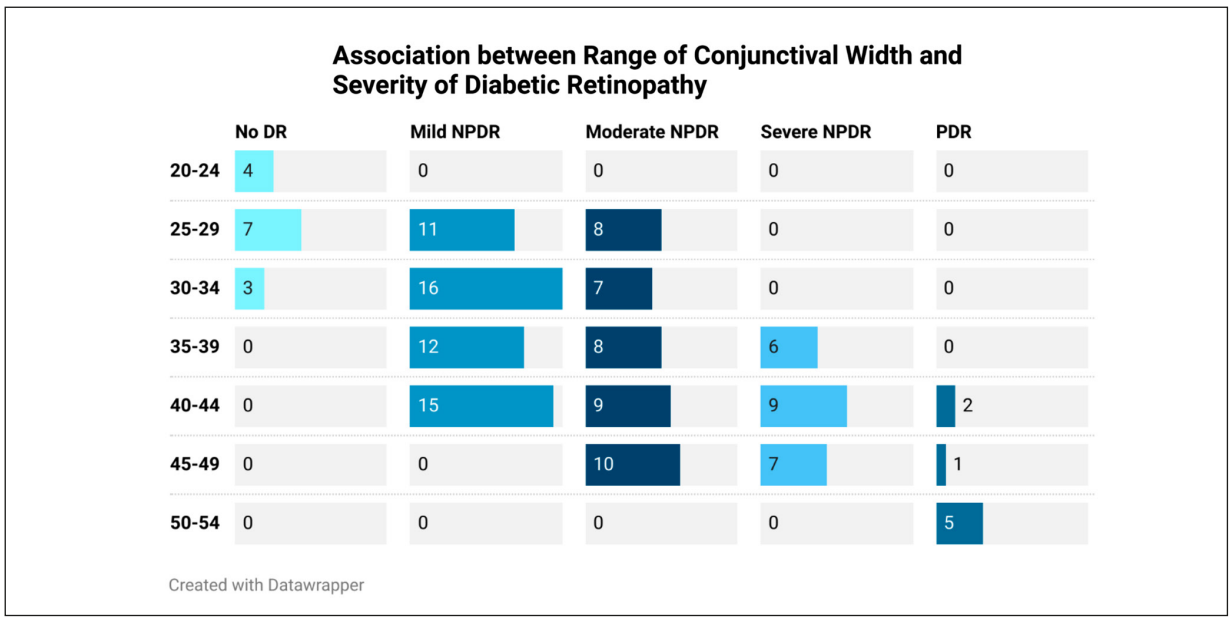

Figure 2. Association between range of conjunctival width and severity of diabetic retinopathy (DR-Diabetic Retinopathy, PDR-Proliferative Diabetic Retinopathy, NPDR-Non-Proliferative Diabetic Retinopathy).

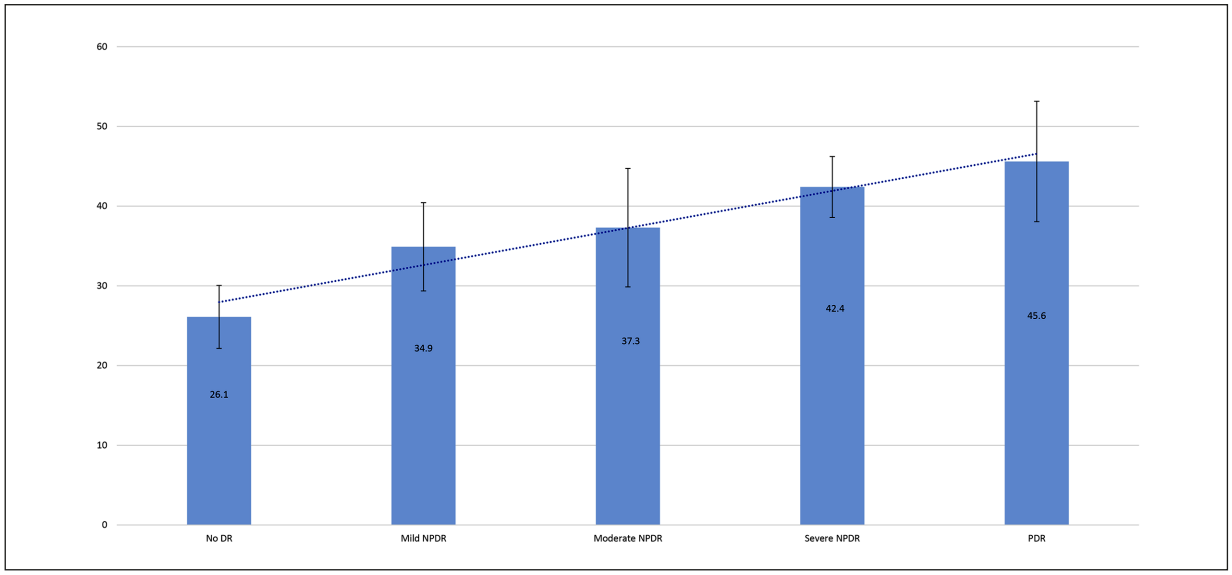

Figure 3. Comparison of mean conjunctival width between different stages of Diabetic Retinopathy (DR-Diabetic Retinopathy, PDR-Proliferative Diabetic Retinopathy, NPDR-Non-Proliferative Diabetic Retinopathy).

tiva (36.7±18.2 cm per unit area against 45.3±9.6 cm per unit area, patients *vs.* control participants). The results from this research agree with those of Cheung et al[15].

Noninsulin-dependent diabetes mellitus was examined by Largue et al[16] using conjunctival and peri-ungual angioscopy. Diabetics were shown to have significant venous dilatation in their conjunctival veins. The dilatation in diabetes individuals has been emphasized in previous studies[17,18] on conjunctival tortuosity and conjunctival micro-circulation abnormalities. Our results are consistent with these observations.

This investigation not only measured the diameter of conjunctival vessels but also assessed the results in light of the literature on the subject of DR's severity. This research found that the diameter of the conjunctival arteries was larger in individuals with diabetes mellitus and that this increase became statistically significant with increasing DR severity (p-value <0.0001). Similarly, there is a statistically significant (p-value <0.0001) difference in the breadth of the conjunctiva between eyes with mild and moderate NPDR and eyes with severe NPDR and PDR.

Similarly, 96 individuals with type 2 diabetes mellitus had their conjunctival artery widths and tortuous segment lengths photographed and analyzed using the Zeiss Fundus camera 100 and Visupac software (Carl Zeiss Meditec Group, Germany). Statistically significant (p-value <0.01) was a rise from a mean of 34.9 (SD 5.54) in the moderate NPDR group to a mean of 45.6 (SD 7.55) in the PDR group for the breadth of the conjunctival vessels. The length of the convoluted conjunctival vascular segment grew from 711.51 (SD 83.90) to 921.94 (SD 129.26) in the group with moderate NPDR (p-value <0.01). Patients with PDR were the only ones with vessel diameters more than 80, and those with severe grades of NPDR and PDR had tortuosity values greater than 900[19].

Another research[20] found that in arterioles, NDR led to a significant reduction in blood flow (V), wall shear rate (WSR), and wall shear stress (WSS) (p-value <0.01). V was significantly lower in NDR individuals (p-value=0.02) compared to NPDR and PDR patients. D was greater in NDR and NPDR (p-value=0.03), but V was lower in venules in the PDR (p-value=0.04). Higher values for venular V and Q were seen in NPDR participants compared to PDR subjects (p-value=0.04). Since both WSR and WSS were reduced in all stages of DR (p-value <0.05), this finding suggests that WSS might be used as a diagnostic for diabetic microvasculopathy.

Long-term diabetic patients show a reduction in tortuosity and a loss of capillaries, as shown by the vascular analysis technique in infrared pictures of the conjunctiva[8,9,21]. It is well established that the duration of diabetes has a major impact on the microangiopathic consequences

of the conjunctival vasculature[18,22]. Conjunctival hypoxia has been linked to the pathology of acute infectious conjunctivitis, which has been reported[23] to afflict people with diabetes mellitus more often than those without diabetes.

According to the literature, vasodilation in NPDR and PDR subjects is caused by the Vascular Endothelial Growth Factor (VEGF) in the conjunctival macrophages, epithelial, endothelial, and fibroblast cells[24].

The results of this research might also be used to create automated methods for detecting diabetic retinopathy. The screening process might be simplified, and the disease's development slowed as a result. With corresponding values of 78.70%, 69.08%, and 75.15%, a hierarchical multi-tasking network model (HMT-Net) was built to detect diabetes. Reportedly, the model's evaluation of the conjunctival pictures enabled rapid diagnosis of diabetes[25].

Limitations

In the current study, the latent period, if any, associated with the appearance of conjunctival changes was not calculated, as this time-gap would help in anticipating the changes in retinal vessels.

Conclusions

In patients with diabetes mellitus, the caliber of conjunctival arteries increases considerably with the increasing severity of the disease. These two categories have generally different therapeutic regimens, as pan-retinal photocoagulation is routinely performed in cases of severe NPDR and PDR. Conjunctival artery dilatation and increased tortuosity are plainly detectable changes that correlate positively with deteriorating retinopathy in diabetics. This may find wider use as a screening tool for prompt referral.

Conflict of Interest

The authors declare that they have no conflict of interests.

Authors' Contribution

RAS has conceptualized the study and played a primary role in compiling analysis and interpretation of the data. RAS, BV and CJJ did the manuscript preparation. MZA and MAA did the manuscript editing. MBA and MMA approved the final draft. RAS, MZA, and MZA did the data cleaning and data analysis. All the authors take complete responsibility for the content of the manuscript, read, and approved the final version of the manuscript.

Ethics Approval

The ethical approval for the study was obtained from the Institutional Human Ethical Committee, Koppal Institute of Medical Sciences, Koppal, with No. KIMS-Koppal/IEC/53/2021-22.

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Informed Consent

Informed consent was obtained from patients prior to the beginning of the study.

Availability of Data and Materials

The data will be available with the corresponding author and can be accessed on request *via* email.

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