

INTRODUCTION

Proliferative diabetic retinopathy, a blinding complication of diabetes mellitus (DM) is associated with several risk factors namely - duration of diabetes, poorly controlled hyperglycaemia and presence of autonomic neuropathy (1, 2).

The different types of diabetic retinopathy include proliferative retinopathy, nonproliferative background diabetic retinopathy and diabetic maculopathy. Proliferative retinopathy is characterised by the proliferation of new vessels on the disc or retina, maculopathy with exudation of fluid and lipid exudates in the macula and background retinopathy includes presence of dot and blot haemorrhages, retinal microinfarcts and other exudates with microvascular anomalies without the specific changes of the other two types of retinopathy. The pathogenesis of diabetic retinopathy is complex and involves changes related to hyperglycaemia, increased permeability of the retinal vasculature and hypoxia.

The normal choroidal circulation is controlled by autonomic regulation, but the retinal vasculature has autoregulatory mechanisms related to tissue oxygenation, level of carbon dioxide and tissue pH.

Systemic and local autonomic dysfunctions have been identified as associated factors linked with proliferative retinopathy, but the two may not necessarily have a direct cause-effect relationship.

Diabetic autonomic neuropathy is usually asymptomatic and affects 10–40%

of diabetic patients. The eyes, heart and blood vessels can be involved secondary to diabetic autonomic neuropathy (3). Smith postulated that pupillary changes come before cardiovascular changes in diabetic autonomic neuropathy (4). A strong association was also found between the severity of diabetic retinopathy and cardiovascular autonomic dysfunction in diabetic patients (5). Autonomic dysfunction of the pupil can be assessed by two methods (a) estimation of pupil cycle time and (b) pharmacological tests based on the principle of denervation hypersensitivity.

The pupil cycle time, a simple, non-invasive reproducible test, has been shown to be effective in detecting autonomic dysfunction in various diseases including diabetes mellitus. Denervation hypersensitivity, an increased response of a denervated tissue to an agonist neurotransmitter or other chemical agonist after deprivation of its nerve supply, is the basis for another set of tests of ocular autonomic function. Thus, diluted pilocarpine constricts a parasympathetically denervated pupil and diluted phenylephrine dilates a sympathetically denervated pupil but will usually have no effect on a normal pupil (6).

Recent years have witnessed an upsurge of autonomic function assessment to diagnose neural or cardiovascular involvement secondary to diabetic autonomic neuropathy as early as possible. Diabetic retinopathy presents in stages: non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). The cardiovascular autonomic involvement is gradual and the two divisions of the

autonomic nervous system are involved at varying stages of diabetes mellitus. Considering this stage-wise involvement, we proposed to study the overall autonomic function and retinal involvement in type 2 DM in a cross sectional study design.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board. Patients with type 2 DM of more than 5 years duration were screened and selected for the study. A total of 30 patients with different grades of retinopathy (no retinopathy, non-proliferative diabetic retinopathy, proliferative diabetic retinopathy, n=10 each) and 10 age matched normal persons were recruited after excluding subjects with any detectable abnormality which may affect the pupil and those with any other systemic disease or taking any medication known to affect autonomic function. Patients with uveitis, rubeosis iridis, glaucoma, ocular trauma or ocular surgery were also excluded. Informed consent was obtained from all the patients included in the study.

Fasting and post-prandial blood glucose and glycosylated hemoglobin levels were assessed in all patients. A detailed ophthalmological evaluation was done including testing of visual acuity, refraction, slit lamp examination, measurement of intraocular pressure and detailed fundus examination by ophthalmoscopy.

Ocular autonomic function tests

Pupillary tests :

Pupil diameter was recorded according to the method described by Sharma et al (7)

by using a slit lamp mounted anterior segment camera and taking photographs under standard background illumination. The same distance between the camera and the eye of the subject was maintained in every case by using a metallic rod placed over the frontozygomatic suture. Same magnification was used for all the patients. Patients were asked to look at a target in the distance to minimize any constriction due to the accommodative reflex (7). Horizontal pupil diameter was then assessed by placing a translucent grid accurate upto 0.1 mm over the photograph of the pupil and measured using a standard magnifier. Measurements were verified by a masked observer.

(a) Denervation hypersensitivity test : Parasympathetic test.

One drop of 0.125% pilocarpine was instilled in the conjunctival sac of both eyes. Photographs were obtained before and 45 minutes after instillation of pilocarpine. Pilo-pupil ratio average was obtained by taking the average of the ratio of the two eyes.

$$\text{Pilo-pupil ratio} = \frac{\text{Pupil diameter after pilocarpine}}{\text{Pupil diameter before pilocarpine}}$$

(b) Denervation hyper sensitivity test : Sympathetic test.

Phenylephrine pupil ratio average test is considered as a measure of sympathetic dysfunction. In this test one drop of 0.5% phenylephrine was instilled in the two eyes and photographs were taken before and 45 minutes after instillation of 0.5% phenylephrine.

$$\text{Phenylephrine pupil ratio} = \frac{\text{Pupil diameter after 0.5\% phenylephrine}}{\text{Pupil diameter before 0.5\% phenylephrine}}$$

Phenylephrine pupil ratio average was obtained by taking the average of two eyes.

(c) *Pupil cycle time (PCT)*

Pupil cycle time was measured in both eyes of all subjects using the method described by Miller and Thompson (8). The patient was seated at a slit lamp and was asked to look at a distance in a dimly illuminated room. A thin horizontally aligned beam of light of moderate intensity and 0.5 mm width was focused from below at the inferior pupillary margin to initiate cyclic constriction and dilation. The pupil cycle was measured by a hand held electronic stopwatch measuring 1/100th of a second. The time taken by 100 cycles (two runs of 50 cycles each) in seconds was multiplied by ten to obtain the PCT in milliseconds.

- d) The pupils of all patients were fully dilated after instilling one drop of Tropicacyl plus (a combination of 0.8% tropicamide and 5% phenylephrine) in each eye three times at 5 minutes interval. The pupil photograph was taken after 45 minutes and pupil size measured by the above mentioned method to rule out any myogenic pathology in the iris.

Systemic autonomic function tests

A standard set of cardiovascular tests was performed in all the subjects. These

tests included (a) deep breathing test (b) Isometric hand-grip test (c) Head-up tilt and (d) cold face test.

After sitting in a chair, subjects performed deep breathing at the rate of 6 minutes. After taking rest for 10 minutes in supine position, head-up tilt test (70° tilt over 15 seconds) was done. Hand-grip test was done by squeezing hand dynamometer at 30% of maximum voluntary contraction for 4 minutes. In cold face test a bag containing ice was placed over the forehead for 30 seconds (9). ECG and respiration were recorded and monitored continuously. With deep breathing E:I ratio (ratio of R-R interval during expiration and inspiration) and difference in heart rate (Inspiration - Expiration) were calculated. With head up tilt 30:15 ratio (ratio of 30th beat and 15th beat) and maximum fall in systolic blood pressure was measured. With hand grip test maximum rise of diastolic blood pressure was calculated. In cold face test, change in heart rate was calculated.

Statistical analysis was carried out by applying Kruskal-Wallis one way analysis of variance (ANOVA) and multiple range test was applied to see significant difference between various pairs. A cut off level of 0.935 (<2SD of normal mean) value of pilopupil ratio average was taken as an indication of ocular autonomic neuropathy.

Huber et al found that the diabetic pupil dilates poorly after laser therapy compared to prelasered diabetic pupils (10). Hence, in patients with proliferative diabetic retinopathy we recorded the pupil cycle time and did the phenylephrine test before panretinal photocoagulation was performed

as indicated. The pilocarpine and systemic tests were done later. This was done to avoid delay in laser treatment of the retinopathy.

RESULTS

There was no significant difference for age, duration, blood glucose level and glycosylated haemoglobin in the different groups of diabetic patients (Table I).

There was a significant difference in expiration: inspiration ratio, difference in

heart rate; 30th beat :15th beat ratio, difference in diastolic blood pressure between normal controls and diabetics with retinopathy. No significant difference was found in cold face test and fall in systolic blood pressure during head-up tilt test (Table II).

Pilo-pupil ratio average and pupil cycle time showed significant difference among different levels of retinopathy (Table III). Both tests did not show any significant difference between normal controls and

TABLE I: Clinical and biochemical parameters in controls and patients with varying severity of diabetic retinopathy (values are mean \pm SD).

	<i>Control</i> (1)	<i>No Retinopathy</i> (2)	<i>NPDR</i> (3)	<i>PDR</i> (4)	<i>P value</i> •
Age (yrs)	48.9 \pm 8.79	56.0 \pm 9.94	57.7 \pm 6.58	52.2 \pm 8.75	0.118
Duration (years)		11.9 \pm 4.41	12.7 \pm 4.88	11.7 \pm 4.45	0.876
FBS (mg%)	84.4 \pm 6.72	152.2 \pm 67.9	146.7 \pm 30.94	153.5 \pm 48.04	<0.001*
PPBS (mg%)	98.4 \pm 10.4	180.1 \pm 76.34	204.6 \pm 45.80	199.6 \pm 51.38	<0.001*
HbA ₁ C (%)		9.4 \pm 3.2	9.3 \pm 2.63	9.7 \pm 1.89	0.939

•By Kruskal-Wallis ANOVA test

*(1, 4) (1, 3) (1, 2) these pairs contribute p value <0.001 by multiple range test

FBS : Fasting blood sugar; PPBS : Post-prandial blood sugar; HbA₁C : Glycosylated haemoglobin

TABLE II: Systemic Autonomic Function.

<i>Test parameters</i>	<i>Control</i> (1)	<i>No Ret</i> (2)	<i>NPDR</i> (3)	<i>PDR</i> (4)	<i>P value</i>	<i>MRT</i>
E:1	1.393 \pm 0.065	1.212 \pm 0.09	1.186 \pm 0.19	1.132 \pm 0.04	<0.001	(1,4) (1,3) (1,2)
DHR	27.8 \pm 5.83	17.4 \pm 5.22	17.6 \pm 7.29	13.3 \pm 3.13	<0.001	(1,4) (1,3) (1,2)
30:15	1.21 \pm 0.148	1.08 \pm 0.05	1.12 \pm 0.075	1.10 \pm 0.06	<0.014	(1,4) (1,3) (1,2)
CFT	1.087 \pm 0.04	1.042 \pm 0.024	1.038 \pm 0.065	1.043 \pm 0.079	0.244	NS
Δ dBp	12.8 \pm 2.86	12.0 \pm 1.27	6.0 \pm 4.9	3.6 \pm 3.24	<0.001	(1,4) (1,3) (1,2)
Δ sBP	8.0 \pm 2.83	8.4 \pm 2.63	11.8 \pm 6.7	14.4 \pm 8.79	=0.95	NS

Parasympathetic test - E :1 : Expiration : Inspiration; DHR : Difference in heart rate; 30:15:30th beat; 15th beat; CFT : Cold face test; Sympathetic test - Δ dBp : difference in diastolic blood pressure; Δ sBP : difference in systolic blood pressure; MRT : Multiple range test, shows that significant p value is contributed by the pairs mentioned in the column.

TABLE III: Ocular Autonomic Function (Values are in mean±SD).

<i>Test of autonomic functions</i>	<i>Control (1)</i>	<i>No Ret (2)</i>	<i>NPDR (3)</i>	<i>PDR (4)</i>	<i>P value</i>	<i>MRT</i>
Test of parasympathetic functions						
Pilo-pupil ratio average	0.991±0.028	0.957±0.042	0.873±0.089	0.792±0.092	<0.001	(1,4) (1,3) (2,4) (2,3) (3,4)
Pupil cycle time	860.0±17.14	874.6±26.15	946.7±81.25	1079.7±101.78	<0.001	(1,4) (2,4) (3,4) (1,3) (2,3)
Test of parasympathetic functions						
Phenylephrine pupil ratio average	1.184±0.174	1.25±0.128	1.412±0.21	1.57±0.328	<0.01	(1,4) (2,4)

MRT – Multiple range test results show significant P value is contributed by the pairs mentioned in the column.

diabetic a with no retinopathy, Phenylephrine pupil ratio average showed a significant difference between normals and proliferative diabetic retinopathy and between no retinopathy and proliferative diabetic retinopathy (Table III).

Taking the pilo-pupil ratio average value that was below the 95% confidence limit as abnormal, we found abnormal pilo-pupil ratio average in 100% of patients with proliferative diabetic retinopathy; 80% of patients with non-proliferative diabetic retinopathy; 30% patients with no retinopathy and 10% of normal controls (Table IV).

TABLE IV: Patients with values outside 95% confidence limits of normal mean of different pharmacologic pupillary autonomic function tests.

	Normal	No retinopathy	Non-proliferative diabetic retinopathy	Proliferative diabetic retinopathy
Pilo-pupil ratio average	10%	30%	80%	100%
Phenylephrine pupil ratio average	0%	0%	30%	50%

DISCUSSION

The different groups of diabetics were comparable regarding duration of diabetes, level of glycosylated hemoglobin, fasting and post-prandial blood sugar, systolic and diastolic blood pressure. We are not sure about the level of hyperglycaemia in the preceding years and therefore cannot comment on the past levels of blood sugar and long term control accurately. As far as the duration of diabetes is concerned all patients had a recorded history of diabetes mellitus for 5 years. However, we are not sure about the exact time of onset of diabetes mellitus as type 2 diabetes mellitus is initially asymptomatic. We excluded patients with known hypertension from our study.

Our study clearly suggests that there is an association of severity of retinopathy with severity of autonomic neuropathy in type 2 DM. This confirms the finding of Smith et al (5) and Krolewski et al (11) and also shows that different types of autonomic function performed on the same subjects give similar results.

The pupil cycle time (PCT), also known as edge light pupil cycle time, depends on the structural integrity of the iris and pupillary reflex. It is a good detector of the parasympathetic efferent limb of the reflex arc (12). In this study we found significantly prolonged pupil cycle time in patients with non-proliferative and proliferative retinopathy compared to that of normal controls and patients with no retinopathy. This agrees with the findings of Clark who found prolonged pupil cycle time in patients with proliferative retinopathy (13). Our study has additionally shown that pupil cycle time (PCT) in PDR is more prolonged than that of NPDR. Since there may be deposition of glycogen, lipid and vacuole formation in the iris of diabetic patients (14), all subjects were fully dilated with Tropicacyl plus (0.8% tropicamide and 5% phenylephrine combination) and pupil size measured to rule out iris myopathy. There was no significant difference between normal and diabetic patients, indicating that there was no clinical anatomical iris pathology in the group of patients included in this study.

It is well established that ocular sympathetic and parasympathetic denervation occurs in diabetes (4, 13, 15, 16, 17). Increased response to diluted sympathomimetic and parasympathomimetic is the characteristic of denervation hypersensitivity.

Clark found 38.4% of patients with proliferative diabetic retinopathy had sympathetic denervation (13). The phenylephrine test performed in this study with results expressed in terms of the phenylephrine pupil ratio average gave similar results. We found the phenylephrine pupil ratio average to be a useful method

for recording and analyzing ocular sympathetic function.

Pilo-pupil ratio average showed significantly different values between different groups of diabetics: between normal and proliferative diabetic retinopathy and between normal and non-proliferative diabetic retinopathy. Sharma et al, found good correlation of pilo-pupil ratio average with proliferative retinopathy (7) and Clark found parasympathetic neuropathy in 36% of patients with proliferative retinopathy (13). We selected only type 2 diabetic patients in whom the incidence of autonomic neuropathy is known to be higher (18). Clark included patients with type 1 and type 2 diabetes mellitus. 10% of normals in our study had pilo-pupil ratio average values outside the 95% confidence limits (Table IV) which is similar to Clark's findings of abnormal parasympathetic values by 0.125% pilocarpine test in 5% of normals (mean age of normals in Clark's study was 53.1 ± 10.1 years).

Our study also suggests that ocular and systemic parasympathetic involvement appears earlier than sympathetic involvement. This coincides with the results of Lanting et al (17) though Sigsbee et al (15), and Alio et al (16) had found ocular sympathetic involvement to be earlier than parasympathetic. A recent study suggested that QT interval dispersion is related to sympathetic & vagal dysfunction in type 2 diabetic patients (19). It will be worthwhile to include such parameter in further studies. This parameter may have significant prognostic value for autonomic involvement of ocular and other systems.

Our study also confirmed by several different tests performed on the same subjects the findings of Smith et al (4) and Lanting et al (17) that pupillary changes come before the systemic changes.

Further studies on a larger number of patients are required to prove the efficacy of the pilo-pupil ratio average as an ocular autonomic function test. It can be used as a research tool to select patients at high risk for developing

proliferative diabetic retinopathy in the investigation of the pathogenesis of diabetic retinopathy.

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REFERENCES

- Fong DS, Warram JH, Aiello LM, Rand LI, Krolewski AS. Cardiovascular autonomic neuropathy and proliferative diabetic retinopathy. *Am J Ophthalmol* 1995; 120: 317-321.
- Rand LI, Krolewski AS, Aiello LM, Warram JH, Baker RS, Maki T. Multiple factors in the prediction of risk of proliferative diabetic retinopathy. *New Engl J Med* 1985; 313: 1433-1438.
- Deepak KK, Godbole SA, Kochhar KP, Shah P, Kochupillai N. Autonomic dysfunction and peripheral vasodilatory response in diabetes. *Indian J Physiol Pharmacol* 1996; 40: 325-329.
- Smith SE, Smith SA, Brown PM, Fox C, Sonksen PH. Pupillary signs in diabetic autonomic neuropathy. *Br Med J* 1978; 2: 924-927.
- Smith SE, Smith SA, Brown PM. Cardiac autonomic dysfunction in patients with diabetic retinopathy. *Diabetologia* 1981; 21: 525-528.
- Cannon WB. A law of denervation. *AM J Med Sci* 1939; 198: 737-750.
- Sharma S, Mott AH, Benstead T, Maxner C. Correlation of the pilo-pupil ratio average, a new test for autonomic denervation, to the severity of diabetic retinopathy. *Can J Ophthalmol* 1997; 32: 170-174.
- Miller SD, Thompson HS. Edge-light pupil cycle time. *Br J Ophthalmol* 1978; 62: 495-500.
- Trouerbach J, Drupez D, Buyzere MD, Sutler JD, Clement D. Cardiovascular responses elicited by different stimulated diving manoeuvres. *Eur J Appl Physiol* 1994; 68: 341-344.
- Huber MJE, Smith SA, Smith SE. Mydriatic drugs for diabetic patients. *Br J Ophthalmol* 1985; 69: 425-427.
- Krolewski AS, Barzilay J, Warram JH, Martin BC, Pfeifer M, Rand LI. Risk of early onset proliferative retinopathy in IDDM is closely related to cardiovascular autonomic neuropathy. *Diabetes* 1992; 41: 430-437.
- Martyn CN, Ewing DJ. Pupil cycle time : a simple way of measuring an autonomic reflex. *J Neurol Neurosurg and Psychiatry* 1986; 49: 771-774.
- Clark CV. Ocular autonomic nerve function in proliferative diabetic retinopathy. *Eye* 1988; 2: 96-101.
- Fujii T, Ishikawa S, Uga S. Ultrastructure of iris muscles in diabetes mellitus. *Ophthalmologica* 1977; 174: 228-239.
- Sigsbee B, Torkelson R, Kadis G. Parasympathetic denervation of the iris in diabetes mellitus. *J Neurol Neurosurg and Psychiatry* 1974; 37: 1031-1035.
- Alio J, Hernandez I, Millan A, Sanchez J. Pupil responsiveness in diabetes mellitus. *Ann Ophthalmol* 1989; 21: 132-137.
- Lanting P, Bos JE, Aartsen J, Schuman L, Reichert-Thoen J, Heimans JJ. Assessment of pupillary light reflex latency and darkness adapted pupil size in control subjects and in diabetic patients with and without cardiovascular autonomic neuropathy. *J Neurol Neurosurg Psychiatry* 1990; 53: 912-914.
- Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, Wilson DM, O'Brien PC, Melton LJ 3rd, Service FJ. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort. The Rochester Diabetic Neuropathy Study. *Neurology* 1993; 43: 817-884.
- Takahashi N, Nakagawa M, Saikawa T, Watanabe M, Ooie T, Yufu K, Shigematsu S, Kara M, Sakino H, Katsuragi I, Tanaka K, Yoshimatsu H. Regulation of QT indices mediated by autonomic nervous function in patients with type 2 diabetes. *Int J Cardiol* 2004; 96: 375-379.