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Original article

Development of dynamic pupillometry apparatus to quantify pupil light reflex for assessment of autonomic dysfunction in patients with type 2 diabetes

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ABSTRACT

Background: Diabetes is a chronic non-communicable disease leading to various microvascular and macrovascular complications. Autonomic dysfunction is one of the early-onset complications of diabetes which goes undetected. The quantitative Pupillary light reflex (PLR) is a sensitive indicator of autonomic failure, which helps to identify these high-risk patients to reduce morbidity, mortality, and economic burden on diabetic care.
Objectives: To record and determine altered pupil light reflex variables for evaluating autonomic dysfunction in type 2 diabetic patients.
Materials & methods: The study was conducted during 2018–2020. 400 participants were recruited, divided into the diabetic group (n = 200), and healthy volunteer group (n = 200). All participants were evaluated for autonomic status evaluation using Pupillary light reflex and Heart rate variability.
Results: All PLR variables are highly significant between the diabetic and healthy volunteer participants including parasympathetic variables (R.L, ACA, MPD, and MCV), sympathetic variables BPD, ADA, RPD, and MDV). All the HRV parameters were within the range of normative data from the Taskforce (1996). The RMSSD, NN50, and pNN50 % significantly differed between the two groups, whereas all frequency domain parameters showed statistically similar results.
Conclusion: Autonomic dysfunction in diabetic patients evaluated by PLR, especially parasympathetic dysfunction was detected, which delays the constriction phase and its variables. It is also evidenced by reduced SDNN, RMSSD, and NN50. However, the frequency domain has not shown any variation between the two groups. Thus, the evaluation of PLR aids in the early detection of autonomic dysfunction and the extent of parasympathetic and sympathetic contribution to inadequate PLR response.

1. Background

Diabetes Mellitus (DM) is a well-known metabolic disorder that occurs due to the inability of the pancreas to produce enough insulin or receptor failure to insulin, known as insulin resistance. DM is a heterogeneous, polygenic, and metabolic disease characterized by abnormal glucose homeostasis. Diabetes is one of the insidious threats to public health in the 21st century.[1] There is an enormous transformation in the human environment, and lifestyle in association with globalization leading to an intense upsurge in the incidence of diabetes.[2] The International Diabetes Federation (IDF) has estimated the global burden of diabetes is approximately 463 million, which accounts for a prevalence

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of 9.3 % among the population.[3] It has been rising swiftly in high and middle-income countries. India has 77 million, which is 8.9 % of the whole population, so India is considered the "Diabetic capital of the world".[4] The World Health Organization (WHO) has emphasized the significance of research on the epidemiology of diabetes to empower the development of appropriate diagnostic procedures and interventions.[5] DM is expected to emerge as a significant public health problem due to its serious complications. The primary long-term complications are categorized into microvascular, macrovascular, and non-vascular problems. DM is also common for microvascular complications, as Tripathy includes neuropathy, nephropathy, and retinopathy.[6] Autonomic neuropathy is one of the most common complications of diabetic neuropathy, often undetected by patients and physicians, which affects the autonomic nervous system (ANS). It is a heterogeneous disorder, that encompasses a wide range of abnormalities in the ANS, leading to significant morbidity, mortality, and substantial economic burden for Diabetic care.[7] Diabetic autonomic neuropathy (DAN) is the common cause of hospitalization compared with other known causes of complications. The pathogenesis of neuropathy in diabetic patients is still not precisely known. The current hypothesis concerning multiple etiologies for the occurrence of diabetic neuropathy includes altered metabolic activities, oxidative stress, microvascular insufficiency, endothelial damage, and loss of neurotrophins.[8] Diabetic Autonomic Neuropathy (DAN) prevalence varies widely due to inconsistent definitions, characterization, and differences in methodological protocols.[9]

Autonomic function tests (AFT) are performed to confirm the clinical diagnosis of autonomic dysfunction and to assess the involvement of sympathetic and parasympathetic nerves in the process of autonomic failure. The American Neurology Academy (ANA) has recommended and categorized autonomic testing into three types: cardiovagal, adrenergic, and sudomotor.[10] The conventional AFT is also known as Ewing's battery of tests, widely used to assess autonomic failure.[11] The heart rate variability (HRV) analyzes cardiac beat-to-beat variation, which indicates the fine balance between the two divisions of ANS. So the HRV has been accepted as one of the gold standard tests to evaluate autonomic dysfunction.[12] The pupil is the central aperture of the iris that controls light falling on the retina. Two antagonistic muscles control the pupil size, i.e., the Sphincter pupillae is an inner circular and dilator pupillae, an outer radial muscle fibers that are innervated by the ANS's two divisions.[13] A bright light stimulus near vision decreases pupil size, mediated by parasympathetic action. Cognitive load, attention, alertness, and darkness, the other stimulants, cause pupil dilation through sympathetic stimulation.[14] So pupil size and reactivity provide potential evidence for the evaluation of ANS balance. A clinician routinely offers a pen torch test to visualize the pupil size and response for pupil light reflex (PLR). However, it is a subjective assessment that provides qualitative information.[15] Dynamic Pupillometry (DP) is a quantitative measure of pupil size and reactivity, which is not routinely performed during a clinical examination.[16] It is a non-invasive diagnostic practice emerging expeditiously in neuroscience, convenient, and more effective than invasive procedures.[17] It has played a crucial role in diagnosing several diseases using computer-aided imaging devices.[18] A pupillometer is a self-contained camera that objectively measures static pupil diameter and dynamic pupil response to a standard light stimulus using infrared (IR) videography.[19] Recently the utility of computerized or portable, handheld pupillometers has been expanding in health care systems.[20] The earlier pupillometers from the 1960s took a lot of time and had poor precision because of their slower frame rate. The latest automated Pupillometers have developed and generated precise PLR results because of sophisticated digital imaging technology with superior resolution and frame rate.[21] There has been considerable development in digital imaging tools in the past few years due to cameras' accessibility with optimal resolution and frame rate.[22] Digital cameras' improved, special optics have made it easier to capture high-quality photos. The pupil's response to various light wavelengths can be captured by these cameras.[23,24] The mesopic vision causes pupil dilation captured under Infrared illumination, and photopic vision leads to pupil constriction under bright light illumination.[25] The entire PLR can be recorded, which includes maximum dilation to maximum constriction. The entire video can be analyzed for pupil diameter using image analysis specifying amplitude, time duration, and constriction rate. The PLR is divided into several static and dynamic variables that reflect autonomic activity status.[26] Thus a Dynamic Pupillometry system can be indigenously developed from a standard web camera to capture and quantify PLR, divided into several static and dynamic variables to evaluate ANS balance. Therefore, the current study aimed to design and develop an inexpensive, user-friendly dynamic pupillometry system to quantify pupil light reflex as an indicator of autonomic function in diabetic patients.

2. Materials and methods

It is a multicentric cross-sectional study carried out in patients with type 2 diabetes and compared the data with apparently healthy participants. The study protocol has been reviewed and approved by the institutional Ethics Committees of the host institutions (REF: IEC-NI/18/JAN/63/10 & REF: IEC-17/SEP/19/01). The study participants were employed using a simple randomized sampling method. Type 2 diabetic patients were preliminarily evaluated for the appropriate information about their occurrence, duration, and diabetes glycemic index. A fundamental questionnaire was used to collect the socio-demographic variables from the participants. For both autonomic disturbances, diabetic comorbidities, and other non-communicable conditions, if any, they were also interviewed. To detect some peripheral conductive deficits, a comprehensive evaluation of the sensory and motor system was also conducted. We have not noticed their refractive errors, however, as they do not interact with the result. Participants having a history of certain psychiatric illnesses, and patients who have non-diabetic neuropathy excluded. The study protocol, purpose, and intention of the research were outlined to them and obtained written informed consent as per the guidelines of the Helsinki Declaration (1975). They were directed to abstain until 24 h of data collection from centrally-acting medications and caffeinated beverages, which could influence the study findings. It is a multicenter experiment, and the data are collected from two host institutions' Autonomic Experimental Laboratories.

2.1. Sample size

The sample size for this study was calculated based on the mean difference 0.56 significance level – 95 % (1 -alpha, i.e.,0.05), power of 0.8 (1- beta, % chance of detecting) which makes up a total of 298 participants. Among type 2 diabetic patients (n = 149) and healthy participants (n = 149) as per the study conducted by A G Learner et al., 2015.[27] Considering possible outliers and non-respondent values, 25 % more participants were recruited to make up n = 400 (200in each group).

2.2. Demographic characteristics

The necessary demographic variables like weight and height were measured before subjecting to dynamic Pupillometry and HRV. Weight is estimated from the digital weighing scale and height from the stadiometer. The BMI was calculated using the standard formula weight/height^2. The blood pressure and heart rate were recorded in a resting state to confirm the regular cardiac activity. Later the participants were subjected to dynamic Pupillometry for Pupillary Light Reflex (PLR) and lead II ECG for HRV simultaneously in a comfortable sitting posture in a less noisy room.

2.3. Dynamic pupillometry

The measurement of pupil size and response with time is known as

Dynamic Pupillometry. Accommodation reflex and pupillary light reflex (PLR) evaluate Dynamic pupillary response. The accommodation reflex is excluded from the protocol because the stimulus application is a challenging response. The PLR was selected, and its pattern can be quantified and divided into various indices that reflect the fine balance between autonomic nerves.

2.3.1. Pupillary light reflex (PLR)

The modified flexible wearable technology box fits all and induces dimness as well. The advantage of this box is that the ambient light of the pupillary reflex does not affect the result. On a wide computer screen, the real-time pupil can envision, which is not feasible on portable handy pupillometers. Videography performed with the Debut app (NCH app edition 5.09) which is a free app that can capture and display real-time pupillometry. With a logical interface, it's user-friendly and easy to navigate. Using the video splitter tool, the video footage is divided into pictures (jpeg) (Video to jpeg conversion Ver.5.0.101.201). Later, these photographs (ImageJ ver.1.43u National Institution of Health, USA) were subjected to an image analysis application. In previous research carried out by the authors, all the analysis protocols were described.[28,29]

2.3.2. Image analysis

The Image J software was employed to evaluate various quantitative indices of pupil frames using a macro plugin, which is a Java-based application as per the necessity. The macro plugin was programmed to calculate the immediate pupil area and diameter for the entire batch of pupil images from a pupillary light reflex. To eliminate inter-subject variability of pupil dimensions, automatic batch processing, To plot the graph, the outcomes may be transferred to an Excel sheet. These observations may help distinguish the PLR into different parameters, such as Reflex Latency (R.L.), Minimum Pupil Diameter (MPD), Baseline Pupil Diameter (BPD), Maximum Constriction Velocity (MCV), Maximum Redilation Velocity (MRV), Total Constriction Amplitude (ACA), Total Dilation Amplitude (ADA), Constriction Period (D.C.), and Dilation Period (D.D.). The size of the pupil and the light reaction represent the combination of sympathetic and parasympathetic nerves.[30]

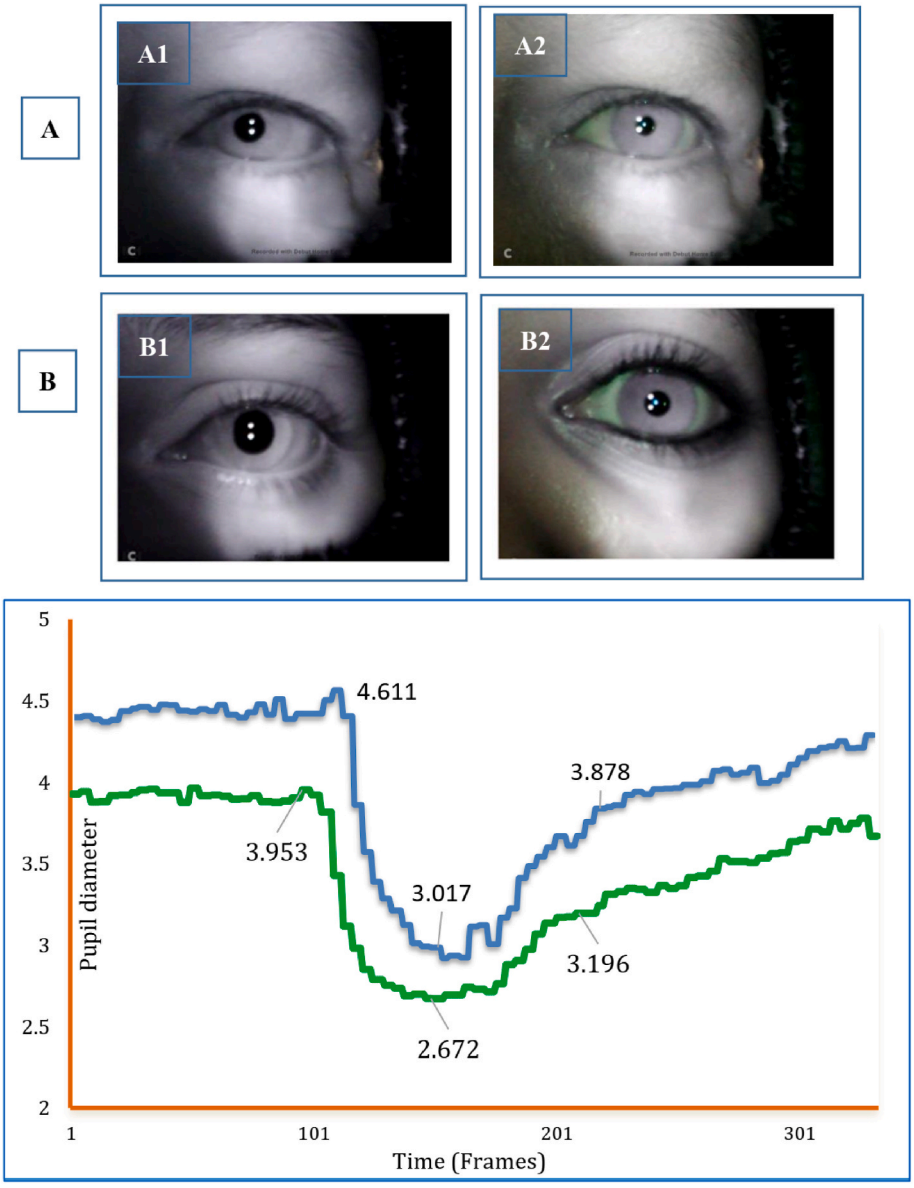

Fig. 1. Randomly selected pupil images A1 & B1 in IR light (mydriasis) and A2 & B2 in response to bright light (miosis) and their PLR graph from diabetic patient (A) and healthy participant (B).

Fig. 1 shows the images of pupils selected randomly from each group of participants. 'A' was taken from a healthy participant and 'B' from a diabetic patient. In each group, the maximum pupil (A1&B1) was obtained in total darkness under IR illumination and the minimum pupil was obtained in response to a flashlight (A2&B2). The A1 is significantly larger than A2 indicating that the resting pupil in diabetic patients was smaller. Furthermore, the constriction amplitude was much higher in healthy participants which showed in terms of smaller pupils (A2) than in diabetic patient (B2).

Fig. 1 also showed the graphs from each group depicting all static and dynamic PLR variables that were significantly reduced in diabetic patients except latency, constriction, and dilation. The 'X' axis represents time in terms of frame rate (30fps/sec), and the 'Y' axis represents pupil diameter in millimeters. The three values mentioned in the above graph are BPD, MPD, and RPD, respectively. The BPD is the resting pupil size reduced in diabetes even in total darkness either due to loss of sympathetic tone or muscle compliance indicating neuropathic pupil changes. When exposed to light, the reflex latency was also prolonged compared to healthy participants due to improper conduction. As the BPD is less, the amplitude of constriction and dilation is also less. However, the time taken for each phase was prolonged, which indicates that compromised PLR is an indicator of autonomic dysfunction, which can be quantified using Dynamic Pupillometry.

2.4. Heart rate variability (HRV)

HRV is the physiological beat-to-beat variation that reflects the sympathovagal balance. The R.R. interval obtained from ECG short-term recording [5min] helps to calculate the quantitative cardiac autonomic balance. PC-based sound card module measures two-channel ECG continuously via three electrodes. The monitoring system consisted of an LM 1000 Life monitor sensor electronic module (SEM), Life monitor belts of varying sizes, an SEM lead and charging dock, a blue tooth USB dongle for laptop/PC, and an analog-to-digital converter to configure SEMs and to download and export data. Raw ECG data from EQ (02) was analyzed using a lab chart modular physiological monitoring and analysis platform. EQ (02) unit provided two leads of ECG measurements that shared a common reference. These are denoted as SEM_ecg1 (primary raw ECG signal) and SEM_ecg2 (secondary raw ECG signal) respectively. We use SEM_ecg1, which is then scaled, and filtered, by a lab chart, as the primary source ECG for the derivation of RR, HR, and HRV parameters.[31,32]

In the time domain, the standard deviation of the NN interval (SDNN), the square root of the mean squared differences of successive NN intervals (RMSSD), the number of interval differences of successive NN intervals greater than 50 ms (NN50), and the proportion derived by dividing NN50 by the total number of NN intervals (pNN50) were used. In frequency domain power spectral density (PSD) analysis in the nonparametric method (fast Fourier transform) will be used. They were low frequency (LF, 0.04–0.15 Hz) and high frequency (HF, 0.15–0.40 Hz) power expressed in normalized units (LF nu and HF nu, respectively), and LF/HF ratio.

3. Statistical analysis

The data variables represented as mean ± standard deviation (mean ± S.D.) are evaluated using 'R' software. The normality of the test results was revealed using the Kolmogorov-Smirnov test. The normal Gaussian distribution was shown by a p-value >0.05. The Man-Whitney *U* test was used to compare the variations in PLR dimensions in two pupils as the data sets were distorted. For the correlation coefficient (CC), the Spearman correlation (ρ) was conducted to find relations between the desired parameters. For the study of linear relationships between different factors, a simple regression test was carried out using two approaches.

4. Results

The present study includes 400 subjects [n = 400] among 200 were healthy volunteers (males = n135, females = n65), and 200 were type 2 diabetic patients (Males = n115, females = n85). The demographic variables of the participants are shown in Table 1. In both groups, all demographic parameters like height, weight, and BMI were statistically not significant. Baseline resting cardiovascular parameters in both groups were not showing any significant ($p > 0.05$) difference between the diabetic and control groups except SBP, and P.P. were found to be significant in both groups. However, they were within the normal range. Though SBP and PP differed in the baseline, MAP did not show any variation, and hence we can consider normal cardiac behavior in these groups. In both groups, except for age, other parameters were not significantly different. The age difference observed between the groups was less than five, and it won't cause any significant variation in the autonomic function. Height, weight, and BMI are statistically not significant. Baseline resting cardiovascular parameters in both groups did not show any significant ($p > 0.05$) difference between the diabetic and control groups except SBP and PP were found to be significant in both groups. HR in the diabetic group and the control group did not show any variation. Though SBP and PP differed in the baseline, MAP did not show any variation, and hence we can consider normal cardiac behavior in these groups.

The PLR is categorized into various static and dynamic parameters, as shown in Table 2. However, the PLR indices are highly variable, and there is no normative data for the Indian population to the best of our knowledge. All PLR variables are highly significant between the two groups. All static and dynamic PLR variables showed a significant difference between the diabetic and the healthy group. The static and dynamic PLR parameters indicate independent autonomic fiber activity. The PLR variables including RL, MPD, ACA, DC, and MCV are the predictors of PNS activity in response to light exposure, which was significantly reduced except the time factors in diabetic patients suggest PNS dysfunction in both pupils. There is a significant percentage reduction in all PNS variables in diabetic patients than in the control group. Similarly, BPD, RPD, ADA, DD, and MDV are predictors of SNS activity, which were also statistically reduced in diabetic patients as shown in Table 2. However, there was no association between glycemic index and PLR variables of diabetic patients (r > 0.3 results were not displayed).

The HRV spectral analysis is expressed as time domain and frequency domain variables, as shown in Table 3. All the HRV parameters were within the range of normative data from the Taskforce (1996). However, The RMSSD, NN50, and pNN50 % were significantly different between the two groups, whereas all frequency domain parameters have shown

Table 1
Demographic variables between diabetic patients and healthy participants.

Demographic variables	Diabetic patients (n = 200)	Healthy participants (n = 200)	*p*-value
Age (years)	44.63 ± 8.15	43.54 ± 5.54	0.12
Male/Female	115/85	135/65	0.02
Height (cm)	158.4 ± 8.32	160.4 ± 9.43	0.15
Weight (kg)	62.32 ± 13.52	61.48 ± 11.23	0.47
BMI (kg/m^2)	24.94 ± 5.10	24.59 ± 4.5	0.39
SBP (mmHg)	127.3 ± 9.88	124.3 ± 13.15	**<0.05**
DBP (mmHg)	80.58 ± 7.13	80.85 ± 8.17	0.72
PP (mmHg)	46.64 ± 13.15	42.86 ± 14.6	0.02
MAP (mmHg)	96.13 ± 6.89	95.27 ± 9.12	0.22
HR (bpm)	85.38 ± 22.48	84.12 ± 11.16	0.91
Duration of Diabetes	5.32 ± 1.55	NA	–
FBS	142 ± 25.57	822 ± 15.57	**<0.01**
PPBS	136 ± 17.23	116 ± 17.23	**<0.01**
HbA1C	8.0 ± 1.38	5.2 ± 0.52	**<0.01**

NA – Not Applicable.
P value <0.05 significant, <0.01 Highly significant.

Table 2
Comparison of the static and dynamic PLR parameters in both pupils between diabetic patients and healthy participants.

PLR variables	Left pupil		*P*-value	%	Right pupil		*P*-value	%
	Diabetic patients	Healthy participants			Diabetic patients	Healthy participants		
RL (ms)	275 ± 34.27	263 ± 24.74	**0.04**	4.3	292 ± 47.53	257 ± 28.53	**<0.0001**	11
BPD (mm)	4.32 ± 0.26	5.02 ± 0.45	**<0.0001**	13	4.30 ± 0.35	4.99 ± 0.48	**<0.001**	13
MPD (mm)	2.59 ± 0.15	2.72 ± 0.30	**0.01**	4.7	2.63 ± 0.21	2.86 ± 0.29	**0.01**	8
RPD (mm)	3.67 ± 0.22	4.69 ± 0.66	**<0.0001**	21.7	3.65 ± 0.30	4.55 ± 0.54	**<0.0001**	19
DC (sec)	1.18 ± 0.06	1.02 ± 0.11	**<0.0001**	13.5	1.26 ± 0.06	0.95 ± 0.13	**0.01**	24
DD (sec)	1.92 ± 0.17	1.98 ± 0.25	**0.05**	3	2.20 ± 0.26	2.28 ± 0.32	**0.03**	3.5
ACA (mm)	1.72 ± 0.29	2.26 ± 0.56	**<0.0001**	23	1.66 ± 0.39	2.16 ± 0.39	**<0.001**	23
ADA (mm)	1.07 ± 0.25	1.96 ± 0.57	**<0.0001**	45	1.02 ± 0.35	1.68 ± 0.49	**<0.001**	39
MCV (mm/sec)	1.45 ± 0.26	2.24 ± 0.64	**<0.0001**	35	1.32 ± 0.31	2.27 ± 0.52	**<0.0001**	41
MDV (mm/sec)	0.57 ± 0.27	0.85 ± 0.24	**<0.0001**	32	0.47 ± 0.18	0.87 ± 0.28	**<0.0001**	45

%- Percentage difference of PLR variables in diabetic patients compared with healthy participants.

Table 3
Comparison of Time and frequency domain variables of HRV between diabetic patients and healthy participants.

HRV variables	Diabetic patients	Healthy participants	*p*-value
Time-domain variables			
Mean RR (ms)	700 ± 90	701 ± 79.6	0.87
(SDNN) (ms)	41.8 ± 24.55	47.2 ± 13.0	**<0.001**
Mean HR (1/min)	90.8 ± 11.9	87.9 ± 9.9	0.43
STD HR (1/min)	6.25 ± 18.37	7.8 ± 7.6	0.26
RMSSD (ms)	32.78 ± 30.33	36.3 ± 17.2	**<0.001**
NN50 (count)	30.3 ± 44.40	38.7 ± 27.4	**<0.001**
pNN50 (%)	10.57 ± 13.2	13.1 ± 12.0	**<0.05**
Frequency domain variables			
Power _VLF (ms2)	978 ± 1145	951 ± 731	0.13
Power _LF (ms2)	1136 ± 975	1118 ± 746	0.37
Power _HF (ms2)	1178 ± 1796	1008 ± 1046	0.22
VLF (%)	31.8 ± 16.37	33.32 ± 15.73	0.25
LF (%)	38.44 ± 13.41	37.39 ± 12.68	0.39
HF (%)	29.84 ± 15.36	29.44 ± 14.31	0.78
Total power	3144 ± 2445	3031 ± 1714	0.25
LF/HF Ratio	1.54 ± 0.89	1.51 ± 0.88	0.62
LF (n.u.)	61.81 ± 13.94	60.62 ± 13.33	0.36
HF(n.u.)	38.16 ± 13.42	40.21 ± 12.19	0.10

RR, normal-to-normal interval; SDNN, the standard deviation of the normal-normal interval; RMSSD, root-mean-square of the successive normal sinus RR interval difference; NN50 count = Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording; pNN50, percentage of absolute differences between successive normal RR intervals that exceed 50 ms. Frequency domain variables are VLF, Very low-frequency power. HF, high-frequency power; LF, low-frequency power; LF/HF, the ratio of low frequency to high-frequency power; n.u, normalized unit.
P value <0.05 significant, <0.01 Highly significant.

statistically similar results. Most of the time domain short-term heart rate variability parameters significantly differed among two groups like SDNN, RMSSD NN50, and pNN50. The R-R interval has not shown any variation, but both are within the normal limit. However, all frequency-domain variables have shown similar results in the two groups, including L.F., H.F., and VLF power and percentage. Most of the time domain short-term heart rate variability parameters significantly differed among two groups like SDNN, RMSSD NN50, and pNN50. The R-R interval has not shown any variation, but both are within the normal limit. The frequency-domain analysis did not show any variation among the HRV variables between the two groups as shown in Table 3. It indicates that the degenerative change could be picked up more easily in the pupil than in HRV as the confounding factors were eliminated.

The categorization of diabetes is based on the duration of the disease. The data was categorized as <5 or>5 years of duration of diabetes, and data sets were compared. However, this did not show any variation among the PLR variables from the current data. This may be due to insufficient data distribution in both groups. However, there was a trend in the decline of PLR variables with prolongation of the duration of diabetes. Further studies are warranted to establish the relationship between autonomic dysfunction and the duration of diabetes.

5. Discussion

Autonomic dysfunction is one of the earliest consequences of diabetes; most often remains subclinical, which is not detected early-stage and leads to severe complications of diabetic neuropathy. The constellation of signs and symptoms of autonomic dysfunction remains asymptomatic for a few years, but it affects the vital organs. Poor glycemic index results in synaptic ganglionic transmission failure, which delays sensory integration and motor performance.[33] Pupil defects from autonomic neuropathy are often seen in diabetes. The deficiency of sympathetic fibers to dilator pupillae often causes diminished night vision in diabetic patients. Parasympathetic impairment on the sphincter muscle compromises accommodation and pupil light reflexes. It has been demonstrated that diabetic patients with mild autonomic dysfunction have substantially decreased pupil diameters than healthy subjects. Diabetic neuropathy may develop due to microvascular complications, coagulopathy, and increased endothelial growth factors that could become clinically evident after a certain period of years. Thus it is necessary to detect DAN, which has cranial autonomic effects on the cardiovascular and digestive tract in patients with diabetes. Autonomous dysfunction is associated with higher mortality and has been reported before the onset of cardiovascular autonomous function disorders by detecting pupil function deterioration.[34] The two limbs of the PLR graphic pattern represent the independent autonomic fiber action. Therefore, evaluation of PLR aids in the detection of autonomic dysfunction and the extent of parasympathetic and sympathetic contribution to inadequate PLR response.

The current study reports that all PLR variables of type 2 diabetic patients are significantly different from the healthy volunteers. The Baseline pupil diameter (BPD) in scotopic vision is less than controls which indicates an imbalance between the pupil's antagonistic muscles. Further, these muscles become stiff and unable to maintain the pupil tends to decrease in size. The reflex latency is the period from the point of stimulus to the onset of response, which initiates pupil constriction. Autonomic dysfunction caused by diabetes mellitus delays the transmission of action potentials to the brainstem centers, delaying the onset of PLR[35]]. Thus the latency and BPD indicate the precise balance between the two antagonistic autonomic fibers. There is strong evidence of reduced BPD, and prolonged latency reflects parasympathetic dysfunction. The amplitude of constriction and dilation (ACA, ADA) also reduced significantly due to compromised initial pupil diameter. However, the duration of constriction is quite long, indicating parasympathetic dysfunction in type 2 diabetic patients. However, the duration of dilation is longer due to the sympathetic hyperactivity on subsequent exposure to light. The constriction velocities, and dilation (MCV, MDV) were significantly reduced in diabetic patients than in the control group. Similar results were found by A. G. Lerner et al. (2015).

There was strong evidence for significant differences in the baseline pupil radius and amplitude of the pupil reaction to being the potential indicators of autonomic dysfunction.[36] Similar results were postulated by Karki et al. (2020) stated that the PLR indices are significant compared to non-diabetics, especially parasympathetic variables, which are highly different from those of sympathetic variables. They concluded that parasympathetic dysfunction has more tendency than sympathetic in diabetic patients.[37] Our results are also in the same line with Yuan et al. (2014) concluded that Parasympathetic and sympathetic pupil Dysfunction tends to be a typical characteristic of the autonomic impairment of diabetes relative to non-diabetic factors of autonomous deficit.[38] However, the duration of diabetes and the glycemic index did not impact PLR variables, but as the duration increases, it affects PLR variables.

The power spectral analysis has reported that most of the parameters are within the normal range for age and gender. The time-domain HRV variables have shown significant differences, especially in SDNN, RMSSD, and NN50, indicating less HRV due to parasympathetic dysfunction and sympathetic hyperactivity. However, the frequency domain variables are similar and do not show any significant difference between the two groups. Similar results were found in Gnindjio et al. (2018) and Siva kumar et al. (2022) concluded that an impaired glycemic index might have a negative impact on cardiovascular autonomic modulation as observed by decreased HRV in the absence of signs and symptoms. Reduced HRV is a predictor of cardiac autonomic dysfunction.[39,40] The dynamic Pupillometry has shown a predictive value for positive (Autonomic neuropathy) is 96.2 % and a predictive value for negative is 93.6 %. Thus it can be used as a surrogate method for HRV to evaluate autonomic dysfunction to reduce morbidity, mortality, and substantial economic burden on diabetic care.[41–43]

6. Conclusion

Pupillometry can produce reliable, precise pupil measurements with no observer bias. The accuracy and precision of the system are considerably higher than that of the manual pupil examination. Infrared light increases pupil acquisition efficiency in complete darkness and does not initiate pupil light reflex. We have developed a cost-effective, dynamic pupillometer with a simple web camera to capture the pupil light reflex using open-source software. This approach is cost-effective, simple, and removes the bias of the examiner and the ambiance. Dynamic Pupillometry evaluates autonomic balance through a quantitative analysis of PLR. Among the measured dynamic pupillometry indices, BPD, MCV, ACA, and MPD were the most robust parasympathetic activity parameters showing significant association with standard HRV indices. It is a simple method that can assess the sympathetic and parasympathetic arm of the ANS independently. Hence, dynamic Pupillometry could be used as a complementary tool to HRV when evaluating ANS balance. Autonomous dysfunction is the earliest consequence of diabetes. Poor glycemic index results in synaptic ganglionic transmission failure, which delays sensory integration and motor performance. Pupil defects from autonomic neuropathy are typical in diabetes. Autonomous dysfunction has been reported before the onset of cardiovascular autonomic function disorders by detecting pupil function deterioration. Evaluation of PLR aids in the detection of autonomic dysfunction and the extent of parasympathetic and sympathetic contribution to inadequate PLR response. In this study, Diabetic patients who had autonomic dysfunction demonstrated using Dynamic Pupillometry compared to healthy participants. Thus, Dynamic Pupillometry can be used as a non-invasive screening tool in various clinical evaluations. It is a novel technique that offers a promising approach portable, inexpensive, and simple to measure the static and dynamic pupil response.

Ethical considerations

The study protocol has been reviewed and approved by the institutional Ethics Committees of the host institutions (REF: IEC-NI/18/JAN/63/10 & REF: IEC-17/SEP/19/01).

Data availability

Blinded.

Author contribution

1. A.V. Siva Kumar - Data collection and analysis, Drafting of the manuscript.
2. R. Padmavathi - Conceptualization of study and drafting of the manuscript.
3. Shriraam Mahadevan - Interpretation of data and clinical insights.
4. K. N. Maruthy - Design of the apparatus, Manuscript editing and corrections.
5. K. Mahesh Kumar - Data collection and analysis.
6. B. Sowjanya - Manuscript editing and corrections.

Declaration of generative AI and AI-assisted technologies in the writing process

While preparing this work, the authors used the Grammarly AI tool to improve the quality of the language. After using this service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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