Original Article

Evaluation of Macular Ganglion Cell Layer in Diabetic Eyes with Normal Visual Acuity: Impact of Metabolic Control

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Abstract

Aims: To evaluate the relation between metabolic control and ganglion cell-inner plexiform layer (GC-IPL) thickness in diabetic eyes.

Methods: This is a single centre cross-sectional study. Consecutive patients of type 2 DM with normal visual acuity were included as per study protocol. Serum lipid levels and serum HbA1c values were recorded. Central macular thickness (CMT) and GCL-IPL thickness were obtained with standard automated segmentation by optical coherence tomography of the macula. Correlation between GCL-IPL thickness and metabolic control was evaluated and multivariate logistic regression was used to evaluate true impact of metabolic control on GCL-IPL thickness.

Results: Eighty six eyes of 43 patients were included. Correlation analysis detected significant relation of GCL-IPL thickness with age (R= -0.248, p=0.022) and CMT (R=0.425 (p=0.000), but not with any other variable including metabolic control (serum lipid profile and HbA1c). There was no significant impact of retinopathy on GCL-IPL thickness. Regression analysis for sectoral GCL-IPL thinning revealed high adjusted odds ratio for HDL cholesterol (5.744), deranged total cholesterol (4.733), and deranged HbA1c (1.575); though with large confidence interval.

Conclusion: There is a possible association of GCL-IPL thinning with metabolic control in diabetic individuals. All future analysis of macular ganglion cell layer must account for inherent confounders like age and CMT.

Keywords

Diabetic neuro-degeneration, diabetic neuro-retinopathy, ganglion cell layer in diabetes, hyperlipidemia and retinopathy

Introduction

Diabetic Retinopathy (DR) is the most common microvascular complication of long-standing diabetes mellitus (DM).[1,2] It is a leading health concern and a significant cause of blindness. The pathogenesis of DR and its evolution are strongly related to metabolic factors like the degree of glycaemic control and hyperlipidaemia.[1,3] It is well known that "sustained" blood glucose control is paramount for best outcomes. For these reasons, plasma glycosylated haemoglobin (HbA1c) is regarded as the gold standard indicator for glycaemic control in DM and has been extensively studied for its impact on DR, the central macular thickness (CMT) and the outer retinal

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layers.[4,5]Deranged serum lipids are very often seen in conjunction with DM as a part of a systemic metabolic disease. They have long been thought to be a risk factor for DR. There are several conflicting reports in the literature regarding the effect of lipid profile on CMT, outer retinal layers and DR.[1-3]However, literature lacks in evaluation of the inner retinal layers in this perspective of metabolic control, particularly the ganglion cell-inner plexiform layer (GC-IPL).

Diabetes has an early neurodegenerative effect on the retina, even in stages of no or minimal vascular component of DR.[1-3,6]Studies highlighting diabetic "neuro-retinopathy" (DNR) have found it to precede clinical DR using multifocal electroretinography (MFERG). Infact, studies have indicated DNR to be a possible cause of clinical manifestations of DR.[7-9]MFERG allows evaluation of the photoreceptors and the bipolar cells, but cannot assess the functional aspect of final neuronal cell body of the retina-the ganglion cell.[10]Thus the precise role of GC-IPL in DNR remains to be evaluated. There are very few studies in eyes without clinical retinopathy. Optical coherence tomography (OCT) is more sensitive in evaluating the macula than other modalities, and modern-day OCT allows evaluation of the retina layer by layer, like an optical biopsy.[11,12]The GC-IPL can be quickly evaluated with OCT, particularly in clinically normal eyes before microangiopathy has set in.[9,13,14]

This study aims to evaluate the relationship between metabolic control (HbA1c and serum lipid levels) and GC-IPL thickness in diabetic eyes without vision-threatening DR. In doing so, we assess DNR at the ganglion cell level and ascertain its possible risk factors.

Methods

This is a single-centre, cross-sectional, observational study at a tertiary eye care centre of central India. The study was approved by the institutional review board and ethics committee (LOP-IM0196, date: 16-7-2018). Informed consent was obtained from all patients and the study was in accordance with the tenets of declarations of Helsinki and its later amendments.

Consecutive patients of type 2 DM presenting between June 2018 and January 2019 were evaluated. These patients were recruited from the retina clinic or diabetic retinopathy screening clinic. Patients with ocular disease apart from cataracts, and those with diabetic macular edema (DME) or proliferative DR (PDR) were excluded. Pseudophakic patients and those with a history of prior ocular treatment were also excluded. Barring hypertension and hyperlipidemia, patients with systemic disease, for example, nephropathy, were also excluded. All serological investigations had been done within the study centre at the time of presentation. Duration of DM, detailed serum lipid levels and serum HbA1c values were recorded. Refraction was done for all eyes and only the patients with visual acuity of 20/20 in both eyes were included in the study. A single retina surgeon did a Fundus evaluation of all patients under mydriasis. PDR was ruled out with fluorescein angiography when in doubt clinically.

Each patient underwent macular OCT imaging of both eyes with automated segmentation. All imaging was done by a single technician using a single OCT machine (HDOCT-Cirrus, Carl- Zeiss Meditec, Dublin, USA). Macular cube protocol (512X128) was used for each OCT examination. This protocol allows ganglion cell analysis, where the software generates data of the actual GCL-IPL thickness across six equitable sectors centred on the fovea and colour codes these values as green, yellow and red (depending upon deviation from the normative database). The machine also provides the average GCL-IPL thickness for each eye. Images with signal strength less than 7/10 were removed from analysis. Central macular thickness, average GCL-IPL thickness and the colour coding for various sectors were noted for each eye.

Data was serially entered in a Microsoft Excel worksheet, and statistical analysis was performed with SPSS version 20 software (SPSS Inc., Chicago, Illinois, USA). The correlation between GCL-IPL thickness and non-parametric variables was assessed using Pearson's coefficient for correlation. The sample was divided into two groups depending on the presence or absence of DR. For analysis of GCL-IPL sector, yellow and red-coded sectors were grouped as abnormal. Chi-square test was used for analyzing parametric variables, while the independent t-test was used for comparing non-parametric variables. Binomial multivariate logistic regression was used with the block method to ascertain the true odds ratio (OR) for abnormal sectors of GCL-IPL thickness. Only a 2-tailed p-value < 0.05 was considered statistically significant, and all confidence intervals (CI) described are true for 95% of the sample.

Results

86 eyes of 43 patients were analysed, out of which 32(74.4%) were male. The mean age of the patients was 54.26 years (SD =9.13) and the average duration of DM was 7.94 years. 50 eyes (58.1%) did not have DR, while 36 (41.9%) had NPDR. The mean HbA1c was 8.79%. Other variables including serum lipid profile have been enlisted in Table 1. The average GCL-IPL thickness was 79.23 microns (SD 8.65). 7 eyes (8.1%) had an abnormal average GCL-IPL layer thickness, while 24 eyes(27.9%) had at least one abnormal sector with thinning as centred on the fovea.

Table 1: Distribution of variables

Non-parametric variable	Minimum	Maximum	Mean	Std. Deviation
Age (years)	37	73	54.26	9.13
Duration of diabetes (years)	.50	24.00	7.94	6.29
Total cholesterol (mg/dl)	101.00	283.17	176.58	41.75
Triglycerides (mg/dl)	68.00	556.00	146.43	81.42
High density lipoproteins (mg/dl)	23.00	79.65	45.07	13.27
Low density lipoproteins (mg/dl)	40.71	183.84	99.34	35.24
Serum HbA1c (%)	5.90	14.55	8.79	2.30
Average GCL -IPL thickness of right eye (microns)	46.0	95.0	78.79	9.57
Average GCL -IPL thickness of left eyes (microns)	45.0	96.0	79.67	8.86
Meanaverage GCL -IPL thickness (microns)	45.5	93.5	79.23	8.65

Parametric variable	N (%)
Gender	43 (100)
Male	32 (74.4)
Female	11 (25.6)
Eye	86 (100)
Right	43 (50%)
Left	43 (50%)
Deranged lipid levels	43 (100)
Yes	27 (62.7)
No	16 (37.3)
Retinopathy	86 (100)
No retinopathy	50 (58.1)
Non proliferative diabetic retinopathy	36 (41.9)
GCL-IPL thickness**	86 (100)
Eyes with abnormal average values	7(8.1)
Eyes with at least one abnormal sector	24 (27.9)

*Any lipid level deranged

**Abnormal sectors include both yellow and red as per software

GCL-IPL: Ganglion cell- inner plexiform layer

Table 2 shows the correlation of all variables with GCL-IPL thickness. Age negatively correlated (R=-0.248) with overall average GCL-IPL thickness, the relation being statistically significant (p=0.022). Left eye average GCL-IPL thickness also showed a statistically significant negative correlation with age (R=-0.318 p=0.038) compared to right eye (R=-0.197 p=0.206). The relation of other variables like duration of DM, total cholesterol, Triglycerides, HDL and LDL Cholesterol, and HbA1c was not found to be significant with either the overall average GCL-IPL thickness or with individual eyes. CMT showed a positive and statistically significant ($p<0.05$) correlation with the individual eye, mean and overall average GCL-IPL thickness. Therefore, metabolic control did not show correlate significantly with GCL-IPL thickness in unadjusted analysis.

Table 2: Correlation of variables with ganglion cell-inner plexiform layer thickness

	Right Avg GCL-IPL thickness (n=43)	Left Avg GCL-IPL thickness (n=43)	Mean Avg GCL-IPL thickness (n=43)	Overall Avg GCL-IPL thickness (n=86)
Age	R= -0.197 (p= 0.206)	**R= -0.318 (p=0.038)**	R= -0.248 (p=0.078)	**R= -0.248 (p=0.022)**
Duration of DM	R= -0.097 (p=0.534)	R= -0.195 (p=0.209)	R= -0.154 (p=0.325)	R= -0.105 (p=0.339)
Total cholesterol	R= 0.148 (p=0.342)	R= -0.145 (p=0.354)	R= 0.008 (p=0.960)	R=0.008(p=0.939)
Triglycerides	R= 0.185 (p=0.235)	R= 0.022 (p=0.891)	R= 0.113 (p=0.469)	R= 0.106(p=0.336)
HDL Cholesterol	R= -0.130 (p=0.407)	R= -0.170 (p=0.276)	R= -0.159 (p=0.309)	R= -0.184(p=0.092)
LDL Cholesterol	R= 0.093 (p=0.551)	R= -0.144 (p=0.348)	R= -0.022 (p=0.890)	R= -0.005 (p=0.961)
HbA1c	R= 0.114 (p=0.465)	R= -0.022 (p=0.888)	R= 0.052 (p=0.741)	R= 0.041(p=0.711)
CMT	R= 0.455 (p=0.002)	R= 0.642 (p=0.000)	R= 0.594 (p=0.000)	R= 0.425(p=0.000)

Avg GCL-IPL: Average ganglion cell-inner plexiform layer, DM: Diabetes Mellitus, HDL: High density lipoprotein, LDL: Low density lipoprotein, CMT: Central macular thickness

Table 3: Comparison between patients with and without retinopathy

Non parametric variable		**Mean**	**Standard Deviation**	**P value**
Age (yrs)	No DR	54.24	9.52	0.951
	NPDR	54.11	8.62	
Duration of diabetes (yrs)	No DR	7.87	7.27	0.992
	NPDR	7.85	4.49	
Total cholesterol	No DR	170.96	39.67	0.147
	NPDR	184.59	43.88	
Triglycerides	No DR	128.36	43.84	**0.037**
	NPDR	172.76	111.39	
High density lipoprotein	No DR	45.57	15.17	0.710
	NPDR	44.54	10.09	
Low density lipoprotein	No DR	94.68	32.67	0.172
	NPDR	105.64	38.12	
HbA1c	No DR	8.37	2.26	**0.039**
	NPDR	9.41	2.23	
Central macular thickness (microns)	No DR	239.12	34.43	0.941
	NPDR	238.62	26.28	
Avg GCL-IPL thickness(microns)	No DR	78.42	9.87	0.088
	NPDR	81.34	5.67	
Parametric variable		No DR	NPDR	P value
Gender	male	39	25	0.329
	female	11	11	
Deranged lipid levels	Yes	31	22	0.936
	No	19	14	

Avg GCL-IPL: Average ganglion cell-inner plexiform layer, NPDR: Non proliferative diabetic retinopathy

Table 3 shows the comparison between groups with and without DR. Mean triglyceride level was found to be 128.36 microns in patients with no DR as compared to 172.76 microns in patients with NPDR, the difference being statistically significant (p=0.037).Mean HbA1c was found to be 8.37% in patients with no DR as compared to 9.41% in patients with NPDR (p=0.039). None of the other tested variables (age, gender, duration of diabetes, deranged lipid levels, total cholesterol, HDL and LDL cholesterol, CMT, Average GCL-IPL thickness)varied significantly between these groups.

A multivariate binomial logistic regression analysis for abnormal GCL-IPL sectors was performed to judge the true impact of metabolic variables on GCL-IPL layer (Table 4). The adjusted OR was found to be high for deranged HDL cholesterol (5.744), deranged total cholesterol (4.733), and deranged HbA1c (1.575). However, the confidence interval was very large for all these variables, and the lower confidence bound was below 1.

Table 4: Multivariate Binomial logistic regression analysis for abnormal GCL-IPL sector

Variable	Adjusted OR	P value	95% CI
Age > 50 yrs	0.173	0.016	0.041-0.726
Male gender	0.232	0.080	0.045-1.188
Duration of DM > 5 yrs	0.303	0.084	0.078-1.174
Deranged TC	**4.733**	0.193	0.456-49.111
Deranged TG	0.247	0.159	0.035-1.730
Deranged HDL	**5.744**	0.225	0.341-96.739
Deranged LDL	0	0.999	0.000-
Deranged HbA1c	**1.575**	0.578	0.318-7.8000
Deranged lipid level	0.350	0.490	0.018-6.898
Presence of DR	0.679	0.576	0.175-2.632

GCL IPL: Ganglion cell-inner plexiform layer, OR: Odds ratio, CI: confidence interval, DM: Diabetes mellitus, TC: Total cholesterol, TG: Triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, DR: Diabetic retinopathy

Discussion

We evaluated the relation between metabolic control (serum lipids and HbA1c) and GCL-IPL thickness in diabetic eyes without vision-threatening DR. We could not establish a significant correlation between these in univariate analysis, which revealed a weak-moderate relation between age and CMT(Table 2). Similarly, we could not find any impact of early retinopathy on GCL-IPL thickness (Table 3). However, on negating the impact of confounding variables on GCL-IPL thickness with regression analysis, we detected high adjusted OR for decreased sectoral GCL-IPL thickness in patients with high serum total cholesterol and HDL, albeit with limited statistical precision (Table 4). To our knowledge, analysis for the relationship of different lipid levels with inner retinal layers in DM has yet to be documented in current literature.

There are very few studies evaluating the impact of metabolic control on inner retinal neurons in early DR. At the same time, there is no such dedicated study in south Asia.[14-18]Further most of the previous studies analyse inner retinal layers with a custom segmentation technique or study ganglion cell complex (GCC=GCL + IPL+ peripheral macular RNFL) thickness as a whole. Evaluating automated GCL-IPL thickness instead of GCC or "custom" inner retinal layers is advantageous in giving more accurate results and reducing noise.[14,15]The available literature studying GCL-IPL thickness in DM has been summarized in Table 5.While most studies have shown decreased GCL-IPL thickness in DM eyes in comparison to controls,[14,16,17]Vujosevic et al could not find a significant difference in their study of 74 diabetic Italian eyes.[15] Some of these studies evaluated metabolic control partially for its impact on GCL-IPL thickness using Hba1c,[14,16] and like us did not find a significant relation upon univariate analysis. Like our results, these studies did not reveal impact of early DR on GCL-IPL thickness on univariate analysis.[14,16,17] However, none of these studies has performed multivariate analysis to negate confounding impact of other variables, and some of them have not commented upon vision loss in their sample (Table 5).[14,15,17]

Table 5: Comparison with existing literaturein context of GCL-IPL changes in early DR

Study design	Place- Year, sample size	Findings	Remarks
Vujosevic S. et al[15] Case- control	Italy-2013, 74 diabetics	Significant decrease of GCL-IPL documented at macula not documented in comparison to controls.	Visual acuity of patients with NPDR was less than that of no DR group Adjustment for CMT and age not done
Santos AR et al[16] Cross-sectional	Italy- 2017, 449 diabetics	Thinning of GCL-IPL in eyes with early DR as compared to normal healthy individuals, 79.4 u vs. 82.1 u	No difference in GCL-IPL between patients with different grades of DR Adjustment for CMT and age not done
Li S. et al[17] Cross-sectional	China- 2017, 175 eyes	1. GCL-IPL less in DM compared to controls, but not much difference between no DR and mild DR 2. No correlation of GCL-IPL with HbA1c.	Automated segmentation of layers of retina done to study GCL in 9 ETDRS sectors GCL and IPL studied individually Eyes up to vision loss of 6/19 included. Adjustment for age and CMT not done Lipid profile not evaluated
Francis AW et al[18] Case-control	USA- 2018, 44 mice	IRT was 2% lower in Akita mice than in wild type mice.	Akita mice were genetically engineered diabetic mouse with mutation in insulin 2 gene causing sustained hyperglycemia. IRT was defined as NFL+GCL+IPL +INL
Borooah M. et al[14] Cross-sectional	North East India-2018, 120 eyes	1. Reduction of mean GCL + IPL in T2DM patients, 79.95 u vs. 84.66 u 2. No correlation of GCL + IPL thickness with duration of diabetes and HbA1c.	No difference between no DR and mild DR groups Visual acuity, CMT and age not taken into account. Multivariate analysis not done Lipid profile not evaluated
Present study	India- 2019, 86 eyes	Lack of significant impact of DR status and metabolic control on GCL-IPL thickness on univariate analysis.	Multivariate regression analysis revealed some association between reduced sectoral GCL-IPL thickness and metabolic control (HDL, total cholesterol and Hba1c) after adjusting for variables like age and CMT.

IPL: Inner Plexiform Layer; DR: Diabetic Retinopathy; RNFL: Retinal Nerve Fibre Layer; GCL: Ganglion Cell Layer; SD OCT: Spectral domain Optical Coherence Tomography; ERG: Electroretinogram; DM: Diabetes Mellitis; IRL: Inner Retinal Layers; FBS: Fasting Blood Sugar; IRT: Inner Retinal Thickness; ORT: Outer Retinal Thickness; TRT: Total Retinal Thickness; T2DM: Type 2 Diabetes Mellitus; NPDR: Non Proliferative Diabetic Retinopathy, ETDRS: Early treatment of diabetic retinopathy study

Despite a multivariate analysis, we failed to find any impact of DR status on GCL-IPL thickness in eyes without vision loss. Chhablani et al studied GCL-IPL thickness in diabetics, but had included PDR and all grades of NPDR, while number of eyes without DR was very less. The authors could not find any impact of DR status per se on inner retinal neurons in their study on DNR.[19]In contrast, van Dijk et al had studied macular GCL thickness in patients with Type 1 DM and no or minimal DR in 2010, and had found DR status to be the most crucial associate of “peri-central GCL thickness” after regression analysis. However, that author group had used manual software-assisted segmentation to measure GCL thickness.[20]Two years later, the author group evaluated type 2 DM patients with same methodology and found similar results.[21]In 2016 a study in Chinese subjects found GCL-IPL thinning in type 1 diabetics but not in type 2 diabetics, however as only

patients without retinopathy were included, the authors could not analyse impact of DR on GCL-IPL thickness.[22] Ng et al studied retinal ganglion cell thickness with OCT in 2016 and found the average ganglion cell thickness to decrease with advancement in DR.[23]As mentioned in table 2, inherent variables like age and CMT have an obvious relation with numerical GCL-IPL values, and their impact must be adjusted for while assessing results. It has been noted during studies on glaucoma and in normal subjects that ganglion cells decrease with age.[24-26] Use of regression analysis and age normative database provided in the OCT machine instead of simple univariate analysis with numerical values can help obviate such confounders, as shown in Table 4. This is the likely reason behind the varying results of the studies described before, and adjustments for such factors must be made in any future analysis of average macular ganglion cell thickness. Other possible reasons for varying results could be the actual impact of metabolic control on GCL-IPL, which these studies have not accounted for.

The chief limitation of this study is lack of an analysis of retinal nerve fibre layer and functional indicators like microperimetry (not available to us), which would have aided in patients with no clinical vision loss. As mentioned prior, MFERG indicates function of outer and middle retina. These instruments must be considered as a simultaneous measure along with the "anatomical" OCT changes in the diabetic retina in future studies.

Understanding the actual degree of impact of metabolic control as seen in our study or in previous studies (Table 5) is crucial as deranged lipids in patients with advanced DR have been shown to be associated with retinal neuronal loss.[27]Some authors have shown retinal nerve fibre layer defects to be an early manifestation of DM, even before clinical vision loss.[28] The possible reason behind DNR is a deranged balance of neuroprotective-neurotoxic elements before setting of clinical microangiopathy.[15] Accelerated apoptosis of glial cells due to altered neurotransmitters may also account for this loss.[29] Experimental studies have revealed hyperplasia and hypertrophy of muller cells in the early course of DM,[30] which may cause their activation and further course of neurodegeneration and angiopathy as contemplated by Vujosevic et al.[15]For these reasons, Li et al. have suggested analysis of segmented intraretinal layers rather than just CMT or other subjective indicators to understand pathology in early stages of DR.[17]We have noted possible association of HDL, total cholesterol and deranged Hba1c with reduced GCL-IPL thickness in diabetic patients in table 4. However, despite high adjusted OR following logistic regression, we recommend further studies on the impact of metabolic control on GCL-IPL as some statistical indicators of our analysis make this association debatable (large confidence interval and high p values).

To conclude, we studied the impact of metabolic control on macular ganglion cell thickness as an indirect indicator of DNR in patients without vision loss. We found a possible association of GCL-IPL thinning with deranged HDL, total cholesterol and Hba1c in our sample after adjusting for age and CMT. Based on our results, we recommend future studies on DNR with simultaneous electrophysiology, microperimetry and OCT segmentation.

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