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Original Article

Transorbital ultrasound measurement of optic nerve sheath diameter in normal rabbits

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Abstract: Objective: This study aimed to investigate the feasibility and reliability of transorbital ultrasound (TOUS) measurement of optic nerve sheath diameter (ONSD) in healthy rabbits and evaluate the influence of body weight and head circumference on the ONSD biometry. Methods: TOUS was performed to measure the ONSD 3 mm behind the optic disk in 31 adult male New Zealand White rabbits independently by two investigators. The values of the same animal measured by two investigators were compared with paired *t* test. Relationships between paired samples and the correlations of ONSD with body weight and head circumference were evaluated by Pearson correlation coefficient (*r*). The intra-observer reliability and agreement between two investigators were evaluated with Cronbach's Alpha and Bland-Altman analysis, respectively. Results: Sixty-two optic nerves from 31 rabbits were well imaged by two observers. The mean ONSD was 1.17±0.01 mm (95% confidence interval (CI), 1.15-1.19 mm). No significant difference in ONSD was observed between left and right eyes and between two observers. The Pearson's correlation coefficient between two observers was 0.81 and 0.84 for the right and left eye, respectively. Cronbach's alpha was high (0.92~0.97). The difference between the values obtained by two investigators was 0.02. ONSD had no relationship with body weight (r=0.01, P=0.98) and head circumstance (r=0.02, P=0.56). Conclusion: ONSD measurement with TOUS is feasible and reliable in rabbits, which provides evidence on diagnosis of disorders affecting ONSD.

Keywords: Transorbital ultrasound, optic nerve sheath diameter, intra- and inter-observer reliability, rabbit

Introduction

Increased intracranial pressure (ICP) is a potentially life-threatening condition that usually occurs due to head injury, intracranial hemorrhage, inflammation, or space-occupying lesion. Elevated ICP may result in a series of clinical signs and symptoms including impaired consciousness and cardiopulmonary arrest. Therefore, rapid recognition and adequate treatment are of great importance. The gold standard for ICP measurement is invasive ICP monitoring, which, however, is inapplicable in patients with coagulation disorders or intracranial infection. Several noninvasive strategies have been developed for the ICP measurement, including computed tomography (CT), magnetic resonance imaging, fundoscopy, transcranial Doppler ultrasound, and transorbital ultrasound (TOUS) [1-4]. Among these strategies, TOUS is highly favored because of its simplicity, accuracy, and high repeatability [5, 6].

TOUS is used to evaluate the ICP by measuring the optic nerve sheath diameter (ONSD). It is based on the principle that the optic nerve sheath is a continuation of the subarachnoid space, and cerebrospinal fluid freely flows through the subarachnoid space between the cranium and orbit [7]. When the ICP is elevated, the increased ICP will be transmitted to the fluid surrounding the optic nerve, leading to the inflation of the optic nerve sheath. This change can be detected as an increased ONSD. A variety of studies have shown a significant correlation between ONSD and ICP [8-10]. As a reliable, portable and affordable technique, ultrasound has been widely used in the Department of Emergency and Intensive Care Unit (ICU) for the ICP measurement.

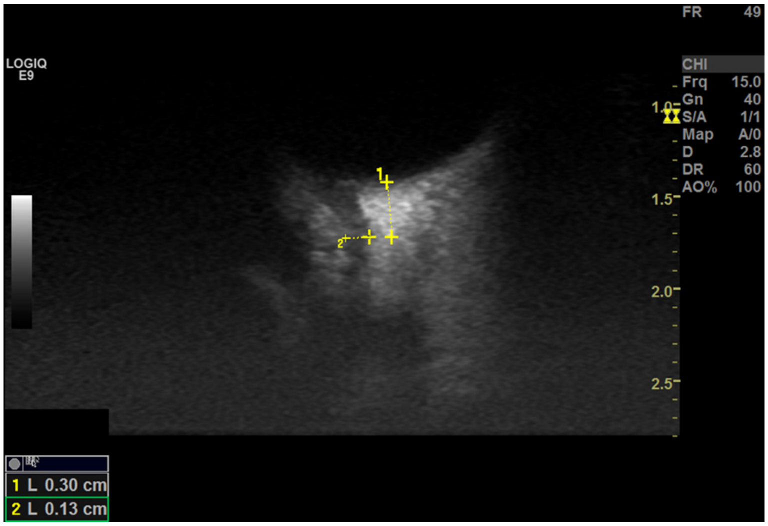

Figure 1. TOUS images of the rabbit optic nerve. ONSD was measured 3 mm behind the optic disc in the longitudinal section of the nerve.

Significant difference in ONSD has been found between species. The mean ONSD measured by ultrasound was 3.68±0.36 mm in adult humans, 4.8±0.4 mm in horses, and 1.85±0.22 mm in dogs [11-13]. Although rabbits are commonly used in ophthalmic experiments, few studies report the ultrasonic measurement of normal ONSD in them. The present study was to (1) assess the feasibility of TOUS measurement of ONSD in healthy rabbits, (2) determine whether TOUS has acceptable inter- and intra-observer reliabilities, and (3) identify the correlations of ONSD with body weight and head circumference.

Materials and methods

Following approval of Animal Care and Use Committee of the Guangxi Medical University, 31 New Zealand White rabbits were purchased and used in this study. All procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All rabbits were examined by a senior veterinarian (Rui-Hui Pan) for ophthalmic and physical health before experiments. The body weight and head circumference of each rabbit were measured and recorded.

After the hair was trimmed around the eyes, rabbits were placed in a box equipped with head-fixing bolt. A LOGIQ E9 Ultrasound machine (General Electric Company, Fairfield, CT) with a 6-15 MHz linear transducer was used in this study. No sedatives or anesthetics were used. The mode was set to small part, and the scanning depth was 3 cm. Gain and time gain compensation were accommodated to clearly visualize the retrobulbar tissues. After applying sufficient acoustic gel to the upper eyelid, the transducer was gently placed on the nasal side in a longitudinal position. When the muscle cone was imaged at the middle of the monitor, the zoom button was pressed to acquire a clear image of the tiny optic nerve within the muscle cone. The probe was slightly adjusted until the best longitudinal image of the optic nerve was achieved. ONSD was measured 3 mm behind the optic disc (**Figure 1**). Three measurements were made and recorded, and the average was calculated.

Experiments were carried out by two investigators (Xi-Yue Yang and Jin-Ping Yang) with rich experience in human ophthalmic examination. Both performed the measurement independently. The investigators consecutively performed the measurement and the inter-observer reliability was evaluated. Investigator 1 (Xi-Yue Yang) performed measurement twice in the same rabbit in an identical manner with the interval of more than 1 hour, and the intra-observer reliability was assessed.

All statistical analyses were processed by SPSS version 17.0 (IBM Corporation, Armonk, NY) and MedCalc 10.1 (MedCalc Software, Ostend, Belgium). Measurement data are expressed as mean ± standard deviation (SD) and 95% confidence intervals (CIs). Histograms and Shapiro-Wilk tests were used to determine the normality of these data. Paired *t* test was used to compare ONSDs between two eyes, acquired by two observers, and measured by the same investigator. If there was no difference in ONSD between two eyes, a mean was used for further analyses. When there was a significant difference, analysis was done with the individual measurement of each eye. Pearson correlation coefficients (*r*) were determined to assess the inter-observer reliability, as well as the relation-

Table 1. ONSD (mm) in 31 rabbits

	Right eye (n=31)			Left eye (n=31)		
	Investigator 1I	Investigator 2	Investigator 1II	Investigator 1I	Investigator 2	Investigator 1II
Mean	1.18	1.16	1.18	1.19	1.17	1.17
SD	0.11	0.12	0.10	0.13	0.12	0.12
95% CI	1.14-1.22	1.12-1.21	1.15-1.22	1.14-1.24	1.12-1.21	1.14-1.20
	Both eyes (n=62)			Both eyes (n=124)		
	Investigator 1I	Investigator 2	Investigator 1II	Both Investigators		
Mean	1.18	1.16	1.18	1.17		
SD	0.12	0.12	0.10	0.12		
95% CI	1.15-1.21	1.14-1.19	1.15-1.21	1.15-1.19		

ONSD, optic nerve sheath diameter; Investigator 1I, values obtained by investigator 1 in the first time; Investigator 2, values obtained by investigator 2; Investigator 1II, values obtained by investigator 1 in the second time.

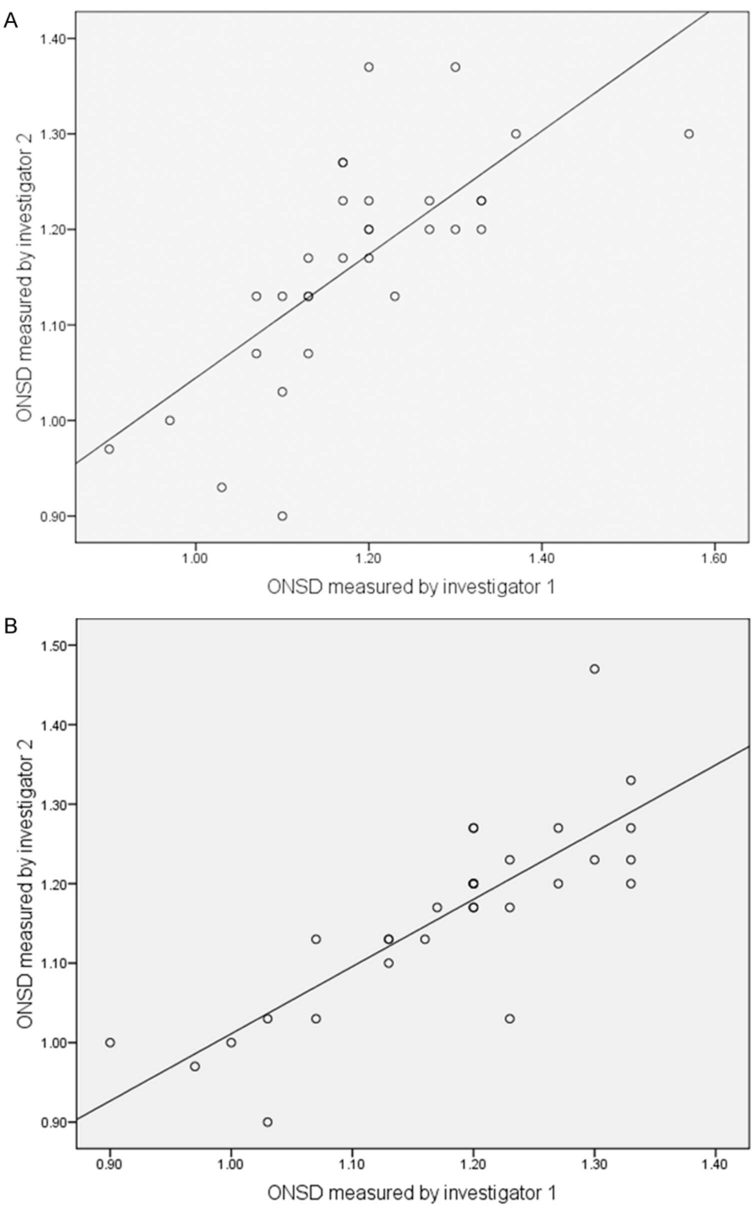

Figure 2. Correlations of ONSD between two investigators for the left (A) and right (B) eyes. The first measurements obtained by investigator 1 and measurements obtained by investigator 2 were used.

ships of ONSD with body weight and head circumference. Cronbach's Alpha was calculated to assess the intra-observer reliability with a value of 1 indicating perfect agreement. Bland-Altman analysis was performed to evaluate the agreement between measurements acquired by two investigators. A value of $P<0.05$ was considered statistically significant.

Results

ONSDs of 62 optic nerves from 31 rabbits were measured. All of the rabbits were 6 months old and males. The mean body weight was 2.49±0.11 kg (95% CI, 2.45-2.53 kg), and the average head circumference was 19.00±0.57 cm (95% CI, 18.80-19.21 cm). Ultrasound imaging of the rabbit optic nerve revealed a dark strip behind the globe (**Figure 1**). All of the optic nerves were clearly imaged, and all ONSDs were successfully measured. The average ONSD measured

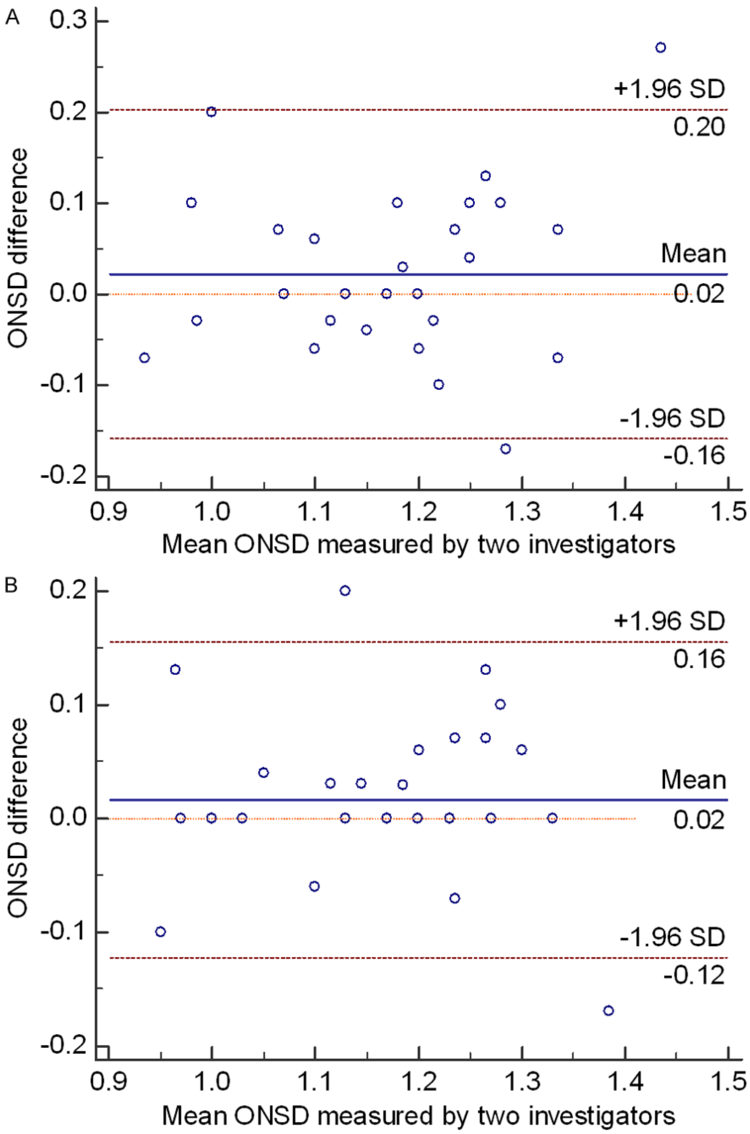

Figure 3. Bland-Altman plots show significant agreement between two investigators for the left (A) and right (B) eyes.

3 mm behind the optic disc was 1.17±0.01 mm (95% CI, 1.15-1.19 mm). There was no difference in ONSD between left and right eyes obtained by two investigators (all $P>0.05$). No significant difference in ONSD was noted between two investigators (all $P>0.05$). Additional descriptive statistics are shown in **Table 1**. The Cronbach's Alpha for investigator 1 was 0.969 and 0.945 for the right and left eyes, respectively. The Pearson's correlation coefficient was 0.811 and 0.721 for the right and left eyes, respectively, between ONSDs obtained by two investigators (**Figure 2**). Bland-Altman plots confirmed a significant agreement between investigators (**Figure 3**). **Figure 4** shows that ONSD had no significant correlations with body weight ($r=-0.132$, $P=0.479$) and head circumstance ($r=0.061$, $P=0.745$).

Discussion

Our results demonstrated that TOUS could obtain clear images of the rabbit optic nerve. The examinations were easy and could be quickly completed (both investigators completed a measurement within 5 minutes), sedation was not needed, and there was no complication. The mean rabbit ONSD measured by TOUS was 1.17±0.01 mm, and ONSD had no significant correlations with body weight and head circumference. Good intra- and inter-reliability were attained in both eyes.

Both human and animal studies have confirmed the ONSD enlargement immediately after ICP elevation [6, 14]. ONSD measurement by TOUS is a simple and convenient assessment for the elevated ICP, particularly in the department of emergency and ICU. How ever, the relationship between ONSD and ICP has not been fully elucidated. Most studies show that ONSD correlates linearly with ICP ($r=0.41$-0.97) [5, 15, 16], but one animal study notes that a fractional polynomial regression model is more suitable [17]. More studies should be done to clarify the correlation between ONSD and ICP.

Rabbits are common animals used in experimental models, but few studies have assessed the ultrasonic appearance of rabbit optic nerve. In our ultrasonic images, the optic nerve appeared as a dark strip running serpiginously and medially in the muscle cones. Unlike larger animals and humans, clear boundary was not observed between the nerve proper and the dura mater [5, 11]. This might be due to the fact that the rabbit optic nerve is very small and

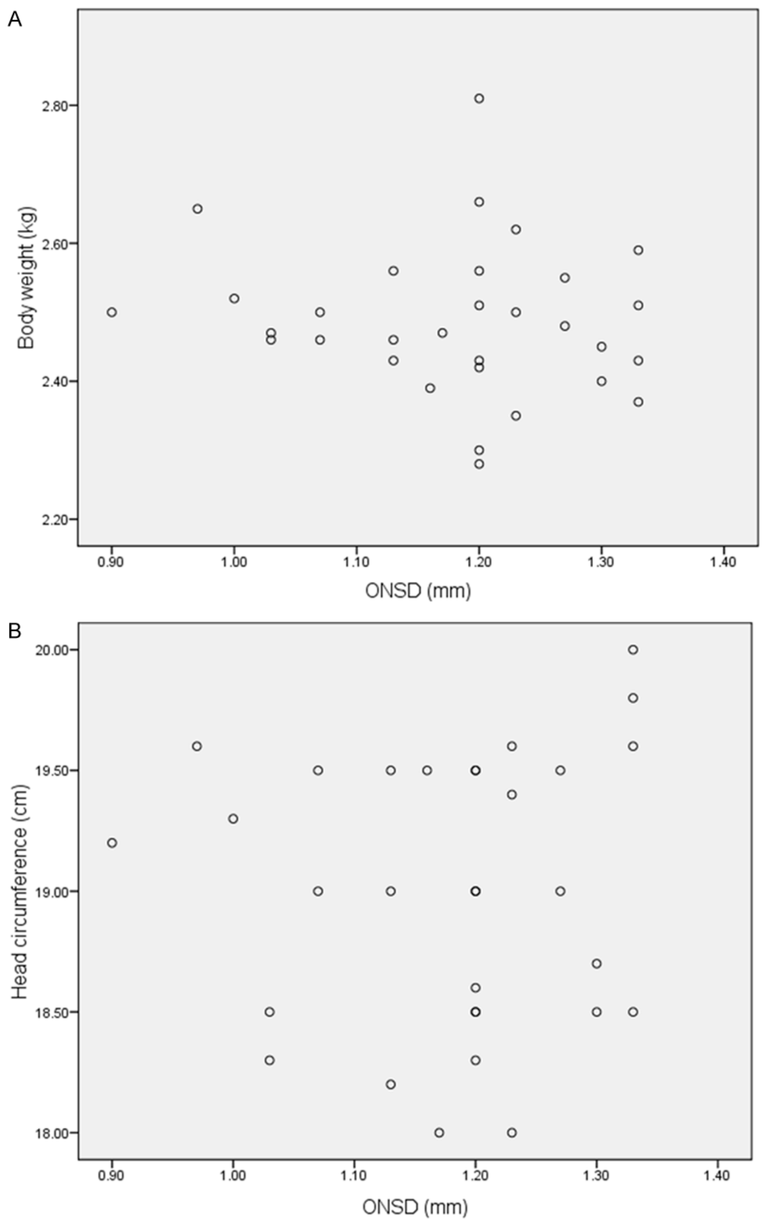

Figure 4. Scatter plots show no significant correlations of ONSD with body weight (A) and head circumference (B). The first measurement obtained by the first investigator was used.

beyond the resolution of ultrasound. In the present study, the mean ONSD of 62 rabbit eyes was 1.17±0.01 mm, similar to the size reported in an *in vitro* study [18], but smaller than that reported by Kasapas et al [19]. In their report, 20 rabbits were used to establish the experimental epidural hematoma model and the baseline ONSD measured by ultrasound was 1.73±0.22 mm. This apparent discrepancy might be the difference in the measurement site. In our study, ONSD was measured 3 mm behind the optic disk, which is consistent with the recommended protocol for humans. Conversely, Kasapas and colleagues did not measure ONSD at a specific site, which might result in a larger variation as the diameter increases around the papilla.

The intra- and inter-observer reliabilities were also tested. To our knowledge, no ultrasound-based study has dealt with this issue. Intra-observer agreement for two eyes was higher than in humans [20], probably because the optic nerve is much smaller in rabbits than in humans, and there was no need to distinguish the nerve from the sheath [21]. No significant difference was found in ONSD between two investigators. Pearson correlation coefficients revealed significant associations between the measurements, and Bland-Altman plots confirmed a good agreement between two investigators. There was no evidence on the systematic bias as demonstrated by the mean difference nearing zero.

In addition, ONSD had no significant correlations with body weight and head circumference. Similar findings were reported in some human studies [13, 22], but Wang et al [23] reported that ONSD was associated with body mass index (correlation coefficient: 0.042). We speculate that this difference may be ascribed to the relatively small sample size and narrow body weight range. Further investigations involving larger and more diverse samples are needed to clarify factors relevant to normal ONSD measured by ultrasound in rabbits.

Several limitations of the present study should be noted. Firstly, the gaze position of each rabbit was not uniform, which might affect the measurements [24]. Secondly, two investigators performed TOUS independently, but investigator 1 could not be blind to their own results, which might affect the intra-observer agreement. Thirdly, ONSD was only measured in young, male rabbits, and controversy has been reported on the relationship of ONSD with sex and age [13, 23, 25, 26]. To rule out other factors affecting the ONSD measurement, a large study involving animals of both sexes and different ages should be performed.

In conclusion, TOUS is a reliable method to measure rabbit ONSD. Our findings provide basic knowledge about the optic nerve in this important experimental species. They will be helpful for future investigations regarding rabbit optic nerve disorders, especially when rabbits are used in animal models of elevated ICP.

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Disclosure of conflict of interest

None.

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