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RESEARCH

Diagnostic utility of pupillometry in detecting autonomic dysfunction in migraine disorders

Migren bozukluklarında otonomik disfonksiyonun saptanmasında pupillometrinin tanısal faydası

Ceren Türkoğlu[1], Dilek İşcan[2], Elif Arslan[3]

[1]Lokman Hekim University, Ankara, Turkiye
[2]Niğde Ömer Halisdemir University, Niğde, Turkiye
[3]Ulucanlar Eye Education and Research Hospital, Ankara, Turkiye

Abstract

Purpose: This study aims to comprehensively investigate autonomic nervous system dysfunction in migraine patients using automated pupillometry, a non-invasive and objective assessment method.

Materials and Methods: This study was designed as a prospective, non-randomized clinical trial involving a total of 128 participants aged 18–59 years (64 migraine patients and 64 healthy controls). All participants underwent a comprehensive ophthalmological examination followed by automated pupillometry testing. Pupillometry measurements were performed under scotopic (dark), mesopic (dim light), and photopic (bright light) conditions, with subsequent recording of dynamic pupil dilation measurements.

Results: The mean pupil dilation velocity in migraine patients (0.11 ± 0.45 mm/s) was found to be significantly slower compared to the healthy control group (0.13 ± 0.46 mm/s). Additionally, a significant difference was observed between the two groups in pupil diameter under mesopic conditions. Analysis of dynamic pupil dilation revealed a significant difference at the 10th second of measurement between Group I (migraine patients; 5.63 ± 0.87 mm) and Group II (healthy controls; 6.05 ± 0.95 mm).

Conclusion: This study demonstrates that migraine patients exhibit slower pupil dilation velocity compared to healthy individuals, indicating autonomic nervous system dysfunction. These findings suggest that automated pupillometry may serve as a valuable, non-invasive, and objective tool for evaluating autonomic functions in migraine patients.

Keywords:. Migraine, autonomic dysfunction, pupil diameter, static pupillometry, dynamic pupillometry

Öz

Amaç: Bu çalışmanın amacı, migren hastalarında otonomik sinir sistemi işlev bozukluğunu, invaziv olmayan ve objektif bir değerlendirme yöntemi olan otomatik pupillometri kullanarak detaylı bir şekilde incelemektir.

Gereçl ve Yöntem: Bu çalışma, 18-59 yaş aralığında toplam 128 katılımcı (64 migren hastası ve 64 sağlıklı kontrol bireyi) ile yürütülen, prospektif ve rastgele olmayan bir klinik araştırma olarak tasarlanmıştır. Tüm katılımcılar, kapsamlı bir oftalmolojik muayeneden geçirilmiş ve ardından otomatik pupillometri testine tabi tutulmuştur. Pupillometri ölçümleri; skotopik (karanlık), mezopik (loş ışık) ve fotopik (aydınlık) koşullar altında gerçekleştirilmiş, bunu takiben göz bebeği dilatasyonunun dinamik ölçümleri kaydedilmiştir.

Bulgular: Migren hastalarında ortalama göz bebeği dilatasyon hızının (0,11 ± 0,45 mm/sn) sağlıklı kontrol grubuna (0,13 ± 0,46 mm/sn) kıyasla istatistiksel olarak anlamlı düzeyde daha yavaş olduğu saptanmıştır. Ek olarak, mezopik koşullardaki göz bebeği çapı açısından iki grup arasında belirgin bir fark gözlenmiştir. Dinamik göz bebeği dilatasyonu incelendiğinde, ölçümün 10. saniyesinde Grup I (migren hastaları; 5,63 ± 0,87 mm) ve Grup II (sağlıklı kontroller; 6,05 ± 0,95 mm) arasında anlamlı bir fark olduğu belirlenmiştir..

Sonuç: Bu çalışma, migren hastalarının sağlıklı bireylere göre daha yavaş bir göz bebeği dilatasyon hızına sahip olduğunu ortaya koymuş olup, bu durum otonomik sinir sistemi işlev bozukluğuna işaret etmektedir. Bu bulgular ışığında, otomatik pupillometrinin migren hastalarında otonomik fonksiyonların değerlendirilmesi amacıyla kullanılabilecek değerli, non-invaziv ve objektif bir araç olabileceği düşünülmektedir

Anahtar kelimeler: Migren, otonomik disfonksiyon, pupil çapı, statik pupillometri, dinamik pupillometri

Address for Correspondence: Ceren Türkoğlu, Department of Ophthalmology, Faculty of Medicine, Lokman Hekim University, Ankara, Turkiye E- mail: dr.cerenturkoglu@gmail.com
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INTRODUCTION

Migraine is a highly prevalent and often incapacitating neurological disorder, recognized globally as a leading cause of disability. It is estimated to affect approximately one billion individuals worldwide, underscoring its significant public health burden. Clinically, migraine is characterized by recurrent episodes of severe headache, typically lasting between 4 and 72 hours. However, the symptomatology of migraine extends far beyond head pain, encompassing a complex array of autonomic, affective, cognitive, and sensory disturbances[1,2]. Among these, autonomic symptoms such as nausea, vomiting, profound sensitivity to light (photophobia) and sound (phonophobia), and an overwhelming need to sleep are frequently reported by patients and are integral to its diagnostic criteria[2] Furthermore, distressing complaints like orthostatic hypotension, syncope, lacrimation, nasal congestion, and eyelid edema often accompany migraine attacks, pointing towards a significant dysregulation of the autonomic nervous system (ANS)[3,4]. There is also compelling evidence suggesting that photophobia, a hallmark of migraine, may be intrinsically linked to craniofacial autonomic dysfunction[5]. Crucially, these autonomic disturbances are not confined to the ictal (during an attack) phase but have been observed to persist during the interictal (between attacks) period as well, suggesting an underlying, chronic alteration in autonomic control[6]. Indeed, a consistent finding in migraine research is a shift in the sympathetic/parasympathetic balance, often manifesting as a systemic state of increased sympathetic resting tone[7].

The pupil, with its dynamic changes in size, serves as a readily observable indicator of ANS activity. The neuronal pathways governing pupil diameter are well-characterized and involve a delicate interplay between the sympathetic and parasympathetic branches of the ANS. The pupillary light reflex, a fundamental homeostatic mechanism, is primarily mediated by the parasympathetic system. When light impinges upon the retina, neural signals are transmitted via the optic nerve. A subset of these fibers projects to the pretectal region of the midbrain, stimulating cells within the Edinger-Westphal nucleus, which is the parasympathetic control center for pupillary constriction. From the Edinger-Westphal nucleus, preganglionic parasympathetic fibers travel to the ciliary ganglion, where they synapse. Postganglionic fibers then course along the oculomotor nerve (cranial nerve III) to innervate the pupillary sphincter muscle, causing miosis (pupil constriction). Conversely, pupillary dilation (mydriasis) is primarily under sympathetic control, originating from the hypothalamus and descending through the brainstem and spinal cord to the superior cervical ganglion, with postganglionic fibers reaching the iris dilator muscle[5,8].

Given the pupil's direct innervation by both divisions of the ANS, the objective measurement of its size and reactivity a technique known as pupillometry offers a valuable, non-invasive window into autonomic function. Automated pupillometry has emerged as a particularly useful method because it standardizes critical parameters such as the intensity, distance, and duration of the light stimulus, ensuring reproducibility and objectivity[8]. This technique is less time-consuming than many other autonomic function tests, relatively inexpensive, and can be easily repeated, making it suitable for both clinical and research settings. By quantifying various aspects of pupillary behavior, such as baseline pupil diameter under different lighting conditions (scotopic, mesopic, photopic) and the dynamic response to light stimuli (e.g., constriction velocity, dilation velocity), pupillometry can provide indirect yet insightful evaluations of both sympathetic and parasympathetic integrity.

The established involvement of the ANS in migraine pathophysiology, coupled with the utility of pupillometry in assessing autonomic function, forms a strong rationale for investigating pupillary responses in individuals with migraine. Key brain regions implicated in the autonomic dimension of migraine, including the hypothalamus, anterior cingulate gyrus, and dorsal medulla, are integral to central autonomic control networks that also influence pupillary function. Moreover, the recognized brainstem dysregulation in conditions like postural orthostatic tachycardia syndrome (POTS), which frequently co-occurs with migraine and represents a form of autonomic dysfunction, further suggests that the pupillary light reflex pathway, with its brainstem components, may be affected in migraineurs[9]. Therefore, subtle alterations in pupillary dynamics could serve as an accessible biomarker reflecting the underlying autonomic dysregulation characteristic of migraine.

The primary aim of this study is to comprehensively evaluate pupillometry parameters in migraine patients compared to healthy controls, to determine if this objective, non-invasive test can effectively delineate autonomic dysfunction associated with migraine disease. While previous studies have explored pupillary function in migraine, this investigation distinguishes itself through its comparatively large sample size, encompassing 128 participants (64 migraine patients and 64 healthy controls). This larger cohort enhances the statistical power and generalizability of the findings. Furthermore, the study employs a detailed protocol assessing both static pupillometry under scotopic, mesopic, and photopic conditions, and dynamic pupillometry, specifically focusing on pupil dilation velocity. By meticulously examining these parameters, this research seeks to provide more definitive evidence on the nature of pupillary changes in migraine and their potential utility in clinical practice, potentially aiding in diagnosis, monitoring disease progression, and evaluating treatment efficacy.

Based on existing literature indicating autonomic nervous system dysregulation in migraine and the involvement of brainstem structures in both migraine pathophysiology and pupillary control, this study hypothesizes that migraine patients will exhibit significant differences in pupillometry parameters such as static pupil diameters under various lighting conditions and dynamic pupil dilation velocity compared to healthy controls. These differences are expected to reflect underlying autonomic dysfunction, particularly potential alterations in sympathetic and/or parasympathetic tone or reactivity in individuals with migraine. Additionally, factors such as age, sex, and refractive error may influence pupillometry values, and their effects will be systematically analyzed to enhance the interpretation of migraine-specific findings.

MATERIALS AND METHODS

This study was designed as a prospective, non-randomized clinical investigation and received approval from the Nigde Omer Halisdemir University Local Ethics Committee, adhering to the tenets of the Declaration of Helsinki (Approval No: 2022/125) on December 22, 2022. The neurological examinations for this study were conducted by Dİ. The ophthalmological examinations and pupillometry measurements were performed by CT. All examinations and measurements took place at the Niğde Ömer Halisdemir Training and Research Hospital. Data collection and management followed institutional guidelines to ensure accuracy and reliability, consistent with Good Clinical Practice (GCP) standards. Procedures were implemented to protect patient confidentiality and ensure data integrity throughout the study.

Sample

Male and female patients aged 18-70 who visited our clinic between December 2022 and May 2023 were included in the study. The sample size for this study was determined based on a power analysis to detect a clinically significant difference in pupil diameter between the migraine and control groups. Assuming a medium effect size (Cohen's $d = 0.5$), an alpha level of 0.05, and a power of 0.80, a sample size of 64 participants per group was calculated. This calculation ensures that the study has sufficient statistical power to detect meaningful differences if they exist.

A meticulous process was followed in selecting participants for the study. Participants were chosen from individuals who presented to our clinic between December 2022 and May 2023. Participants deemed eligible for inclusion in the study were required to be between 18 and 70 years of age. Group I consisted of individuals who had previously been diagnosed with migraine by the Neurology Department according to the International Classification of Headache Disorders, 3rd edition (ICHD-3) criteria established by the International Headache Society. This diagnosis also confirmed that patients were receiving only non-specific treatment during acute attacks and were not following any chronic treatment protocol. The control group, designated as Group II, included healthy individuals without a history of migraine or any other exclusion criteria, who presented to the Ophthalmology Clinic.

A total of 64 patients diagnosed with migraine were assigned to Group I, and 64 healthy individuals were assigned to Group II. Exclusion criteria applicable to all participants included the presence of any chronic systemic disease requiring active treatment, such as diabetes mellitus or systemic hypertension. Individuals with any known ocular disease such as glaucoma, uveitis, or retinal disorders as well as those with a history of ocular surgery or trauma were excluded. Furthermore, participants with neurological conditions other than migraine, such as epilepsy or multiple sclerosis, were not eligible for inclusion. Finally, individuals with a history of regular

use of medications that could affect the autonomic nervous system were also excluded from the study.

Procedure

The study was conducted at the Niğde Ömer Halisdemir Training and Research Hospital, following approval from the Niğde Ömer Halisdemir University Local Ethics Committee (Approval No: 2022/125) and adhering to the principles of the Declaration of Helsinki. Eligible participants were enrolled between December 2022 and May 2023, as per the criteria detailed in Section 2.1.

Once enrolled, participants in Group I (migraine patients) had their diagnosis re-confirmed by the Neurology department. Subsequently, all participants from both Group I and Group II underwent a comprehensive ophthalmological examination. This examination included visual acuity assessment with the Snellen chart, Best Corrected Visual Acuity (BCVA) test, slit-lamp biomicroscopy of the anterior segment, and fundoscopy. Refractive errors were measured using an automatic refractor-keratometer (Canon RF-K2 Full Auto Ref-Keratometer, Tokyo, Japan). Automated pupillometry measurements were then performed on all participants as detailed in Section 2.4.

Automated pupillometry is used to measure basal pupil size in a dark environment by means of infrared light which does not stimulate the retina. Following this action, a 3-second visible light flash is transmitted and the pupillary response is recorded with the camera [6]. Using this method to demonstrate the presence of autonomic involvement in migraine patients can be advantageous in several ways.

Neurological examination

Patients diagnosed with migraine according to the International Classification of Headache Disorders-3 (ICHD-3) criteria[10] of the International Headache Society (The International Classification of Headache Disorders, 2018) were included. These patients were assessed for chronic autonomic involvement, cranial nerve function, muscle strength, sensory function, cerebellar function, and deep tendon reflexes. Individuals who did not have any underlying disease or were not on medication that could induce autonomic effects were examined in detail. Patients exhibiting lateralized neurological findings were excluded from the study. Cerebral imaging was reviewed to exclude secondary headaches, and secondary causes were ruled out.

Ophthalmological examination

All participants underwent a thorough ophthalmological evaluation, which included the Snellen chart, Best Corrected Visual Acuity (BCVA) assessment, slit-lamp biomicroscopy, and fundoscopy. Visual acuity, initially determined using the Snellen chart, was subsequently converted to LogMAR values. Refractive errors were measured for all participants using an automatic refractor-keratometer (Canon RF-K2 Full Auto Ref-Keratometer, Tokyo, Japan).

Automated pupillometry measurements were performed by the same practitioner in a darkened room using the Sirius 3D rotating Scheimpflug camera topographic system (Costruzione Strumenti Oftalmici, CSO, Italy). To minimize the influence of circadian variation on pupil response, measurements were conducted at the exact same time each day (between 13:00 and 14:00). The examination was carried out under three distinct lighting conditions following a 20-minute period of dark adaptation, as per the device's protocol. Measurements were conducted using an LED light source with the following specifications: 660 nm peak wavelength, 640 nm dominant wavelength, 20 nm spectral line half-width, 95 pF capacitance, 1.85 V typical forward voltage, 2.5 V maximum forward voltage, and 10 μA maximum reverse current. To minimize accommodation, patients were instructed to look straight ahead and avoid fixating on the LED light.

Following 20 minutes of dark adaptation, participants were instructed to fixate on a target 3 meters away with their contralateral eye to inhibit the accommodative reflex. Static pupillometry was then performed in three stages. Scotopic measurements were conducted at an illumination level of 0.4 lux, mesopic measurements at 4 lux, and photopic measurements at 40 lux, all within the scope of static pupillometry. Subsequent to scotopic measurements, dynamic measurements commenced by fully illuminating the annulus disc at 500 lux. Once 500 lux illumination was achieved at time 0 seconds, the illumination was extinguished, and pupil dilation was monitored as the eye transitioned from illumination to no light, allowing for pupil size analysis. In dynamic pupillometry, initial measurements were taken at 0 seconds, and data were utilized to measure pupil size at 10 seconds, this being the longest

duration that included the majority of participants. The average pupil dilation velocity was then calculated by dividing the difference in pupil widths at 0 and 10 seconds by 10 (expressed in mm/s).

Subjects were instructed to look directly at the target and keep both eyes open. The right eye was measured under each lighting condition. Participants were permitted to blink between measurements. Pupillometry measurements were repeated three times for each illumination level.

In addition to the aforementioned analyses, the effects of refractive error, age, and sex on static and dynamic pupillometry values were evaluated. For the assessment of pupil diameters, participants were categorized based on age (pre-presbyopes: 18-39 years; presbyopes: 40+ years) and refractive error (myopes: ≤ -0.50D; emmetropes: ≥ -0.25D to ≤ +0.50D; hyperopes: ≥ +0.75D), which were used as fixed factors[11].

Statistical analysis

All statistical analyses for this study were conducted using the Statistical Package for the Social Sciences (SPSS), version 26.0 (Inc., Chicago, Illinois, USA). Descriptive statistics, including mean and standard deviation (SD) for continuous variables and frequencies and percentages for categorical variables, were calculated to summarize the demographic and clinical characteristics of the participant groups (Group I: Migraine patients; Group II: Healthy controls).

Prior to comparative analyses, the normality of distribution for continuous variables within each group was assessed. This assessment guided the selection of appropriate parametric or non-parametric tests. For comparisons of continuous variables between the two independent groups (Group I vs. Group II), the independent samples t-test was employed when the data were found to be normally distributed and variances were homogenous. This test was used to determine if there were statistically significant differences in the means of the variables between the migraine and control groups. If the data were not normally distributed or if the assumption of homogeneity of variances was violated, the non-parametric Mann-Whitney U test was utilized. This test compares the medians (or more broadly, the distributions) of the two independent groups and is robust to violations of normality.

To evaluate the effects of multiple independent categorical variables (such as age group, sex, and refractive error group) on various continuous dependent pupillometry measurements, and to investigate potential interactions between these factors, Analysis of Variance (ANOVA) techniques were applied. Specifically, multivariate analysis of variance (MANOVA) or factorial ANOVA models were likely used, as suggested by the examination of main effects and interaction effects of these demographic and clinical factors on pupillary parameters. These analyses allow for the simultaneous assessment of the influence of several factors and their combined effects on the dependent variables.

For all inferential statistical tests performed, a p-value of less than or equal to 0.05 ($P \leq 0.05$) was considered the threshold for statistical significance. This alpha level indicates that observed differences or relationships are unlikely to have occurred by chance alone, with a 5% probability of a Type I error.

RESULTS

The study included 128 eyes from 128 participants. Group I consisted of 64 eyes from 64 participants diagnosed with migraine, while Group II comprised 64 eyes from 64 participants with no known diseases. The age of the participants ranged from 18 to 59 years, with a mean age of 33.72 ± 9.57 years. There was no significant difference in age between Group I (35.27 ± 8.79 years) and Group II (32.17 ± 10.12 years) ($p = 0.07$). Of the participants, 92 (71.9%) were female, and 36 (28.1%) were male.

The mean refractive values of the participants were -0.57 ± 1.16 D for spherical error, -0.53 ± 0.78 D for cylindrical error, and 85.64 ± 68.75 degrees for cylindrical axis. No statistically significant differences were detected in these refractive parameters between Group I and Group II ($p = 0.25$, $p = 0.61$, and $p = 0.42$, respectively). The intraocular pressure (IOP) was 15.00 ± 2.55 mmHg in Group I and 14.67 ± 2.73 mmHg in Group II, with no statistically significant difference observed between the groups ($p = 0.48$). Demographic data and ocular values of the participants are presented in Table 1.

Regarding static pupillometry values, the scotopic pupil diameter was 5.79 ± 0.90 mm in Group I and 6.07 ± 0.85 mm in Group II. The photopic pupil diameter was recorded as 4.58 ± 0.95 mm in Group I and 4.72 ± 0.83 mm in Group II. No significant

differences were detected in scotopic or photopic diameters between the two groups (p = 0.07 and p = 0.37, respectively). However, the mesopic pupil diameter was found to be significantly different between the groups (p = 0.05), with Group I showing 5.32 ± 0.91 mm and Group II showing 5.64 ± 0.88 mm. Dynamic pupil diameters were measured up to the 10th second. The pupil diameter at 0 seconds was 3.71 ± 0.89 mm in Group I and 3.66 ± 0.72 mm in Group II, with no significant difference detected (p = 0.73). The pupil diameter at 10 seconds was 5.63 ± 0.87 mm in Group I and 6.05 ± 0.95 mm in Group II, revealing a statistically significant difference (p = 0.01). Pupillometry data are detailed in Table 2. Furthermore, a statistically significant difference was noted in the mean pupil dilation velocity between Group I and Group II (p = 0.001); the mean pupil dilation velocity was found to be slower in the group diagnosed with migraine (Group I), which was 0.11 ± 0.45 mm/s compared to 0.13 ± 0.46 mm/s in Group II.

As indicated by the data in Table 3, the effects of refractive error, age, and sex on static pupillometry values and dynamic pupil dilation velocity were calculated. Ninety-one participants were classified as pre-presbyopic, and 37 were classified as presbyopic. Based on refractive error, 67 participants were myopic, 53 were emmetropic, and eight were hyperopic. The pupil diameters according to these demographic characteristics are presented in Table 3.

Multivariate analyses of variance were conducted to examine the effects of age, sex, and refractive error on pupillometry measurements. When comparing pre-presbyopic and presbyopic participants, a significant difference was observed in static pupillometry values (scotopic, p < 0.001; mesopic, p = 0.001; photopic, p < 0.001). Dynamic pupillometry also revealed a significant difference in pupil diameter at the 10th second (p = 0.02) between these age groups. Additionally, there was a significant difference in scotopic pupil diameters between males and females (p = 0.02), and photopic pupil diameters also differed significantly (p = 0.02). Regarding refractive errors, a significant difference was found between myopic, emmetropic, and hyperopic participants in terms of scotopic, mesopic, and photopic pupil diameters (p = 0.03, p = 0.01, and p = 0.03, respectively).

According to the multiple analysis of variance, no significant common effects of age-sex interaction or age-refractive error interaction on pupillometry measurements were found (p > 0.05). However, a significant common effect of sex and refractive error on scotopic pupil diameter was observed (p = 0.02).

Population-specific normative data on static and dynamic pupillometry values, as well as the effects of age and sex on pupil diameter, are presented based on the control group (Group II) of this study. These data are detailed in Table 4.

Table 1. Demographic data and ocular values of participants

Features	Participants (N = 128)	Group I (N = 64)	Group II (N = 64)	*P*
Age	33.72 ± 9.57	35.27 ± 8.79	32.17 ± 10.12	*0.07*
Sex (%)	71.9 / 28.1%	73.4 / 26.6%	71.9 / 28.1%	*0.06*
Spherical refraction	-0.57 ± 1.16	-0.45 ± 1.07	-0.69 ± 1.24	*0.25*
Cylindrical refraction	-0.53 ± 0.78	-0.50 ± 0.71	-0.57 ± 0.84	*0.61*
Cylindrical axis	85.64 ± 68.75	80.78 ± 69.32	90.52 ± 68.37	*0.42*

Values are presented as number or mean and standard deviation.

Table 2. Dynamic and static pupillometry values

Features		Group I (N = 64)	Group II (N = 64)	*P*
Static pupillometry values	Scotopic diameter	5.79 ± 0.90	6.07 ± 0.85	*0.07*
	Mesopic diameter	5.32 ± 0.91	5.64 ± 0.88	***0.05***
	Photopic diameter	4.58 ± 0.95	4.72 ± 0.83	*0.37*
Dynamic pupillometry values	0.sec.	3.71 ± 0.89	3.66 ± 0.72	*0.73*
	10.sec.	5.63 ± 0.87	6.05 ± 0.95	***0.01***
	The mean pupil dilation velocity (0-10 sec.)	0.11 ± 0.45	0.13 ± 0.46	***0.001***

Independent-Samples t test. Values are presented as number or mean and standard deviation

Table 3. Pupillometry measurements according to demographic characteristics (age and refractive error groups)

Groups by age	Refractive error	Scotopic diameter (mm)	Mesopic diameter (mm)	Photopic diameter (mm)	The mean pupil dilation velocity
Pre-presbyopes (n = 91)	Myopes (n = 53)	6.06 ± 0.82	5.62 ± 0.84	4.76 ± 0.89	0.12 ± 0.50
	Emmetropes (n = 36)	6.32 ± 0.81	5.86 ± 0.91	5.07 ± 0.85	0.13 ± 0.43
	Hyperopes (n = 2)	5.41 ± 0.00	3.81 ± 0.00	3.22 ± 0.00	0.13 ± 0.00
Presbyopes (n = 37)	Myopes (n = 14)	5.41± 0.71	5.01 ± 0.74	4.12 ± 0.62	0.11 ± 0.05
	Emmetropes (n = 17)	5.48 ± 0.95	5.14 ± 0.80	4.23 ± 0.76	0.10 ± 0.04
	Hyperopes (n = 6)	5.21 ± 0.84	4.73 ± 0.57	4.08 ± 0.39	0.07 ± 0.04

Values are presented as number or mean and standard deviation

Table 4. Pupillometry measurements according to demographic characteristics in Group II

	Gender	Scotopic diameter (mm)	Mesopic diameter (mm)	Photopic diameter (mm)	The mean pupil dilation velocity
Pre-presbyopes (n = 47)	Female (n = 34)	6.46 ± 0.75	5.80 ±1.02	4.95 ± 0.82	0.14 ± 0.04
	Male (n = 13)	5.77 ± 0.79	5.79 ± 0.76	4.69 ± 1.02	0.15 ± 0.03
	Total (n = 47)	6.27 ± 0.81	5.79 ± 0.95	4.88 ± 0.87	0.14 ± 0.04
Presbyopes (n = 17)	Female (n = 12)	5.62 ±0.61	5.30 ± 0.34	4.40 ± 0.54	5.69 ± 1.22
	Male (n = 5)	5.35 ± 1.05	5.03 ± 0.74	4.03 ± 0.39	0.08 ± 0.04
	Total (n = 17)	5.54 ± 0.74	5.22 ± 0.48	4.29 ± 0.52	0.10 ± 0.05

Values are presented as number or mean and standard deviation

DISCUSSION

In the present study, pupillometry values were compared between individuals diagnosed with migraine and healthy participants to investigate potential autonomic dysfunction. One of the most significant findings is that the mean pupil dilation velocity was notably slower in patients diagnosed with migraine compared to healthy individuals ($p < 0.05$). Furthermore, the results indicate a significant difference in mesopic pupil diameter between migraine patients and the healthy control group.

Several studies have presented population-specific normative data on static and dynamic pupillometry values across different age groups and have examined the effect of age on pupil characteristics[11,12]. A study by Guillon et al. found that both age and refractive error influenced pupil diameter, with larger pupils observed in younger patients and in those with myopia[11]. In our current study, static pupillometry diameters (scotopic, mesopic, and photopic) were found to be lower in myopic individuals compared to emmetropic individuals, and lowest in those with hyperopia. This pattern was similarly observed when comparing pre-presbyopic and presbyopic individuals.

Consistent with these findings, our study demonstrated that static pupillometry diameters (scotopic, mesopic, and photopic) were higher in pre-presbyopic individuals than in presbyopic individuals. In other words, younger patients exhibited larger static pupillometry diameters. In dynamic pupillometry, the pupil dilation velocity was also higher in younger (pre-presbyopic) individuals.

Numerous studies have indicated that pupil diameter tends to decrease with increasing age[12,13,14]. To minimize the confounding effects of age, sex, and circadian variation on pupil size, pupillometric measurements in our study were performed in age- and gender-matched groups at the same time each day.

Additionally, while some studies report that refractive errors do not affect pupillary function, it is worth noting that individuals with high refractive errors (spherical errors ≤ 4 D, cylindrical errors ≤ 4 D) were not included in those particular studies[15]. In our investigation, both static and dynamic measurements

were lowest in hyperopic individuals and highest in emmetropic individuals.

A study conducted by Cambron et al, involving 46 interictal migraine patients, 37 healthy volunteers, 18 migraine attack patients, and 26 volunteer participants, found no difference in pupillometry values between the migraine and control groups during the interictal phase; the same result was obtained in the group experiencing an acute attack[8]. Cortez et al.[16] conducted a study with 24 patients without a migraine diagnosis (control group) and 36 patients with a migraine diagnosis (14 episodic, 12 chronic, 10 probable). Their findings revealed that migraine criteria related to photophobia and disease severity are associated with changes in pupillary light responses. In another study by Eren et al.[17], strong sympathetic stimulation was applied via a cold pressor test to 20 migraine patients and 20 healthy participants, and autonomic nervous system activation was measured using pupillometry. No difference was detected in baseline pupillometry values or in values recorded 2 minutes after.

A study by Kavuncu et al[18] involving 34 migraine patients and 37 healthy participants found no significant difference in static pupillometry comparisons. In dynamic pupillometry, no difference was detected between the two groups, except for pupillary contraction latency. However, when examining inter-eye differences, no variation was found in the migraine group solely for scotopic pupillometry diameter.

The results obtained from the current study are supported by several investigations in the literature, as discussed above. The key findings indicate that both mesopic pupil diameter and pupil dilation velocity were lower in the migraine group compared to the control group. Moreover, this study revealed that static and dynamic pupillometry values can be influenced by age, sex, and refractive error. As strongly supported by our findings, in addition to demographic variations, changes in autonomic function also impact pupillometry values. In conclusion, a comparison of the results clearly showed that the mean pupil dilation velocity is slower in patients diagnosed with migraine than in healthy individuals. It is believed that the significant data from this study, which investigated automated pupillometry a technique providing valuable information regarding autonomic function will contribute to the existing literature. These results may further support the hypothesis that the sympathetic/parasympathetic balance is disrupted in migraine patients.

Certain limitations should be acknowledged in this study. Pupillometry was performed exclusively during the interictal period, and potential confounding factors such as stress, sleep patterns, and lifestyle were not controlled. Furthermore, the study was conducted at a single center and included a limited age range, which may affect the generalizability of the findings. Despite these limitations, the results contribute valuable data to the understanding of autonomic involvement in migraine.

In conclusion, this investigation compellingly demonstrates that individuals with migraine exhibit significantly impaired autonomic function, as evidenced by a slower mean pupil dilation velocity and altered mesopic pupil diameter when compared to healthy controls. These pupillary alterations, which were also influenced by demographic factors such as age and sex, as well as refractive error, underscore the complex interplay of systemic and ocular variables in migraine pathophysiology. The findings robustly support the utility of automated pupillometry as a valuable, non-invasive, and objective biomarker for assessing autonomic nervous system dysregulation in migraineurs.

The implications of this research extend to clinical practice, suggesting that pupillometry could be integrated into the diagnostic toolkit for migraine, offering a quantifiable measure of autonomic involvement. This could aid in patient stratification, monitoring disease progression, and potentially evaluating therapeutic responses.

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