· Clinical Research ·

Noninvasive stiffness assessment of the human lens nucleus in patients with anisometropia using an ultrasound elastography system

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Abstract
● **AIM:** To investigate the significance of ultrasound elastography for evaluating stiffness of the human lens nucleus in patients with anisometropia.
● **METHODS:** A total of 14 patients (28 eyes) with anisometropia were enrolled. The difference in refractive status between two eyes ≥-4.0 diopters (D) and the difference in axial length (AL) of the eyes was ≥3 mm. There were 5 males and 9 females with an average age of 62±3.3y. The test data of the long AL eye of each patient was included in group A (14 eyes), and test data of the eye with relative short AL was included in group B. Lens nuclear stiffness was measured with free-hand qualitative elastography by independent operators. Strain gray scale and color-coded elastography maps were recorded. In each case, three consecutive measurements were performed and strain ratio was used for statistical analysis. Photograph and sectional view of the lens were analyzed and archived by anterior segment image.
● **RESULTS:** In the long AL group, the strain rate in the nucleus of the lens was 0.16%±0.08%; in the short AL group, the strain rate in the nucleus of the lens was 0.54%±0.16%. Independent sample *t*-test analyses showed that the long AL group lens had a significantly smaller nuclear strain rate than the relatively short AL group, $P<0.05$.
● **CONCLUSION:** The relationship between human lens stiffness and different AL is demonstrated by ultrasound elastography. The long AL is associated with lower strain ratio and less resilience of the lens.
● **KEYWORDS:** ultrasound elastography; human lensnucleus; stiffness; anisometropia
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INTRODUCTION

Cataract is the world's leading cause of blindness, among which the incidence of nuclear cataract is high, and the damage to vision is earlier and more severe. With cataract patients becoming younger, the correlation between cataract and myopia has gradually attracted people's attention. The existing cataract patients have been affected by myopia, and the age of surgery in cataract patients has become younger. People with myopia are more likely to suffer from cataract before the age of 60 compared with those with normal vision or hyperopia, while high myopia is often complicated with nuclear cataracts at a younger age[1]. Studies have confirmed that there is a close relationship between the occurrence and development of myopia and nuclear cataract in different races and geographical regions. Longer axial length (AL) is one of the important risk factors for the development of nuclear cataract. Younger patients with long axial and axial myopia develop nuclear cataract and require cataract surgery earlier. In addition, axial myopia increases the risk of cataract surgery, and the longer AL, the higher the probability of posterior capsule rupture during surgery[2-3].

It is commonly seen in clinical practice that high myopia nuclear cataract is often accompanied by anatomical abnormalities

Table 1 The clinical data of ametropia patients

No.	Age (y)	Gender	AL (mm)		UCVA		BCVA		IOP (mm Hg)	
			R	L	R	L	R	L	R	L
1	59	M	23	26	20/25	20/500	20/33	20/33 (-15.00 DS)	15	17
2	60	M	25.8	31.1	2/500	20/333	20/33 (-5.250 DS/-1.50 DC×95°)	20/200 (-20.00 DS)	15	14
3	62	M	28	24.1	20/500	20/200	20/40 (-17.00 DS/2.50 DC×65°)	20/20 (-5.00 DS)	14	12
4	67	M	28.5	23.5	20/1000	20/100	20/50 (-10.00 DS/1.00 DC×70°)	20/33 (-4.20 DS)	17	14
5	60	M	22.9	26	20/20	20/333	20/20	20/33 (-15.00 DS)	15	17
6	59	F	28.0	23.5	20/1000	20/32	20/40 (-10.00 DS/-1.00 DC×70°)	20/20 (-1.25 DS)	18	15
7	67	F	31.1	25.8	20/1000	20/333	20/67 (-12.00 DS)	20/33 (-5.25 DS/-1.50 DC×95°)	16	15
8	57	F	27	23.1	20/500	20/20	20/25 (-14.00 DS)	20/20	14	12
9	63	F	22.8	26	20/20	20/333	20/20	20/33 (-8.25 DS)	15	15
10	66	F	29.0	24.5	20/1000	20/50	20/50 (-15.00 DS/-3.00 DC)	20/20 (-5.25 DS)	18	15
11	66	F	23.0	26.5	20/25	20/333	20/25	20/40 (-9.00 DS)	14	15
12	65	F	22.8	25.5	20/20	20/200	20/20	20/33 (-8.00 DS/-1.50 DC×85°)	14	15
13	67	F	27.0	23.1	20/333	20/32	20/50 (-10.00 DS/-3.00 DC×60°)	20/20 (-1.25 DS)	16	15
14	62	F	29.0	23.5	20/1000	20/32	20/50 (-9.00 DS)	20/20 (-1.25 DS)	16	14

UCVA: Uncorrected visual acuity; BCVA: Best corrected visual acuity; AL: Axial length; IOP: Intraocular pressure; DS: Spherical power; DC: Cylinder power.

such as vitreous liquefaction, poor toughness and elasticity of the lens suspensory ligament, and increased nuclear hardness. The inability of clinicians to recognize the hardness of the lens nucleus in real time makes cataract surgery challenging, leading to postoperative complications. Lens nucleus hardness has become an important indicator for the choice of cataract surgery and the setting of intraoperative surgical parameters. Lens hardness can also provide important information for the choice of cataract surgery methods, and its accurate assessment is a key to the success of cataract surgery. Effective, noninvasive and feasible *in vivo* detection methods are therefore of value to both clinical and scientific researchers. Elasticity is an intrinsic biomechanical property of biological tissues. From a biomechanical point of view, changes in the soft tissue texture means a change of its mechanical properties. Ultrasound elastography has become an area of interest in the field of medical imaging. Ultrasound elastography provides a relatively quantitative representation of the lesions within the tissue by assessing changes in soft tissue volume. In principle, it can be applied to any tissue suitable for ultrasound imaging that can withstand static or dynamic pressure.

In our previous studies[4], by measuring the degree of age-related lens nucleus sclerosis, ultrasound elastography showed excellent diagnostic performance, and was also effective and reproducible, allowing objective evaluation of the nuclear sclerosis of the lens. We selected individuals with transparent lenses at different ages and measured the hardness of the lens nuclei by elastic strain rate. Our findings show that with aging, the lens nucleus strain rate and resilience decrease, demonstrating harder texture. In order to understand the extent of axial-related lens nucleus sclerosis, we included the detection of the lens nucleus ultrasound elastography in patients with anisometropia. For the first time, the relationship between the hardness of the lens and the AL of the eye was analyzed by ultrasound elastography, to evaluate its diagnostic value in high myopia nuclear cataract.

SUBJECTS AND METHODS

Ethical Approval This prospective study protocol was approved by the University Institutional Review Board of the Fourth Military Medical University. Written and verbal consent for participation in the study was obtained from all participants.

Patients A total of 14 patients (28 eyes) with anisometropia who were admitted to the Ophthalmology Department from November 2012 to January 2014 were enrolled. The difference in refractive status between two eyes ≥-4.0 diopters (D) and the difference in length of the eyes was ≥3 mm. There were 5 males and 9 females with an average age of 62±3.3y. The test data of the long AL eye of each patient was included in group A (14 eyes), and test data of the eye with relative short AL was included in group B (14 eyes; Table 1).

Patients with systemic diseases associated with eye diseases were excluded. A corneal curvature examination was performed to rule out the effect of keratopathy on the AL (mainly excluding the keratoconus). Intraocular pressure was 14 mm Hg to approximately 20 mm Hg.

Methods The participants assumed the supine position, and were asked to keep their eyes closed while gazing above. The EUP2L54M 7L ultrasonic probe with a frequency of 8-10 Hz was used, with appropriate coupling agent applied to the probe during B-mode scanning. Conventional ultrasound examined the structure of the eyeball, and the shape, location and echo of the lens. The best section showing the lens was chosen, which is when demonstrating the "smiley face-like graphics". A constant eye pressure was applied to fix the probe and switch to the ultrasonic elastic imaging mode. Region of interest (ROI) was set to the minimum default value of the equipment. The sampling frame was placed at the center of the lens nucleus to read the strain rate. The participants were instructed not to move their eye in order to maintain a steady transection view. The different stiffness properties of the lens nucleus were characterized by pseudocolor imaging. For ultrasonic elastography, green indicated the average stiffness of the tissue in the ROI; red indicated a greater strain rate, as the tissue was relatively soft; and blue indicated a smaller strain rate and greater degree of tissue rigidity. The elastic image was continuously observed, and each eye was measured three times with the average value recorded. The examinations were performed by the same ultrasound physician. Data were analyzed by SPSS version 13 software. The measurement data were expressed by mean±standard deviation, and the ratio of strain rate between groups was analyzed by paired samples test, and differences with $P<0.05$ were considered statistically significant.

RESULTS

Lens Nuclear Hardness Analysis In the long AL group, the strain rate in the nucleus of the lens was (0.16%±0.08%); in the short AL group, the strain rate in the nucleus of the lens was (0.54%±0.16%; Table 2). Paired samples test analyses showed that the long AL group lens had a significantly smaller nuclear strain rate than the relatively short AL group, $P<0.05$ (Figure 1).

Analysis of Ultrasound Elastography and Lens Image The ultrasound elastogram showed that with constant external force, the sampling frame of the lens nucleus of the long axial group was dark blue, the iris was red as the tissue was relatively soft, the cornea was green, and the entire elastic image was mainly dark blue. The nucleus sampling frame of the short axial group lens is shown in green or dark blue, the iris is red, the cornea is green, and the entire elastic image is dominantly green, as shown in Figures 2-4. The results showed that for the same subject, the lens of the long axial eye was relatively harder than the lens of the shorter axial eye. Binocular lens images collected by the anterior segment digital camera system showed the degree of nuclear opacity of the lens in the long AL group was significantly more severe than that in the relatively short AL group.

A 67-year-old male patient. Uncorrected visual acuity (UCVA): VOD 20/1000, VOS 20/100; best corrected visual acuity (BCVA): VOD 20/50 (-10.00 DS/1.00 DC×70°), VOS 20/33 (-4.20 DS); AL: R 28.5 mm, L 23.5 mm. The external force was constant, the lens section was clear, and the area indicated by the arrow was the lens nucleus strain rate. The lens nucleus

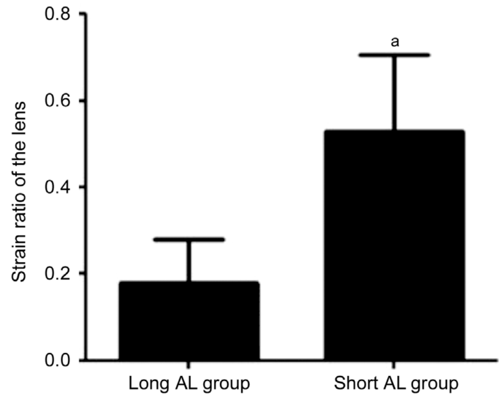

Figure 1 Comparison of strain rates in the lens nucleus area of different AL groups [a]$P<0.05$ compared with the short AL group.

Table 2 Strain rate of lens nucleus in different axial length groups mean±SD

Groups	Cases	Strain rate (%)
Long AL group	14	0.16±0.08
Short AL group	14	0.54±0.16

AL: Axial length.

strain rate of the right eye with long AL was 0.01% (Figure 2A). The lens nucleus strain rate of the left eye with relatively short eye AL was 0.23% (Figure 2B). Photograph of the lens in the anterior segment of the right eye shows the nuclear opacity of the lens (Figure 2C). Photograph of the anterior segment of the left eye shows that the lens was slightly opacification (Figure 2D). Sectional view of the anterior segment of the right eye lens shows that opacity of the lens nucleus was more severe than that of the left eye (Figure 2E, 2F).

A 59-year-old female patient. UCVA: VOD 20/1000, VOS 20/32; BCVA: VOD 20/40 (-10.00 DS/-1.00 DC×70°), VOS 20/20 (-1.25 DS); AL: R 28.0 mm, L 23.5 mm. The external force was constant, the lens section was clear, and the area indicated by the arrow was the lens nucleus strain rate. The lens nucleus strain rate of the right eye with long was 0.12% (Figure 3A). The lens nucleus strain rate of the left eye with relatively short eye AL was 0.64% (Figure 3B). Photograph of the lens in the anterior segment of the right eye shows the nuclear opacity of the lens (Figure 3C). Photograph of the anterior segment of the left eye shows that the lens was transparent (Figure 3D). Sectional view of the anterior segment of the right eye lens shows that opacity of the lens nucleus was more severe than that of the left eye (Figure 3E). Sectional view of the left anterior segment lens shows that the lens nucleus was transparent (Figure 3F).

A 62-year-old female patient. UCVA: VOD 20/1000, VOS 20/32; BCVA: VOD 20/50 (-9.00 DS), VOS 20/20 (-1.25 DS); AL: R 29.0 mm, L 23.5 mm. The external force was constant, the lens section was clear, and the area indicated by the arrow was the lens nucleus strain rate. The lens nucleus strain rate

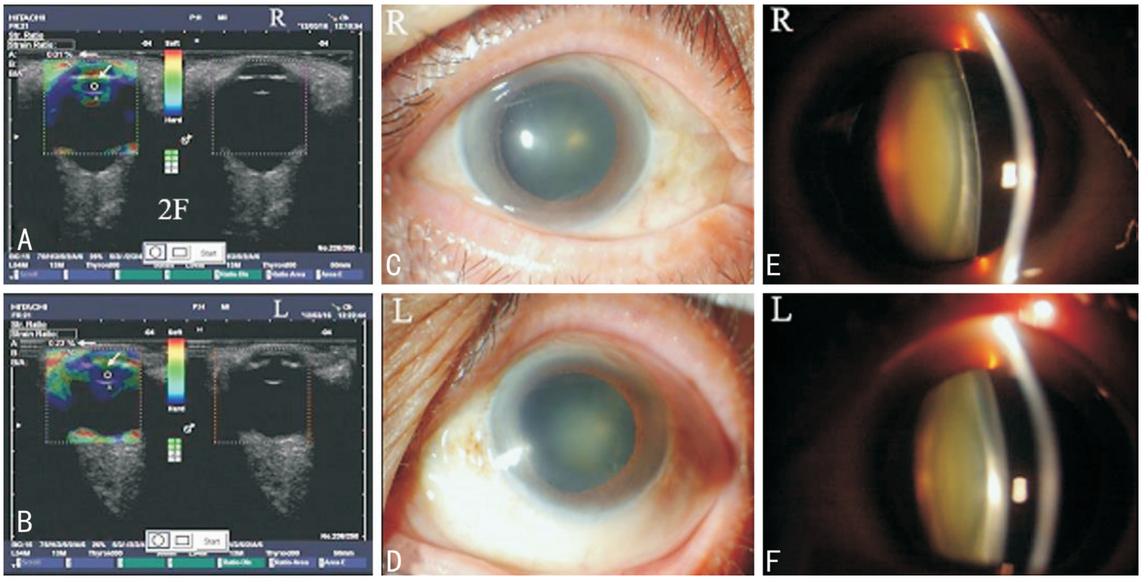

Figure 2 Elastography of the binocular lens in patients with anisometropia The case No.4.

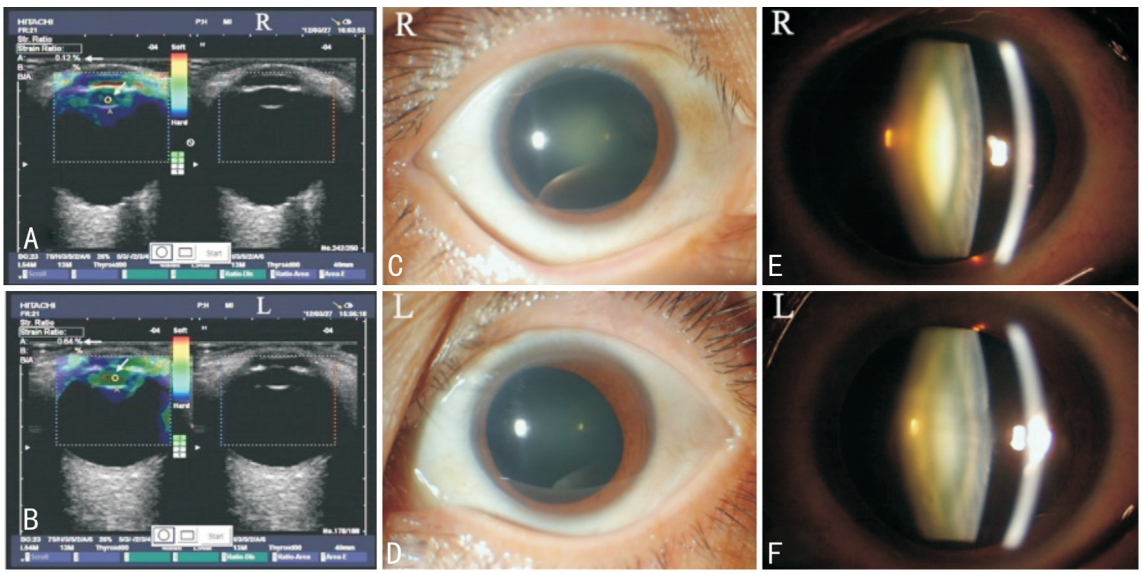

Figure 3 Elastography of the binocular lens in patients with anisometropia The case No.6.

of the right eye with long AL was 0.13% (Figure 4A). The lens nucleus strain rate of the left eye with relatively short eye AL was 0.62% (Figure 4B). Photograph of the lens in the anterior segment of the right eye shows the nuclear opacity of the lens (Figure 4C). Photograph of the anterior segment of the left eye shows that the lens was almost transparent (Figure 4D). Sectional view of the anterior segment of the right eye lens shows that opacity of the lens nucleus was more severe than that of the left eye (Figure 4E). Sectional view of the left anterior segment shows that the lens nucleus was almost transparent (Figure 4F).

DISCUSSION

The lens is an important structure of the intraocular refractive system with no blood vessels or innervation, which has a special metabolic mode. The formation of a nuclear cataract is believed to be caused by aggregation of insoluble proteins in the nucleus of the lens and the compression and coagulation of fibroblasts, resulting in loss of lens transparency, increasing hardness, and decreasing elasticity.

The relationship between axial myopia and nuclear cataract was proposed for the first time by O'Donnell and Maumenee[5] in 1980. Nuclear cataract is considered to be the cause of decreased visual acuity in patients with axial myopia, which is the main reason for the decline in vision of young people with high myopia. Kaufman and Sugar[6] conducted a nine-year observation of 12 patients under 55 years of age with a myocardium greater than 24 mm and high myopia with nuclear cataract, and found that occurrence of nuclear cataract was accompanied by high myopia and axial extension. High myopia and a long AL are important causes of the development of nuclear cataract. Patients with moderate myopia and moderate AL also develop early clinical signs

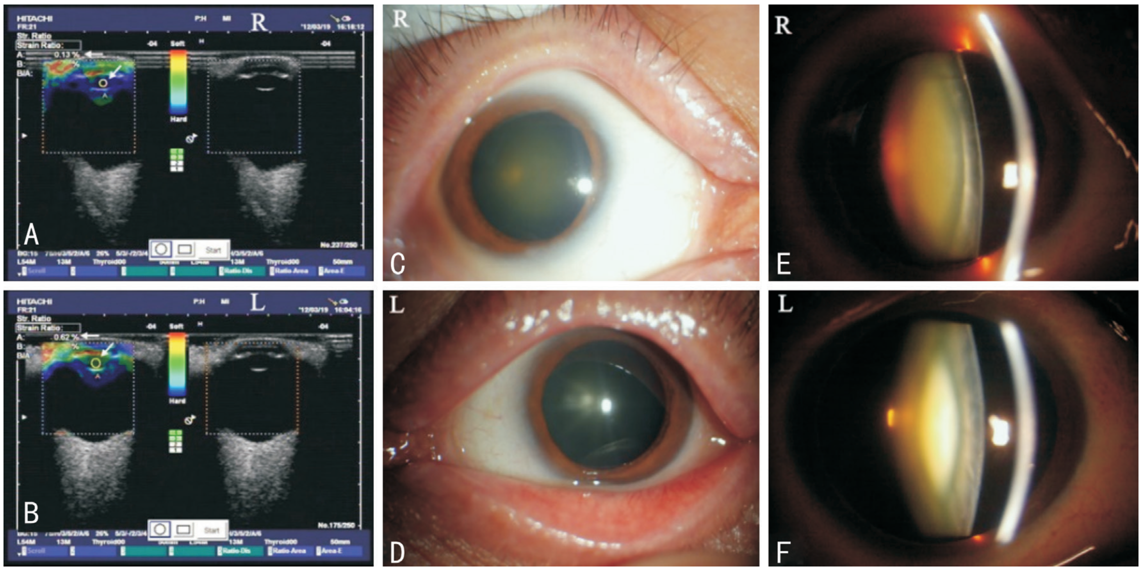

Figure 4 Elastography of the binocular lens in patients with anisometropia The case No.14.

of nuclear cataract. In the study of risk factors related to progressive myopia in the lens by Lin *et al*[7], 35 patients (47 eyes) with nuclear cataract and progressive myopia, from the same hospital, were retrospectively investigated for 5y, comparing preoperative diopter, corneal curvature, and AL between patients with progressive myopia and senile cataract. The results suggested that the AL of patients with nuclear cataract complicated with lens progressive myopia was longer than that of senile cataract patients. It is believed that the longer AL may be one of the important risk factors for the development of nuclear cataract. Kubo *et al*[8] found that young patients with axial myopia developed nuclear cataract earlier and required cataract surgery earlier than elderly patients. It was considered that axial extension and high myopia were important risk factors for the development of nuclear cataract. However, the onset mechanism is unclear, possibly due to the prolonged vitreous cavity reducing the diffusion of metabolites or nutrients at the back of the lens and inhibiting the oxidative defense systems. The prevalence of posterior vitreous detachment in patients with high myopia is higher than that of normal, which is related to the degree of myopia and the length of AL. Holekamp *et al*[9] speculated that the phenomenon of dehydration liquefaction of the vitreous in high myopia occurred early, with wider range, and was exacerbated with increased myopia degree. The liquefied vitreous transported the surface oxygen of the retina into the vitreous cavity, changing the normal oxygen concentration gradient in the eye. The lens is exposed to a high oxygen environment that contributes to nuclear cataract. Therefore, myopia and long AL of the eye contribute to the formation of vitreous liquefaction and nuclear cataract formation. Oxidative damage causes changes in lens proteins and lipids, while vitreous liquefaction increases the risk of developing nuclear cataracts[10].

The detection of lens hardness has value for both clinical and scientific researchers. *In vitro* studies of the lens nucleus hardness have focused on the relationship with aging[11-12]. Pau and Kranz[13] used a device with a conical probe to detect the penetrating power of an *ex vivo* human lens over 20 years of age, and found that the penetrating power of the nucleus was larger than that of the cortex and was age-related manner. The human lens nucleus gradually hardens with age, and the color and age of the nucleus become the basis for clinical nucleus grading[14]. Heyworth *et al*[15] believed that the clinical grade of the lens nucleus was correlated with age. Clinicians can judge the hardness of the lens nucleus according to slit lamp observations, used to guide the surgical operation. Hu *et al*[16] used a device with a conical probe to penetrate the isolated lens nucleus from above, with the penetrating force indicating the hardness of the lens nucleus. It was found that the nucleus turbidity and color of the lens were closely related to the nuclear hardness. The detection of incision lens hardness indirectly assisted in the clinical nucleus grading[17-18]. Compared with *in vivo* studies, the measured mechanical properties may have been greatly affected in the *in vitro* studies. What was encouraging was that researches have investigated the use of elastography to assess age-related changes and biomechanical properties of the lens in animal models and gave a much greater insight of the theories underlying these changes, but these studies are limited to use in animal models[19-20].

Ultrasound elastography has been used in many areas of clinical practice, demonstrating effectiveness and superiority. The important parameter in ultrasound elastography is the strain rate that can indirectly reflect the hardness of the lesion[21]. The ratio of elastic strain rate is a new method for assessing the hardness by ultrasound elastography. By measuring the difference in compliance or hardness between the same normal tissue around the lesion and the lesion, a semi-quantitative relative hardness of the lesion and surrounding normal tissue can be obtained, helping to distinguish the benign and malignant lesions. The normal human lens has a diameter of ~9 mm and a thickness of ~4 mm, and is composed of a lens capsule (A), lens epithelial cells (B), lens cortical fibers (C), and nucleus lens fibers (D) (Figures 5, 6)[22-23]. The lens capsule is a transparent tissue that completely wraps around the lens. There is a layer of lens epithelial cells under the anterior capsule. When the epithelial cells reach the equator, they are elongated, bent, and move into the lens to become the lens fiber cells. Lens fibers grow during the lifetime, and the old lens fibers are gradually squeezed toward the center of the lens and are hardened into the lens nucleus[22]. The lens structure varies in morphology and composition individually and with age. Younger people have more cortex than the elderly, and the nucleus of the elderly is larger than that of the young population[22-23]. As shown in Figures 5 and 6, the central nucleus area is a region of interest for cataract studies. The default minimum frame of interest for B-ultrasound detection is approximately the same size as the nucleus in the B-ultrasound interface. If this exceeds the size of the nucleus, part of the cortex will be included, losing the significance of detection. Therefore, sampling is not possible if the normal tissue around the lesion on the same layer needs to be measured.

In our previous study[4], ultrasound elastography was demonstrated to have excellent diagnostic performance, was effective and reproducible, and could objectively assess the nuclear hardness of the lens in the body by measuring the degree of age-related lens nucleus sclerosis. In order to understand the extent of axial-related lens nucleus sclerosis, we performed lens ultrasound elastography in 14 patients (24 eyes) with refractive errors of ≥-4.0 D and ocular length differences of ≥4 mm. In this study, patients with anisometropia were selected as subjects to minimize individual differences due to age and ethnicity, observing the effect of AL on the hardness of the lens nucleus in the same individual. As age is closely related to lens sclerosis, the age of the enrolled cases was controlled. The age of the 14 patients was 62±3.3 years old, were considered to be within the same age group, thus reducing the bias caused by age. The eyes of each patient were included in the study, which included one eye with the long AL and the other eye with a relatively short AL, which excluded the interference of individual factors such as age, eating habits, altitude, body temperature, etc., and reduced the influence of external factors, making the results more objective. For the first time, this study used lens ultrasound elastography to successfully quantify the hardness of the human lens in eyes of different axes. The elastic strain rate was used to evaluate the hardness of the lens nucleus, which provided an effective supplement for the currently used clinical grading and setting of ultrasonic energy parameters during surgery.

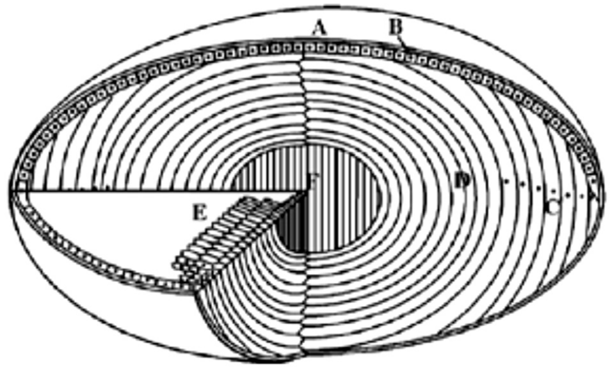

Figure 5 Structural diagram of the lens.

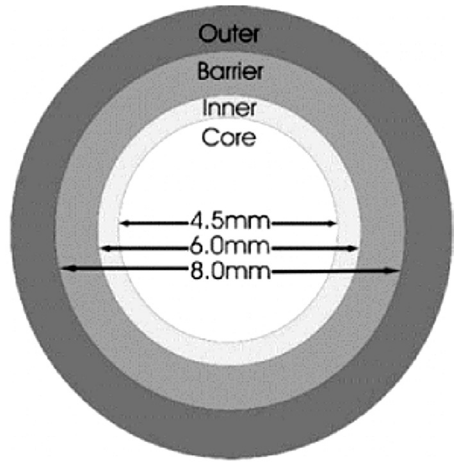

Figure 6 Cross section of the lens.

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Conflicts of Interest: Zhou HY, None; **Yan H,** None; **Yan WJ,** None; **Wang XC,** None; **Li QY,** None.

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