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Original Article

A clinical correlation of conjunctival microangiopathy with grades of retinopathy in type 2 diabetes mellitus

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ARTICLE INFO

Article history:

Received 24 August 2016

Accepted 5 January 2017

Available online 16 February 2017

Keywords:

Conjunctival microangiopathy

Diabetes

Retinopathy

ABSTRACT

Background: Conjunctival microangiopathy has been described among diabetics similar to retinal vessel angiopathy. Correlation of these conjunctival microangiopathy changes with retinopathy may form the basis of screening by external examination without expert fundus evaluation.

Methods: Conjunctival vessels widths and tortuous segment length of conjunctival vessels of 96 patients with type 2 diabetes mellitus were photographed and measured by the Zeiss Fundus camera Visupac software. The measurements were correlated with retinopathy grade in those eyes.

Results: The mean conjunctival vessel width was 40.61 μ (SD 17.25) with a uniform increase from 34.4 μ (SD 8.70) in mild NPDR to 53.50 μ (SD 33.45) in the PDR group which was statistically significant ($p < 0.01$). The tortuous conjunctival vessel segment length increased from 711.51 μ (SD 83.90) in the mild NPDR group to 921.94 μ (SD 129.26) in those with PDR ($p < 0.01$). Vessel width greater than 80 μ was seen only in PDR and tortuosity values greater than 900 μ were seen in severe grades (severe NPDR and PDR). Both conjunctival vessel width and tortuosity showed a positive statistical correlation with increasing severity of retinopathy ($r = 0.386$, $r^2 = 0.149$ and $r = 0.645$, $r^2 = 0.415$).

Conclusion: A positive correlation was seen between conjunctival vessel width and tortuosity with severity of retinopathy. Widths over 80 μ and tortuous segment length over 900 μ are suggestive of severe grades of retinopathy.

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<http://dx.doi.org/10.1016/j.mjafi.2017.01.005>

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Diabetic retinopathy is one of the leading causes of blindness worldwide.¹ It is characterized by microangiopathy of the retinal vasculature resulting in retinal oedema and proliferative retinopathy.² Diagnosis requires fundus examination and in some cases specialized imaging necessitating referral to an ophthalmologist.³ Many patients, however, become aware of their retinopathy only when it starts affecting their vision, resulting in delayed treatment and permanent loss of vision.⁴ Several studies have demonstrated abnormalities in the conjunctival vasculature such as dilatation and tortuosity similar to the retinal vascular changes in retinopathy.^{5,6} Evaluation of conjunctival angiopathy and its correlation with severity of diabetic retinopathy may help in screening out individuals having retinopathy by simple external examination and digital photography, facilitating early referral for evaluation and treatment. Most studies on conjunctival vascular changes among diabetics are on western subjects, and there is no data on conjunctival microangiopathy in the Indian diabetic population. India has one of the highest incidence of diabetes in the world and several studies have shown that the Indian diabetic is a unique demographic subgroup as compared to western subjects.^{7,8} In addition, the previous studies have not clearly documented the correlation of conjunctival changes with various grades of diabetic retinopathy. This study was therefore carried out to describe the conjunctival manifestations in Indian patients with type 2 DM and correlate these changes with various severity grades of retinopathy.

This cross-sectional study was carried out in a tertiary care hospital between December 2013 and September 2015. The subjects were selected from the patients reporting to the outpatient department. Only patients with type 2 diabetes with diagnosed retinopathy of any grade were included in the study. Patients with previous surgery, chronic topical medication, any inflammatory conditions, dry eyes, and chronic ocular allergies were excluded. Also excluded were patients with media opacities precluding good quality fundus photography. A sample size of 96 patients was calculated using an estimated prevalence of 50% for conjunctival abnormality with margin of error of 10%. The study followed the current guidelines of Helsinki declaration and clearance was obtained from the institutional ethical committee. All patients under-

went complete ocular examination inclusive of detailed mydriatic fundus examination with direct and indirect ophthalmoscopy. The level of diabetic retinopathy was assessed clinically based on the Early Treatment of Diabetic Retinopathy Study (ETDRS) classification. On completion of the clinical examination, the subjects were then taken up for the study of conjunctival vessel morphology. The width and tortuosity of the most prominent conjunctival vessel were measured for correlation. The Zeiss Fundus camera FF 450 plus IR with Visupac software was used for both the fundus photo and photography of the conjunctival vessel. The image of the most prominent vessel on the bulbar conjunctiva was located and captured at a field angle of 20° with viewing magnification of 29× and monocular eye piece magnification of 10× by the fundus camera and then subjected to the calculation of the width and tortuosity using the measurement program of the Visupac system (Fig. 1a and 1b). For measurement of width of the vessel, the measure key and line function of the measurement software were used to draw a line across the width of the most prominent vessel, which automatically gave the value in microns. For measuring the tortuosity, the tortuous segment of the most tortuous appearing vessel was marked, then using the 'free hand line' function, a straight line was drawn along the course of this segment. The length of this tortuous segment of vessel was displayed in microns. This was taken as tortuosity of the vessel. The findings of the conjunctival vessels were then correlated with the grades of retinopathy and results were subjected to statistical analysis. One-way ANOVA test was used to measure the intra-group and inter-group variations of width and tortuosity among the severities of diabetic retinopathy. Spearman's correlation coefficient was calculated for correlation of the width and tortuosity with various grades of severity of diabetic retinopathy, which were assigned ordinal ranks from 1 to 4 in ascending grades of severity. Unpaired t-test was used to check the significance of differences in the means of width and tortuosity in males and females.

The demographic profile, distribution of grades of retinopathy and conjunctival vessel width and tortuosity in the study population are summarized in [Table 1](#). The mean age was 60.11 (45–80 years) with a slight male preponderance. Mild and moderate grades of nonproliferative retinopathy (NPDR) accounted for 66.67% and 33% had severe NPDR or proliferative diabetic retinopathy (PDR). The mean conjunctival vessel

Age (years)	Gender, M/F (%)	Severity of retinopathy				Conjunctival vessel width (μ)	Conjunctival vessel tortuosity ^a (μ)
		Mild (%)	Moderate (%)	Severe and very severe (%)	PDR (%)		
^a 60.11 ± 8.9 (58.29–61.93)	54.2/45	37.5	29.17	16.67	16.67	^a 40.61 ± 17.25 (37.68–43.54)	^a 797.92 ± 140.99 (772.75–823.08)
^a Mean, SD and 95% CI.							

Table 2 – Mean conjunctival vessel width and tortuosity length among various grades of retinopathy.

	Mild NPDR	Moderate NPDR	Severe and very severe NPDR	PDR
Conjunctival vessel width in μ (mean, SD and 95% CI)	34.4 $\pm$ 8.70 (31.5–37.3)	39.04 $\pm$ 8.60 (35.58–42.28)	44.47 $\pm$ 11.20 (38.87–50.07)	53.50 $\pm$ 33.45 (36.77–70.23)
Tortuosity length in μ (mean, SD and 95% CI)	711.51 $\pm$ 83.90 (683.54–739.48)	783.82 $\pm$ 77.17 (754.7–812.94)	874.6 $\pm$ 106.75 (821.22–927.98)	921.94 $\pm$ 129.26 (857.31–986.57)

Table 3 – Analysis of variation in width and tortuosity between various grades of retinopathy.

	Mild	Moderate	Severe	PDR
Conjunctival vessel width (μ m)				
Mean and SD	34.44 $\pm$ 8.7	39.04 $\pm$ 8.6	44.47 $\pm$ 11.2	53.5 $\pm$ 33.45
Mild	–	NS	$p < 0.05^*$	$p < 0.01^*$
Moderate	NS	–	NS	$p < 0.01^*$
Severe	$p < 0.05^*$	NS	–	$p < 0.05^*$
PDR	$p < 0.01^*$	$p < 0.01^*$	$p < 0.05^*$	–
Conjunctival vessel tortuosity length (μ m)				
Mean and SD	711.51 $\pm$ 83.88	783.82 $\pm$ 77.17	874.63 $\pm$ 106.75	921.94 $\pm$ 129.26
Mild	–	$p < 0.01^*$	$p < 0.01^*$	$p < 0.01^*$
Moderate	$p < 0.01^*$	–	$p < 0.01^*$	$p < 0.01^*$
Severe	$p < 0.01^*$	$p < 0.01^*$	–	NS
PDR	$p < 0.01^*$	$p < 0.01^*$	NS	–

* One-way ANOVA test; $p < 0.0001$ – significant, for width $F = 11.31$, DoF = 3; for tortuosity $F = 45.29$, DoF = 3. Tukey HSD test for significance between groups.

width was 40.61 μ (SD 17.25), which showed a uniform increase from 34.4 μ (SD 8.70) in those with mild NPDR to 53.50 μ (SD 33.45) in the PDR group (Table 2) and this increase in vessel thickness between various grades of retinopathy was statistically significant (Table 3, $p < 0.01$). The tortuous conjunctival vessel segment length measurement also showed increase from 711.51 μ (SD 83.90) in the mild NPDR group to 921.94 μ (SD 129.26) in those with PDR (Tables 2 and 3, $p < 0.01$). Majority of the patients with mild to moderate retinopathy had conjunc-

tival vessel width between 30 and 40 μ whereas most of the subjects with severe NPDR and PDR had the vessel width in the range of 41–60 μ . Vessel width greater than 80 μ was seen only in cases with PDR. For tortuosity, values greater than 900 μ were more commonly seen in severe grades (severe NPDR and PDR) as compared to mild and moderate NPDR. Conjunctival vessel width showed a positive statistical correlation with increasing severity of retinopathy (Fig. 2, $r = 0.386$, $r^2 = 0.149$) whereas conjunctival tortuosity showed a strong positive

Table 4 – Sub-group analysis gender-wise.

	Male	Female	Significance
Age (mean and SD)	59.2 $\pm$ 9.85	61.04 $\pm$ 8.25	$p = 0.33$
Distribution according to grades of retinopathy			
Mild (%)	20.83	16.67	
Moderate (%)	14.58	14.58	
Severe (%)	7.29	9.38	
PDR (%)	11.46	5.21	
Severity score (mean and SD)	2.17 $\pm$ 1.16	2.05 $\pm$ 1.02	
Conjunctival width (mean and SD)			
Overall	44.19 $\pm$ 21.12	36.42 $\pm$ 9.48	$p = 0.03$
Mild	36.73 $\pm$ 9.78	31.76 $\pm$ 6.30	
Moderate	41.54 $\pm$ 10.56	36.58 $\pm$ 5.18	
Severe	46.5 $\pm$ 8.23	43.33 $\pm$ 13.42	
PDR	58.54 $\pm$ 36.78	39.1 $\pm$ 11.62	
Conjunctival tortuosity (mean and SD)			
Overall	787.25 $\pm$ 126.68	803.85 $\pm$ 120.32	$p = 0.52$
Mild	693.35 $\pm$ 79.47	740.73 $\pm$ 88.15	
Moderate	765.96 $\pm$ 98.93	791.04 $\pm$ 71.34	
Severe	942.41 $\pm$ 118.35	851.22 $\pm$ 114.82	
PDR	886.21 $\pm$ 110.41	1036.5 $\pm$ 259.79

Fig. 1 – (a) Screen shot of the measure function keys used to measure the width of vessel and tortuous segment length. (b) Dilated and tortuous conjunctival vessel in a case of PDR.

Fig. 2 – Graph showing correlation of width of conjunctival vessel with severity of retinopathy.

correlation with increasing severity of retinopathy (Fig. 3, $r = 0.645$, $r^2 = 0.415$).

Table 4 shows the gender-wise analysis of diabetic severity grades and conjunctival microangiopathy. There was no significant difference between males and females in terms of age, distribution of various grades of retinopathy and severity score for retinopathy ($p > 0.05$). There was a higher proportion of subjects with PDR among males but the difference was not statistically significant. The mean conjunctival vessel width was greater among the males compared to females which was statistically significant ($p = 0.03$) but there was no significant difference between the genders for tortuous vessel segment length ($p = 0.52$). The trend of increasing width and tortuous segment length with increasing severity of retinopathy was seen among males and females alike.

Discussion

Conjunctival vessels are easily visible and amenable to imaging and analysis. Several studies since long have

Fig. 3 – Graph showing correlation of tortuosity of conjunctival vessel with severity of retinopathy.

identified distinct morphological changes in the conjunctival vessels as compared to control population.^{9,10} Recent advances in digital photography have now made accurate quantification possible and studies using quantitative analysis have indicated that microangiopathic changes in the retinal vessels are associated with changes in the conjunctival vessels.^{11,12} However the correlation of these conjunctival vascular changes with severity of retinopathy and its utility as predictors of retinopathy severity is not well documented in available literature. In the current study, morphological changes in the conjunctival vessels among type 2 diabetics with various grades of retinopathy were documented and measured using digital photography and correlated with various grades of retinopathy. In our group of patients, significant retinopathy was seen among those in 6th to 7th decade which corroborates with other published studies. According to Wild et al. the increase in number of people with diabetes by 2030 will be primarily due to increase in proportion of people above 65 years age group.¹³ The same

study also showed a higher prevalence of diabetes and its complication in men as compared to women which corroborates with the male preponderance in our sample. Dilatation and increased tortuosity are the commonest conjunctival vessel abnormalities that have been reported among diabetics. These abnormalities were noticed by To et al. in type 2 diabetes even before they had developed significant retinopathy.¹⁴ Cheung et al. videotaped and studied the vessel width using computer assisted intravital microscopy.¹⁵ Lagrue et al. studied conjunctival vessel morphology by conjunctival angioscopy.¹⁶ Both these studies concluded that there was significant increase in conjunctival vessel width among diabetes as compared to non diabetic controls. In this study as well, dilated conjunctival vessels were seen among the diabetic subjects and on comparing between the various grades of retinopathy we noted a trend of increasing vessel width with increasing severity of retinopathy which was statistically significant. Values of more than 80 microns were exclusively seen among subjects with PDR and may be taken as indicative of severe retinopathy. Measurements of tortuous segment length also showed an increasing trend with increasing severity of retinopathy, with tortuous segment lengths of $>900\ \mu$ seen in severe cases (severe NPDR and PDR). In this study we selected the most prominent vessels for measurement of width as well as tortuosity and we were able to demonstrate a positive correlation with increasing severity of retinopathy, unlike Owen et al. who demonstrated straightening more commonly in larger vessels and increasing tortuosity in smaller vessels with increasing duration of diabetes.¹⁷ However Cheung et al.¹⁸ using a novel technique of video taping and computer assisted intravital microscopy also demonstrated a sinusoidal pattern in large conjunctival veins, similar to our study. Compared to conjunctival width, tortuous segment length showed a much stronger correlation with severity of retinopathy in this study. This suggests that tortuosity of conjunctival vessels may be a more important parameter for estimating the severity of retinopathy as compared to width of vessels. Though there was a clear trend towards increasing width with increasing severity of retinopathy there was overlap between different grades of retinopathy making it difficult to define any cut off value for conjunctival width or tortuous length for different grades of retinopathy. Sub group analysis gender wise revealed a similar trend of increasing width and tortuosity in males as well as females with increase in severity of the retinopathy. A higher conjunctival width among males as compared to the females could have resulted from the higher number of PDR cases among the males in the group.

This study for the first time describes conjunctival microangiopathy among Indian diabetics and demonstrates positive correlation with increasing severity of retinopathy. We have not used advanced mathematical models and algorithms for measurement because such complicated methods seldom find wider applicability. However variations resulting from observer bias in selection of the most tortuous vessel segment could be considered a limitation of this method. The use of Fundus camera for capturing images of conjunctival vessels, as done in this study, is not mandatory as digital imaging of conjunctival vessels is possible using any digital camera with optical zoom and appropriate software. These findings can thus be recorded

and interpreted even by technicians and referring doctors other than ophthalmologists.

To conclude, dilatation of conjunctival vessels and increased tortuosity are easily visible changes among diabetics which correlate positively with increasing severity of retinopathy. This may have a potential for wider applicability as a screening method for early referral. Since this study was designed to correlate microangiopathy with severity grades of diabetic retinopathy, controls were not taken. However based on our study we recommend that larger controlled studies may be carried out to further confirm these findings.

Conflicts of interest

The authors have none to declare.

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