Research article

Repeatability and reproducibility of dynamic pupillary parameters using an automated quantitative pupillometer

Yamini Rattan[1], Kawalinder Kaur Girgla[1], Gaurav Mahajan[2], Pawan Prasher[2]

[1]Department of Physiology, [2]Department of Ophthalmology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, India

(Received: October 2023 Revised: November 2023 Accepted: December 2023)

Corresponding author**: Pawan Prasher.** Email: pawanprasher@yahoo.com

ABSTRACT

Introduction and Aim: The examination of the pupils is an integral part of any patient undergoing neuropsychological and ophthalmological evaluation. The manual examination of pupils has its own limitations. A more reliable technique for determining the size and reactivity of pupils is required. Hence, the study was done to examine the repeatability and reproducibility of dynamic pupillary parameters using an infrared pupillometer in the Indian population in routine clinical settings.

Materials and Methods: A total of three paired pupillary measurements were completed within 6 minutes by two observers under identical ambient conditions with NPi-200 pupillometer in 30 healthy participants aged 18 to 60 years, providing a total of 60 paired measurements.

Results: The ICC values for Neurological Pupil Index, Maximum Pupil Diameter, and Minimum Pupil Diameter were greater than 0.90, signifying excellent agreement. For Change in Pupil Size, Constriction Velocity, Maximum Constriction Velocity, Latency, and Dilatation Velocity, the ICC ranged from 0.75 to 0.90, indicating good agreement between measurements by Observer 1 and between measurements (No. 2 and 3) by Observer 1 and Observer 2.

Conclusion: We found excellent agreement regarding repeatability and reproducibility of dynamic pupillary parameters using an automated quantitative pupillometer. In general, the findings of this study affirm the effective performance of automated quantitative pupillometry in routine clinical settings.

Keywords: Pupillometry; pupillary light reflex; repeatability.

INTRODUCTION

Pupil evaluation is a critical component of neuropsychological and ophthalmological evaluation. The typical pupillary examination evaluates the pupil size, shape, and symmetry, as well as the pupillary light reflex (PLR). Nevertheless, due to variations in light intensity, exposure durations associated with different light sources, as well as differences in skill levels and visual acuity among examiners, manual pupil evaluation yields inconsistent results and is susceptible to errors. In manual pupillary light reflex (PLR) examinations, descriptive terms like reactive, non-reactive, dilated, brisk, or sluggish are often subjective and imprecise (1).

Automated pupillometry ensures a consistent distance between the source of light and the eye, along with standardized light stimulus intensity, resulting in measurements that are more accurate, reliable, and reproducible (2). It is possible to quantify the parasympathetic and sympathetic regulation of the pupil, as indicated by various pupillary light reflex (PLR) parameters, through the pupillary constriction and subsequent dilation in response to the standardized intensity and duration of the light stimulation. They measure dynamic pupillary parameters with infrared light, expanding their range of application to patients with a variety of iris colors and lighting conditions. They can be utilized for bedside diagnostics as well as in routine clinical settings because they are portable, easy to use, and rechargeable. Various models of commercial pupillometry are presently accessible. Among these, the NeurOptics Inc., Irvine, CA, USA, pupillometers, particularly the NPi-200 model, and the RAPDx from Konan Medical USA, Irvine, California, USA, have been widely utilized for diagnostic assistance in numerous studies (3-7).

While numerous studies have demonstrated the superiority of automated pupillometry over manual methods (1, 8–10), routine clinical practice still predominantly relies on manual pupillary assessment. The limited awareness of automated pupillometry may constrain its clinical utility and, consequently, affect the decision-making process. Repeatability of an instrument enables us to determine how closely a given outcome or set of data resembles a measurement taken using the same device or instrument under the same conditions. An instrument's reproducibility refers to the degree to which measurements of a single test sample are nearly identical when the same measurement protocols are followed but with different operators, instruments, and/or lab setups. Before advocating the widespread use of automated pupillometry, the reliability and reproducibility of dynamic pupillary parameters should be established. Previous studies have reported the repeatability and

reproducibility of pupillary parameters using different pupillometers like Pupil X (Albomed GmbH) (11), Ideamedical (Copenhagen, Denmark) (12), and Neurolight Algiscan (IDMED, Marseille, France) (1). The repeatability and reproducibility of the NPi-200 model have previously been reported only in one study done on critically ill patients in Germany (8). There is a paucity of data regarding pupillometry measurements in the healthy participants in routine clinical settings. Hence, the current study was done to investigate the repeatability (intra-observer variability) and reproducibility (inter-observer variability) of dynamic pupillary parameters using the NPi-200 pupillometer in the Indian population in routine clinical settings, which would serve as a reference for future clinical and research purposes.

MATERIALS AND METHODS

Following approval from the Institutional Ethics Committee, the pilot study was carried out in the Ophthalmology outpatient department of the institute, adhering to the principles outlined in the Declaration of Helsinki. Thirty healthy adult participants (60 eyes) were consecutively enrolled, with each providing written informed consent. Subsequently, they underwent a standard ophthalmic examination, encompassing tests such as best corrected visual acuity (BCVA) using the Snellen's chart, intraocular pressure measurement, evaluation of eye movements, slit lamp examination, and fundus examination. Participants were considered eligible if they had a BCVA equal to or greater than 6/6 according to Snellen's chart and were free of any physical, mental, neurological, or ophthalmological disorder, except for spherical or cylindrical refractive errors. Participants with a history of use of any systemic or topical medications affecting pupil size, iris and/or pupil abnormalities, head or orbital trauma, or previous ocular or orbital surgery were excluded. The participants were advised to come for pupillometer measurements two days after the initial screening.

Measurements of quantitative pupillometry were conducted utilizing the NPi-200 device from NeurOptics Inc., Irvine, CA, USA (Fig.1a). This device employs an infrared camera to record the pupil's dynamic parameters over the course of 3.2 seconds while integrating a calibrated light stimulus with a fixed intensity of 1000 lux. The images are recorded at a rate of 30 frames per second, providing a temporal resolution of 33 milliseconds. Every frame is automatically processed to evaluate the parameters as a function of time. According to the manufacturer's statement, the device automatically calibrates, focuses, regulates the vertex distance, and omits outliers. In the event of any artifacts brought on by tracking issues due to blinking, the measurements were deleted, and the scan was redone, and only the high-quality measurements were included for further analysis.

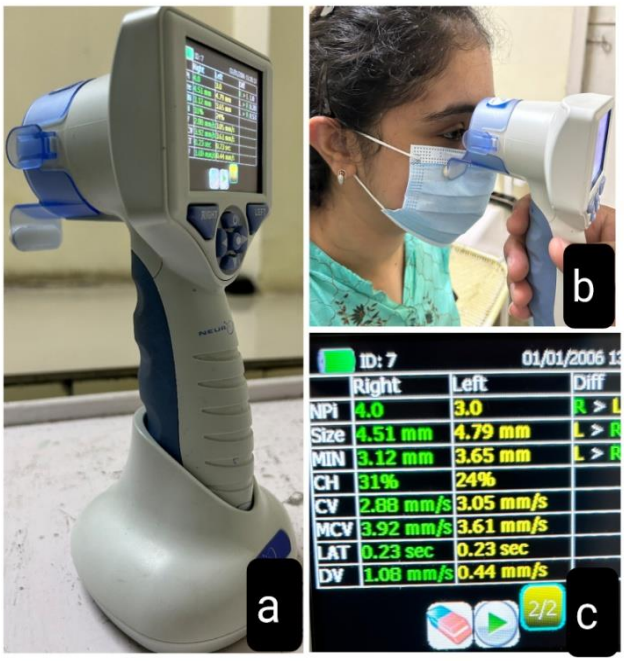

Fig. 1: Device used, and parameters measured. An image of NPi-200 (NeurOptics Inc., Irvine, CA, USA) pupillometer device along with smart guard and charging station (a); An image of measurement being carried out (b); An output of various pupillary parameters measured on the result screen by the device (c).

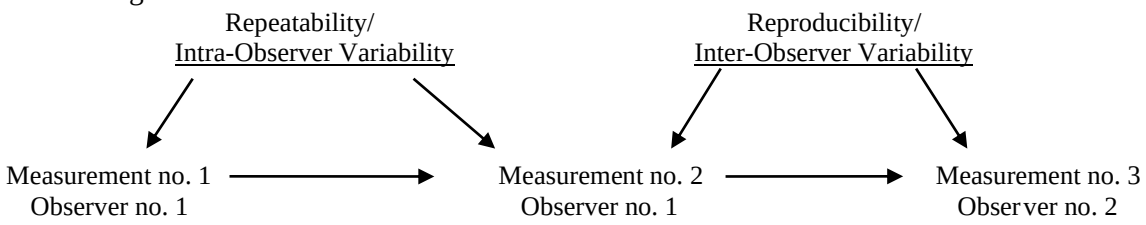

Fig. 2: A diagram illustrating the procedure for assessing participants, outlining a comprehensive series of three measurements conducted on the same participant within a span of 6 minutes.

Statistical analysis

The unprocessed data was obtained from the device and then transferred to an Excel sheet for analysis through Statistical Package for Social Sciences (SPSS) software version 20.0. Statistical significance was established at $p < 0.05$ with a confidence level of 95%. The repeatability (intra-observer variability) was investigated by comparing the first measurement (Measurement No. 1) from the first observer with a repeated measurement from the same observer (Measurement No. 2). Inter-observer variability, or

reproducibility, was assessed by the two observers, with each conducting measurement (Measurements No. 2 and 3) sequentially to each other (Fig. 2). The repeatability and reproducibility were evaluated using the intraclass correlation coefficient (ICC). ICC values below 0.50 were considered poor; those ranging from 0.50 to 0.75 were deemed moderate; values between 0.75 and 0.90 were regarded as good; and those exceeding 0.90 indicated excellent agreement.

RESULTS

The research involved 30 participants in good health, comprising 15 males and 15 females, with ages ranging from 18 to 60 years. The ICC was found to be > 0.90 for the Neurological Pupil Index (NPi), Maximum Pupil Diameter (Size), and Minimum Pupil Diameter (MIN), indicating excellent agreement, whereas the ICC was found to be between 0.75 and 0.90 for Change in Pupil Size (CH), Constriction Velocity (CV), Maximum Constriction Velocity (MCV), Latency (LAT), and Dilatation Velocity (DV), indicating good agreement between the two measurements taken by Observer 1 (Table 1).

The ICC was found to be > 0.90 for the Neurological Pupil Index (NPi), Maximum Pupil Diameter (Size), and Minimum Pupil Diameter (MIN), indicating excellent agreement, whereas the ICC was found to be between 0.75 and 0.90 for Change in Pupil Size (CH), Constriction Velocity (CV), Maximum Constriction Velocity (MCV), Latency (LAT), and Dilatation Velocity (DV), indicating good agreement between the two measurements (Measurements No. 2 and 3) taken by Observer 1 and Observer 2 respectively (Table 2).

DISCUSSION

The examination of the pupils is an integral part of any patient undergoing neuropsychological and ophthalmological evaluation. The manual examination of pupils has its own limitations. The accuracy of manual pupillary tests performed by nurses or physicians has been challenged in recent studies. Couret *et al*., (1) studied the pupils in 200 healthy volunteers using the manual method and an automated pupillometer. The error in manual pupil examination was greater (18%) as compared to an automated pupillometer. Therefore, it is evident that a more reliable technique for determining the size and reactivity of pupils is required. It is believed that the examination of the pupil will become more standardized and reliable with the addition of automated quantitative pupillometry.

Table 1: Observer 1 Measurement No.1 Vs Observer1 Measurement No.2 (Intra-observer variability/repeatability)

Parameter	ICC	95 % CI		p value
		Lower	Upper	
Neurological Pupil Index (NPi)	0.94	0.91	0.96	<0.01**
Maximum Pupil Diameter (Size)	0.93	0.88	0.96	<0.01**
Minimum Pupil Diameter (MIN)	0.95	0.91	0.97	<0.01**
Change in Pupil Size (CH)	0.85	0.76	0.91	<0.01**
Constriction Velocity (CV)	0.88	0.80	0.93	<0.01**
Maximum Constriction Velocity (MCV)	0.86	0.77	0.92	<0.01**
Latency (LAT)	0.81	0.68	0.88	<0.01**
Dilatation Velocity (DV)	0.79	0.65	0.87	<0.01**

**Highly Statistically Significant; Statistical Significance p < 0.05

Table 2: Observer 1 Measurement No.2 Vs Observer2 Measurement No.3 (Inter-observer variability/reproducibility)

Parameter	ICC	95 % CI		p value
		Lower	Upper	
Neurological Pupil Index (NPi)	0.95	0.91	0.97	<0.01**
Maximum Pupil Diameter (Size)	0.95	0.92	0.97	<0.01**
Minimum Pupil Diameter (MIN)	0.97	0.95	0.98	<0.01**
Change in Pupil Size (CH)	0.86	0.77	0.91	<0.01**
Constriction Velocity (CV)	0.90	0.83	0.94	<0.01**
Maximum Constriction Velocity (MCV)	0.90	0.83	0.94	<0.01**
Latency (LAT)	0.76	0.44	0.80	<0.01**
Dilatation Velocity (DV)	0.76	0.60	0.85	<0.01**

**Highly Statistically Significant; Statistical Significance p < 0.05

Table 3: Summary of previous research articles

Author	No. of Participants	Device used	Parameters measured	Results
Kohnen *et al*.,(13) In 2003 Germany	50 healthy subjects (100 eyes)	Colvard infrared pupillometer and Procyon digital pupillometer	Scotopic pupil size	Colvard's digital infrared pupillometer was not as repeatable and agreeable as the Procyon model.
Michel *et al*.,(14) In 2006 California, USA	21 subjects (41 eyes)	NeurOptics, Inc. and P2000D, Procyon, Ltd.	Scotopic pupil size	High repeatability and agreement for repeated measures within each device. Significant differences in variability with the Procyon pupillometer.
Fotiou *et al*.,(15) In 2007 Greece	100 healthy subjects	Custom built fast video pupillography device (Ten measurements for each eye)	Baseline Pupil Radius (R1) Latency (T1) Minimum Pupil Radius (R2) Amplitude (R1-R2) Maximum Constriction Velocity (VCmax) Maximum Constriction Acceleration (ACmax) Time for maximum velocity (T2) Time for maximum constriction (T3) Percentage Recovery (R %) Percentage Amplitude (% AMP)	Satisfactory test-retest reliability for all parameters except T3 and R %.
Schallenberg *et al*.,(16) In 2010 Germany	46 healthy subjects (92 eyes)	Colvard, Procyon, and NeurOptics Pupillometer	Pupil diameter at 0.04 and 0.4 lux	NeurOptics pupillometer showed the largest diameter and had high interobserver agreement and repeatability. Procyon pupillometer performed poorly.
Herbst *et al*.,(12) In 2011 Denmark	10 healthy subjects	IdeaMedical (Copenhagen, Denmark) Blue (470 nm) and red (660 nm) LEDs Infrared video camera (Sony, Japan)	. Baseline pupil size . Maximal Pupil Contraction . Sustained pupil contraction . Summed pupil response or area under curve	For any of the pupil response parameters, there was no discernible change in the recurrent measure.
Schroder *et al*.,(11) In 2018 Germany	91 healthy subjects	Pupil X (Albomed GmbH) Three illumination settings: 0 lux (Scotopic), 1 lux (Mesopic), 16 lux (Photopic)	Pupil Diameter (5 consecutive measurements)	Repetitive measurements under identical brightness conditions showed good repeatability.
McKay *et al*.,(17) In 2020 USA	40 subjects	BrightLamp (Brightlamp Inc., IN, USA) in iPhone 8 And NeurOptics PLR-3000 (NeurOptics, CA, USA)	. Pupil Diameter . Reflex Amplitude . Latency . Constriction Velocity . Dilatation Velocity	Due to its poor repeatability, the iPhone pupillometer is not a useful tool for assisting in clinical decision-making.
Hernandez-Sierra *et al*.,(18) In 2021 Mexico	60 healthy subjects	Mobile pupillography app "Doctor Kit" developed in the Faculty of medicine of the autonomous University of San Luis Potosi, in cooperation with Discomp Mobile inc. (Ing. Jorge Omar Morales)	Three illumination settings: 100 lux, 200 lux, 201 to 300 lux Pupil Diameter at 200 ms (Pb-basal measurement) Pupil diameter at 400, 600, 900, 1200 and 1500 ms (Pi)	Reduced interrater reliability in brighter settings relative to those conducted in less bright settings.
Zheng *et al*.,(19) In 2022 China	30 healthy subjects (60 eyes)	RAPDx® (Konan Medical USA, Irvine, California, USA)	• Amplitude of constriction (AC) • Latency of constriction (LOC) • Velocity of peak constriction (VC) • RAPD score for amplitude and latency	High Repeatability for AC, LOC, and VC. Moderate repeatability for RAPD score for amplitude and latency.

Nyholm *et al*.,(8) In 2022 Denmark	14 subjects (Sedated or comatose)	NPi-200 (NeurOptics, Irvine, CA,USA)	. Maximum Diameter . Minimum Diameter . Percentage change . Constriction Velocity . Maximum Constriction Velocity . Dilatation Velocity . Latency of Constriction . Neurological Pupil Index	Automated pupillometry exhibits outstanding reliability, with twice the reproducibility and repeatability of manual pupillometry.
Current Research In 2023 India	30 healthy participants (60 eyes)	NPi-200 (NeurOptics, Irvine, CA,USA)	. Maximum Diameter . Minimum Diameter . Percentage change . Constriction Velocity . Maximum Constriction Velocity . Dilatation Velocity . Latency of Constriction . Neurological Pupil Index	Excellent agreement regarding repeatability and reproducibility of dynamic pupillary parameters.

Regarding the repeatability and reproducibility of dynamic pupillary parameters, the study's findings indicate excellent agreement when assessed with an automated pupillometer (NPi-200, NeurOptics Inc., Irvine, CA, USA). The use of pupillometer has many advantages over manual pupillary examinations, leading to a more precise evaluation of the pupil's dynamics and better patient care. These findings correlate with the research conducted by Nyholm *et al*., (8), utilizing the identical NeurOptics pupillometer model. In their study, they evaluated manual and automated assessments of pupil size using fifty-six distinct quadrupled sets of measurements from 14 sedated and comatose patients (mean age 70 ± 12 years). The results demonstrated that automated pupillometry exhibits outstanding reliability, with twice the reproducibility and repeatability of manual pupillometry. Additionally, quantitative pupillometry showed lower bias and limits of agreement, as well as a higher intra-class correlation coefficient (ICC) for both intra-observer and inter-device measurements. However, the study was done in a cardiac ICU at a tertiary heart centre in Germany, whereas the current study was done with healthy participants aged 18 to 60 years in routine clinical settings in the ophthalmology outpatient department in India.

The comparison of the current study data with the previous studies reporting the repeatability and reproducibility of pupillary parameters using different devices under specific conditions in healthy subjects shows variable findings, with some devices demonstrating better repeatability and agreement than others (Table 3).Some devices, such as the NeurOptics pupillometer, demonstrated high repeatability and agreement in multiple studies (8,16,17), while others, like the Procyon pupillometer in certain conditions, showed lower reliability (14,16). The custom-built fast video pupillography device (15) and IdeaMedical device (12) also exhibited satisfactory test-retest reliability. The iPhone pupillometer (17) and the mobile pupillography app "Doctor Kit" (18) were reported to have poor repeatability. The studies collectively highlight variations in the performance of different pupillometry devices and emphasize the importance of considering device-specific characteristics and measurement conditions in interpreting pupillometry results. As far as we know, this is the first study to examine the reproducibility and repeatability of dynamic pupillary parameters in routine clinical settings in the Indian population using the NPi-200 model.

The limitation of the study is that we did not address the clinical utility of the device, as only healthy participants were included. The sample size was small as it was a pilot study. Further studies with a larger sample size and the implementation of dynamic pupillary parameters in various clinical scenarios are needed.

CONCLUSION

We found excellent agreement regarding repeatability and reproducibility of dynamic pupillary parameters using an automated quantitative pupillometer. In general, the findings of this study affirm the effective performance of automated quantitative pupillometry in routine clinical settings.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Couret, D., Boumaza, D., Grisotto, C., Triglia, T., Pellegrini, L., Ocquidant, P., *et al*., Reliability of standard pupillometry practice in neurocritical care: an observational, double-blinded study. Crit Care. 2016;20:99.
2. Hsu, C.H., Kuo, L.T. Application of Pupillometry in Neurocritical Patients. J Pers Med. 2023 Jul 5;13(7):1100.
3. Osman, M., Stutzman, S. E., Atem, F., Olson, D., Hicks, A. D., Ortega-Perez, S., *et al.*, Correlation of objective pupillometry to midline shift in acute stroke patients. J Stroke Cerebrovasc Dis. 2019;28(7):1902-1910.
4. Aoun, S.G., Welch, B.G., Cortes, M., Stutzman, S.E., MacAllister, M.C., El Ahmadieh, T.Y., *et al.*, Objective pupillometry as an adjunct to prediction and assessment for oculomotor nerve injury and recovery: Potential for practical applications. World Neurosurg. 2019;121:e475-e480.
5. Miroz, J.P., Ben-Hamouda, N., Bernini, A., Romagnosi, F., Bongiovanni, F., Roumy, A., *et al.*, Neurological pupil index for early prognostication after venoarterial extracorporeal membrane oxygenation. Chest. 2020;157(5):1167-1174.
6. Ong, C., Hutch, M., Barra, M., Kim, A., Zafar, S., Smirnakis, S. Effects of osmotic therapy on pupil reactivity: quantification using pupillometry in critically Ill neurologic patients. Neurocrit Care. 2019;30(2):307-315.

7. Oddo, M., Sandroni, C., Citerio, G., Miroz, J.P., Horn, J., Rundgren, M., *et al.*, Quantitative versus standard pupillary light reflex for early prognostication in comatose cardiac arrest patients: an international prospective multicenter double-blinded study. Intensive Care Med. 2018;44(12):2102-2111.
8. Nyholm, B., Obling, L., Hassager, C., Grand, J., Moller, J., Othman, M., *et al*., Superior reproducibility and repeatability in automated quantitative pupillometry compared to standard manual assessment, and quantitative pupillary response parameters present high reliability in critically ill cardiac patients. PLoS One. 2022;17(7):e0272303.
9. Meeker, M., Du, R., Bacchetti, P., Privitera, C.M., Larson, M.D., Holland, M.C., *et al*., Pupil examination: validity and clinical utility of an automated pupillometer. J Neurosci Nurs. 2005;37(1):34-40.
10. Marshall, M., Deo, R., Childs, C., Ali, A. Feasibility and variability of automated pupillometry among stroke patients and healthy participants: Potential implications for clinical practice. J Neurosci Nurs. 2019;51(2):84-88.
11. Schroder, S., Chashchina, E., Janunts, E., Cayless, A., Langenbucher, A. Reproducibility and normal values of static pupil diameters. Eur J Ophthalmol. 2018;28(2):150-156.
12. Herbst, K., Sander, B., Milea, D., Lund-Andersen, H., Kawasaki, A. Test-retest repeatability of the pupil light response to blue and red-light stimuli in normal human eyes using a novel pupillometer. Front Neurol. 2011;22;2:10.
13. Kohnen, T., Terzi, E., Bühren, J., Kohnen, E.M. Comparison of a digital and a handheld infrared pupillometer for determining scotopic pupil diameter. J Cataract Refract Surg. 2003;29(1):112-117.
14. Michel, A.W., Kronberg, B.P., Narváez, J., Zimmerman, G. Comparison of 2 multiple-measurement infrared pupillometers to determine scotopic pupil diameter. J Cataract Refract Surg. 2006;32(11):1926-1931.
15. Fotiou, D.F., Brozou, C.G., Tsiptsios, D.J., Fotiou, A., Kabitsi, A., Nakou, M., *et al*., Effect of age on pupillary light reflex: evaluation of pupil mobility for clinical practice and research. Electromyogr Clin Neurophysiol. 2007;47(1):11-22.
16. Schallenberg, M., Bangre, V., Steuhl, K.P., Kremmer, S., Selbach, J.M. Comparison of the Colvard, Procyon, and Neuroptics pupillometers for measuring pupil diameter under low ambient illumination. J Refract Surg. 2010;26(2):134-143.
17. McKay, R.E., Kohn, M.A., Schwartz, E.S., Larson, M.D. Evaluation of two portable pupillometers to assess clinical utility. Concussion. 2020;5(4):CNC82.
18. Hernandez-Sierra, J.F., Tellez-Quijada, F., Hernandez Gomez, C.A., Hernandez-Gomez, J.F., Fonseca Leal, P. Estimation and interrater reliability of pupillography by digital mobile app: Digital movilpupillography app validity. Eur J Ophthalmol. 2021;31(4):1779-1784.
19. Zheng, D., Huang, Z., Chen, W., Zhang, Q., Shi, Y., Chen, J., *et al.*, Repeatability and clinical use of pupillary light reflex measurement using RAPDx® pupillometer. Int Ophthalmol. 2022;42(7):2227-2234.