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Elastography acoustic radiation force impulse (ARFI): an investigation of ocular elasticity in dogs with chronic secondary glaucoma

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Abstract: The aim of this study was to evaluate and compare the elasticity of the optic nerve (ON) and retinal-choroid-sclera complex (RCSC) in dogs with healthy eyes and secondary glaucoma using ARFI elastography. ARFI elastography was performed on normal eyes (n = 25) and affected by glaucoma (n = 25). The mean values of shear wave velocity (SWV) of the ON and RCSC were compared between groups by the Student's t-test. The correlation between elastography measurements of the ON and RCSC and the intraocular pressure (IOP) was measured. The SWV of the optic nerve in glaucomatous eyes (4.77 ± 1.59 m/s) was being significantly higher than in healthy eyes (2.34 ± 0.45 m/s) ($p < 0.0001$). RCSC shear wave velocity values in glaucomatous and healthy eye were 4.26 ± 1.28 m/s and 2.36 ± 0.40 m/s, respectively, demonstrating increased stiffness in the glaucomatous group ($p < 0.0001$). RCSC and ON shear wave velocity did not correlate with size of the vitreous chamber and ON as measured by B-mode ultrasound ($p > 0.05$). However, there was a slight positive correlation between RCSC and ON shear velocity in the glaucoma group ($r = 0.38$; $p = 0.05$). The shear wave velocity rate values of ON and RCSC tissues in glaucomatous eyes are greater than in healthy eyes, suggesting that they are less elastic, or harder, in glaucomatous eyes.

Key words: Canine, intraocular pressure, ocular ultrasonography, elastography, ARFI

1. Introduction

Canine glaucoma is a condition associated with a group of neuropathies that commonly raise intraocular pressure (IOP) and cause pain [1]. As a consequence, there is the loss of retina ganglion cells and degeneration of the optic nerve, which leads to irreversible blindness [1]. Chronic cases of glaucoma promote the stretching of collagen fibers of the cornea and sclera, causing buphthalmos [2,3]. Sclera plays an important role in the biomechanics of the optic nerve head, and the organization of its collagen fibers correlates with IOP-induced deformation in the optic disc [4,5]. In humans, sclera rigidity may vary due to age and possible chronic elevations of IOP [6,7].

In veterinary ophthalmology, B-mode ultrasonography is recommended when the clear ocular media is no longer transparent [8]. In the context of glaucoma diagnosis and treatment, 12 MHz probes allow the evaluation of intraocular contents, in addition to measuring globe size, facilitating the follow-up of therapeutic procedures such as pharmacological ablation of the ciliary body [9]. In addition, higher resolution probes (20 MHz), allow the iridocorneal angle and the sclerociliary cleft to be visualized [9]. However, such structures are best measured when ultrasonic biomicroscopy (60 MHz probe) is employed [9].

Elastography is an ultrasound (US) technique that has the ability to evaluate subtle changes in tissue architecture [10], providing quantitative and qualitative measures of tissue stiffness, using short, high-intensity acoustic impulses to deform tissue elements and create a static map (elastogram) of relative tissue stiffness and providing quantitative values reflecting this characteristic [11]. In healthy dogs' eyes, elastography has already been used to assess the degree of rigidity of the ciliary body, optic nerve and lens [12]. This technique is performed in real time, as noninvasive and, most of the time, does not require sedatives and systemic anesthetics [13].

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Considering that canine glaucomas cause changes in optic nerve stiffness and sclera, elastography can be used as an alternative tool to help recognize and prognosticate this condition [5,7,14]. Thus, the present study aimed to evaluate the elasticity of the optic nerve and the retina-choroid-sclera complex in dogs with chronic secondary glaucoma using acoustic radiation force impulse (ARFI) elastography. Such glaucomatous eyes were compared with healthy eyes verifying the accuracy of elastography in the detection of morphostructural changes. To the knowledge of the authors, there are no studies evaluating the use of ARFI elastography in dogs with chronic secondary glaucoma.

2. Material and methods

This study was approved by the institution's ethics committee under the protocol number 000315/19. The owners of the dogs selected for this study authorized the participation of their dogs by signing a free and informed consent form. All dogs included in the study were presented to the Ophthalmology Service of the Veterinary Hospital where the study was performed.

Fifty canine eyes were used/examined in different breeds in this study and they were divided into two groups as the glaucoma (GG; n = 25) and the control (CG; n = 25) group.

Physical, laboratorial [complete blood count, creatinine, and alanine aminotransferase (ALT)], and ophthalmic examinations were performed for all dogs in the study. Ocular examination included direct and consensual pupillary reflexes, menace responses, Schirmer tear tests, slit-lamp biomicroscopic examination, applanationtonometry, binocular indirect ophthalmoscopy, fluorescein and tear-film breakup time, and ocular ultrasonography B-mode (ACUSON S2000, Siemens, Munich, Germany). Dogs of the "healthy" group (CG), presented with no abnormalities on physical, laboratorial, and ophthalmic examinations. All dogs belonging to the chronic secondary glaucoma (GG) had clinical signs such as buphthalmia, increased intraocular pressure, conjunctival hyperemia, corneal edema, blindness and painful eyes.

To perform ocular ultrasonography and elastography, the corneas were desensitized with proxymetacaine hydrochloride (0.5%) eye drop (Anestalcon, Alcon, SP, Brazil). Sterile acoustic gel (Supra Gel, Adlin Plásticos LTDA, Jaraguá do Sul, SC, Brazil) was used as contact medium for a transcorneal ultrasound. Chemical restraint was not needed.

B-mode ocular ultrasound and elastography were performed with the Acuson S2000/Siemens (Siemens Medical Solution USA, Inc., Culver City, CA, USA) device using a 9.0 MHz transducer by an experienced sonographer (GMM). For the B- mode ultrasound, biometric parameters (length of the globe, vitreous chamber and optic nerve measured in mm), echogenicity, and echotexture patterns of vitreous chambers, retinal-choroid-sclera complexes (RCSC) and optic nerve (ON) were evaluated. For the ON measurements the mean of three measurements were used and measured in mm. The elastography ARFI method used Virtual Touch software. For the qualitative study, deformability of the ON and RCSC was assessed (deformable or nondeformable) from the different colors of the elastogram. A blue color indicated fewer rigid structures than those of a green color (intermediate rigidity), and the red color showed the most rigid structures. The quality of the images was tested using a display device in which homogeneous and greenish images indicated high quality, while heterogeneous and yellowish images indicated low quality. After the qualitative analysis of three regions of interest on the ON and RCSC, which were medially and temporally evaluated, a quantitative analysis was performed using a calliper over these areas. Using this methodology, the software automatically calculated the shear wave velocity (SWV) in m/s for each region and the average was calculated for statistical analysis [12].

Data normality was assessed by the Shapiro–Wilk test (GraphPad Prism 5 Software Inc., San Diego, CA, USA). The incidence of glaucoma in relation to gender was assessed by the chi-square test. IOP and age of glaucoma patients and healthy ones were compared by the unpaired and two-tailed Student's t-test. The ON and RCSC shear velocities values of healthy eyes and those with glaucoma were compared by the two-tailed unpaired t-test. Possible correlations between ON shear wave velocities, RCSC, IOP, ON and CV length (measured by B mode) were evaluated by the Person test. At all times, $p \leq 0.05$ values were considered significant. Data are described as mean ± standard deviation.

3. Results

While the mean age for the GG was 7.76 (± 3.39) years, it was 5.68 (± 0.46) in the control group (CG) ($p = 0.015$). GG was composed of Poodle, Shih Tzu and mongrel dogs and CG was composed of healthy Shih Tzu canines.

Of the 25 patients selected for the CG, nine were male and 16 female; in the GG, 11 were male and 14 females (Table 1). Using chi-square test, sex distribution was similar between the two groups ($p = 0.61$). The IOP of canines ranged from 12 to 23 mmHg (17.16 ± 3.07 mmHg) in CG and from 26 to 89 mmHg (46.84 ± 19.87 mmHg) in GG ($p < 0.0001$).

B-mode ultrasonography revealed that the globe size and vitreous chamber of GG was 22.2 ± 1.8 mm and 11.43 ± 1.93 mm, respectively. In CG animals, those

Table1. Details of 25 dogs with secondary glaucoma including intraocular pressure (IOP) and cause of glaucoma**.**

Case #	Age	Breed	Sex	Cause of glaucoma	IOP
1	5	Mongrel	F	Chronic Uveitis	37
2	6	Mongrel	M	Uveitis	28
3	12	Mongrel	F	Chronic Uveitis	39
4	14	Mongrel	M	Cataract	28
5	4	Shih Tzu	M	Uveitis	27
6	11	Mongrel	F	Cataract	39
7	6	Poodle	F	Uveitis	30
8	14	Mongrel	F	Uveitis	26
9	4	Mongrel	F	Uveitis	58
10	6	Shih Tzu	F	Uveitis	42
11	11	Shih Tzu	M	Uveitis	79
12	12	Mongrel	F	Cataract	69
13	8	Mongrel	F	Uveitis	82
14	9	Mongrel	F	Uveitis	62
15	5	Poodle	F	Uveitis	40
16	2	Mongrel	F	Uveitis	50
17	9	Poodle	M	Cataract	75
18	8	Mongrel	M	Uveitis	89
19	6	Mongrel	M	Chronic Uveitis	26
20	7	Shih Tzu	M	Uveitis	28
21	6	Mongrel	M	Uveitis	42
22	7	Mongrel	F	Uveitis	32
23	2	Mongrel	F	Uveitis	44
24	9	Shih Tzu	M	Cataract	67
25	11	Mongrel	M	Cataract	32

IOP: intraocular pressure; M: male; F: female.

measurements were 19 ± 1.35 mm and 8.5 ± 1.35 mm respectively. Both measurements differed significantly between the two groups ($p < 0.0001$).

The elastogram was able to verify increased stiffness of ON and RCSC in patients of the GG. It was verified that both ON and RCSC presented as homogeneous and rigid structures (greenish to reddish shades), when compared to the adjacent tissue (blueish shades), in eyes with glaucoma (Figure 1). For CG animals, ON and RCSC were presented with homogeneous and less rigid structures (bluish tones), compared to the adjacent tissues (Figure 2).

By means of the quantitative ARFI, the values of SWV for RCSC in GG and CG were 4.26 ± 1.28 m/s and 2.36 ± 0.40 m/s, respectively, demonstrating increased stiffness in the affected animals ($P < 0.0001$) (Figure 3). Additionally, the shearing speed of the ON was significantly higher (4.77 ± 1.59 m/s) in GG compared to CG (2.34 ± 0.45 m/s) ($p < 0.0001$) (Figure 4).

In glaucomatous patients, the shear velocity of the ON and RCSC were not correlated with the measurements obtained with B-mode ultrasound (size of globe, vitreous chamber and ON) and IOP ($p > 0.05$) (Table 2). However, in the GG a slight positive correlation between the shear velocity of ON and RCSC was observed ($r = 0.38$, $p = 0.05$) (Figure 5).

4. Discussion

B-mode ultrasonography and elastography are imaging methods applicable in veterinary ophthalmology. In chronic glaucoma, to visualize intraocular structures such as anterior chamber, lens, vitreous and retina, using of the ultrasonic methods is absolutely necessary. In this study,

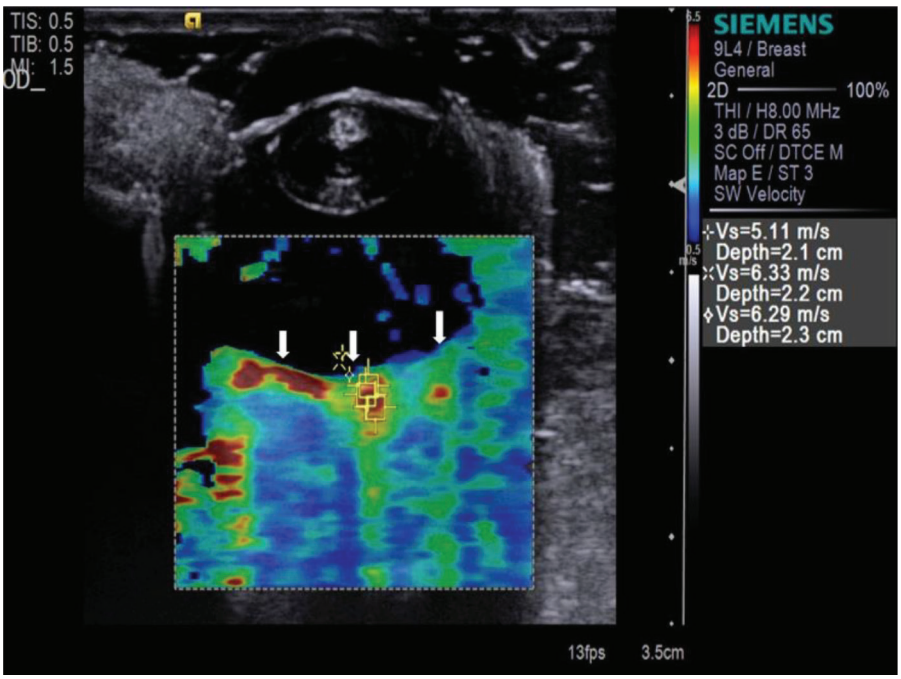

Figure 1. Image of elastogram and quantitative ARFI of a dog eye with chronic secondary glaucoma. A homogeneous and rigid structure is observed [green to reddish shades (white arrows)] compared to the adjacent tissue (blueish shades). In the quantitative elastography we observed increased shear wave velocity values, with an average of 5.91 m/s. Case # 6 (Table 1).

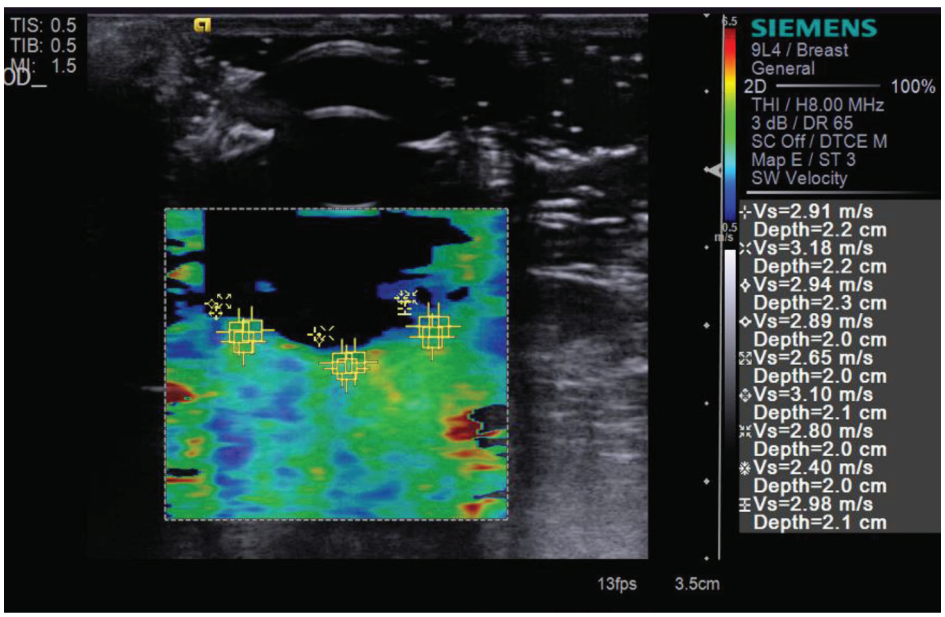

Figure 2. Image of elastogram and quantitative ARFI of a healthy dog eye. Patient with intraocular pressure of 13 mmHg. In the qualitative elastogram one observes a homogeneous and soft structure (greenish tones) in the optic nerve and retino-choroid-sclera complex. The quantitative elastogram shows the shear speed with an average shear wave velocity of 2.97 m/s.

elastography was shown to be a technique that assists in the evaluation of eyes with chronic glaucoma, lending itself to new applications in the early diagnosis and intervention of glaucoma.

Biometric values for globe size in B-mode ultrasound for healthy dogs are similar to those described in the literature [8,12,15,16]. An increase of 3.2 mm in the diameter of the globe and 2.93 mm in the vitreous

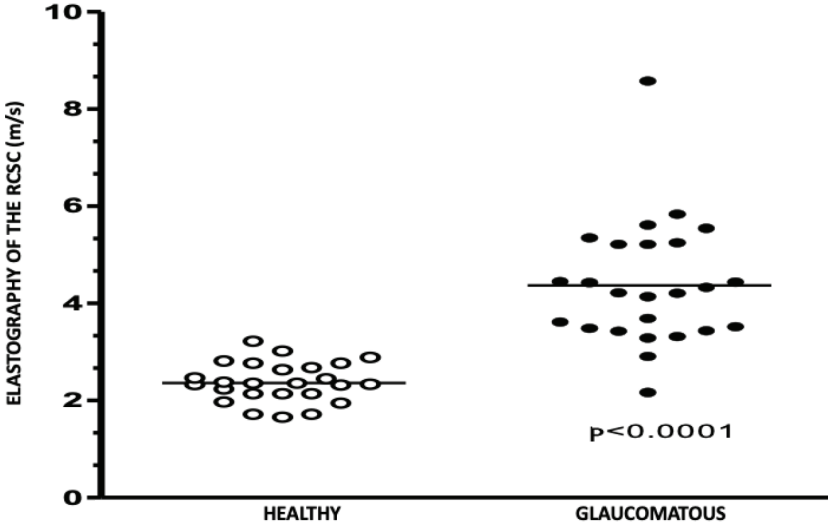

Figure 3. Illustrative graph of the mean (trace) and individual dispersion (circles) shear velocities obtained from elastography of the retino-choroid-sclera complex (RCSC) in healthy and glaucomatous patients ($p < 0.0001$).

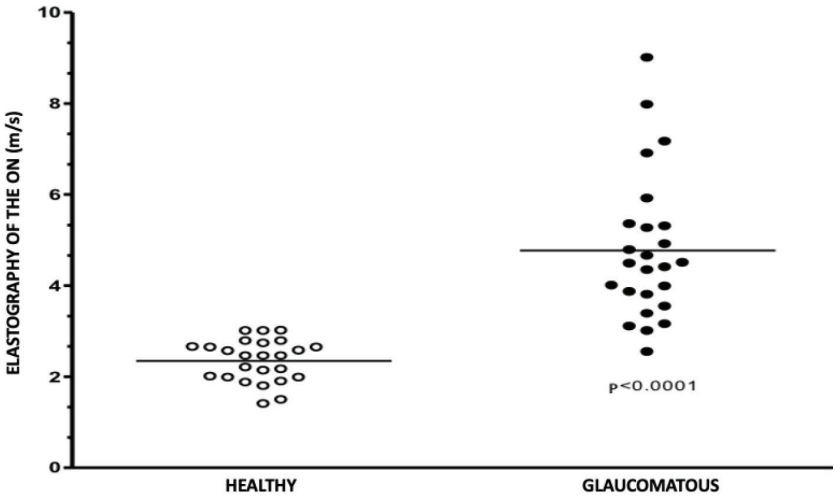

Figure 4. Illustrative graph of the mean (trace) and individual dispersion (circles) shear velocities obtained from elastography of the optic nerve (ON) in healthy and glaucomatous patients ($p < 0.0001$).

chamber were observed in the GG when compared to the CG. Along with the thinner sclera and the constitution of the collagen fibers, the vitreous chamber is the intraocular structure that undergoes the most changes in the process of buphthalmia during chronic glaucoma [8,17].

Studying intraocular pressure (IOP) was not the aim of this study, but as described in several studies, the IOP of glaucomatous patients was significantly higher than in healthy ones [8,18]. This literature only corroborates the findings in patients selected for the current study. High intraocular pressure can cause retinal ganglion cell damage [9]. Retinal damage results in fibrosis especially in optic nerve [19]. Even though in the present study we did not find a correlation between IOP and increased stiffness of the ON and RCSC, we believe the chronic exposure to high IOP and the morphological effects it has on such structures are directly related to higher stiffness observed in the GG.

In the present study, the shear velocity values in the RCSC and ON of patients with glaucoma were higher than in control group ($p < 0.0001$). In the veterinary literature there are no studies demonstrating the use of ARFI elastography to evaluate the biomechanical properties of RCSC and ON in patients with chronic secondary glaucoma. A shear wave elastrographic study on human patients with primary open-angle glaucoma showed no

Table 2. Correlations of the optic nerve and retino-choroid-sclera complex (RCSC) with the intraocular pressure (IOP), vitreous chamber size (VC size) and optic nerve size (ON size) obtained by B-mode ultrasonography.

Shearing speed	IOP	VC size	ON size
Optic nerve	r = 0.05 p = 0.77	r = –0.003 p = 0.98	r = 0.02 p = 0.91
RCSC	r = –0.03 p = 0.86	r = –0.11 p = 0.57	r = 0.07 p = 0.73

IOP: intraocular pressure; VC size: vitreous chamber size; ON size: optic nerve size; RCSC: retino-choroid-sclera complex.

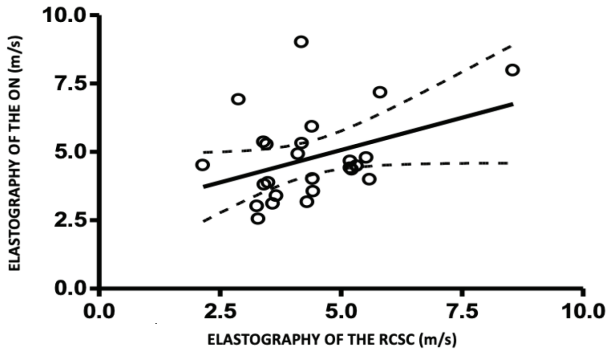

Figure 5. Graphic illustration of the linear regression curve between the shear velocity of the optic nerve (ON) and the retina-choroid-sclera complex (RCSC) of the glaucoma group. Significant Pearson test (p = 0.05, r = 0.38).

significant difference between healthy eyes and eyes with glaucoma [5]. However, other authors observed increased rigidity in both ON and RCSC in glaucomatous eyes [19,20]. Experimental research in monkeys with glaucoma found that sustained high IOP causes damage in the optic nerve, as a scar in the extracellular matrix, which leads to a stiffness of the optic nerve [21].

The increase in IOP and its chronicity may increase the structural rigidity of RCSC and lead directly to deformation of the lamina cribrosa [22]. Deformative changes in the RCSC and ON due to stress through the course of the disease may play an important role in glaucoma pathogenesis [20]. In addition, increased chronic IOP may cause biomechanical changes in the RCSC and ganglion cell axons [7,22]. Similarly, the present study showed reduced elasticity of the ON and RCSC in dogs who had a persistently elevated IOP such as in chronic glaucoma.

5. Conclusion

ARFI elastography is an advanced, noninvasive ultrasound method, a potentially valuable tool for clinical evaluation that is being developed in the area of veterinary ophthalmology. In the present study, patients with chronic secondary glaucoma showed increased shear velocities in ON and RCSC, i.e. the tissues were less elastic, or harder, compared with adjacent tissues or healthy eyes. The use of elastography could be utilized in the future as an early assessment tool in tissues such as the ON and RCSC to detect subtle changes in elasticity to promote early intervention and may ultimately be used to improve clinical outcomes. Further clinical studies are warranted to demonstrate the clinical utility of ARFI elastography in canine glaucoma.

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