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Original paper

Measurement of intraocular pressure in clinically normal Turkish Shepherd Dogs with the rebound tonometer (TonoVet®) and the applanation tonometer (Tono-Pen Vet®)

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Summary

The current study was undertaken to establish reference values of intraocular pressure in clinically normal Turkish Shepherd Dogs with the rebound tonometer (TonoVet[®]) and the applanation tonometer (Tono-Pen Vet[®]). Twenty-eight dogs (14 male and 14 female, aged 9 months-9 years, 56 eyes) were used in this study. All animals were healthy and showed no abnormalities in ophthalmic examinations, which consisted of the Schirmer tear test, slit lamp bio-microscopy, and fluorescein staining. The animals were restrained manually without using systemic anesthetics or tranquilizers. IOP was measured in both eyes with a TonoVet[®] and a Tono-Pen Vet[®]. In normal dogs, the mean IOP values for all eyes were 17.63 \pm 3.34 mmHg according to the TonoVet[®] and 14.95 \pm 2.92 mmHg according to the Tono-Pen Vet[®]. There was no effect of sex, age, or eye side (right vs. left) on the results obtained with either the TonoVet[®] or the Tono-Pen Vet[®]. The mean IOP obtained with the rebound tonometer was 2-3 mmHg higher than that measured with the applanation tonometer. The reference data obtained in the present study may assist in diagnostic testing for ophthalmic diseases in Turkish Shepherd Dogs and may promote further studies in this area.

Keywords: applanation tonometry, rebound tonometry, dog, eye, intraocular pressure

Intraocular pressure (IOP) is defined as the balance between aqueous humor production and drainage (3, 20). Assessment of IOP is important for the diagnosis and management of ophthalmic diseases in animals (29). Increased IOP triggers optic nerve degeneration and retinal ganglion cell dysfunction (29), which results in permanent blindness in some chronic ocular diseases, such as glaucoma (20). Glaucoma is associated with severe ocular pain and is one of the most common causes of ocular enucleation in dogs (33). In North America, the incidence of primary and secondary glaucoma in the total dog population was reported as 0.80% and 0.89%, respectively (6). More than 42 canine breeds were also reported to be predisposed to primary glaucoma (33). IOP measurements are performed by manometric and tonometric methods.

Manometry, that is, direct cannulation of eyeballs, is accepted as a gold standard in IOP measurement, but, due to its invasiveness, it is not a preferable technique in clinical practice. Therefore, for human and animal IOP measurement practices, tonometric (indirect) methods are more frequently applied. For this purpose, a number of tonometers operating by different principles, namely, identification (e.g. Schiotz tonometer), applanation (e.g. pneumatograph, Goldmann, Mackay-Marg, Tono-Pen) and rebound (TonoVet[®], TonoLab[®]) have been developed (35). Nowadays, applanation and rebound tonometers are widely used. The principle of operation of the applanation tonometer is based on the force required to flatten the curvature of the corneal surface (21). However, such factors as the central corneal thickness, corneal curvature, corneal

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rigidity and even the pre-corneal film may influence the measurement of IOP values (29). Tono-Pen Vet[®] (Reichert Inc., Depew, NY, USA) is an applanation tonometer widely used in veterinary ophthalmology (32). In rebound tonometry, a magnetic probe contacts the corneal surface and measures IOP (35). A popular rebound tonometer is TonoVet[®] (ICare Finland Oy, Helsinki, Finland) (32) featuring different calibration modes (,h' mode for horses, ,d' for cats, and ,p' for dogs) that facilitate species-specific IOP measurements in animals (30). This technique is becoming very popular among veterinary ophthalmologists because it provides quick and accurate measurements, is easy to use and does not require topical anesthesia before application (35).

IOP value may vary according to species, age, sex, measurement technique, researcher's experience, circadian rhythm, stress, and anesthesia application (30).

Kangal is one of Turkish shepherd dogs, just like the Çatalburun, Kars and Akbas breeds (5, 15, 27). It takes its name from a district in Sivas Province in Turkey, from which it originates and where it is bred (5). It is hypothesized that this breed was brought to Anatolia by Turkish tribes as they migrated from Asia (5). The Kangal dog has distinct phenotypic characteristics, including a large strong body, short dense hair ranging from gray to steel gray, a very large head, and a black mask on various parts of the body (27). The average weights and lengths are 40.5-41.00 kg and 66.2-71.1 cm for males and 32.4-35.8 kg and 60.8--66.9 cm for females, respectively (15). It is estimated that there are about 1500 Kangal dogs in the province of Sivas alone (14).

The phenotypic and genotypic characteristics of the Kangal dog are well known in the world. It has been reported that Kangals, which serve as livestock guards in America, can well cope with many wild animals. The Kangal dog (26), recognized by The United Kennel Club in the United States and national dog clubs in Australia, New Zealand, and South Africa, is also appreciated by pet owners in Belgium, France, the Netherlands, Germany and the United Kingdom (1).

In our literature review, we found no information about the average IOP for Kangal dogs. The purpose of our study was to determine the average IOP value with the latest generation tonometers, the applanation tonometer Tono-Pen Vet[®] and the rebound tonometer TonoVet[®], and to investigate the relationship between this parameter and such variables as age, sex, and eye side.

Material and methods

The research material consisted of 28 Kangal dogs (14 males and 14 females, aged 9 months – 9 years, 56 eyes) kept at a private Kangal breeding kennel in Sivas. Prior to the study, all dogs underwent ophthalmic examinations: the Schirmer Tear Test, slit-lamp biomicroscopy, indirect ophthalmoscopy and fluorescein staining. Only dogs with



Fig. 1. IOP measurement with TonoVet®



Fig. 2. IOP measurement with Tono-Pen Vet®

healthy eyes were used for the study. The research was conducted with an official approval from the Sivas Cumhuriyet University Animal Experimental Ethics Board (15.03.2017/24) and in accordance with guidelines of the Association for Research in Vision and Ophthalmology. During the study, no feed or water restriction was applied. IOP in both eyes of each animal was measured once with a rebound tonometer (TonoVet®; Icare Finland Oy, Helsinki, Finland) (Fig. 1) and an applanation tonometer (Tono-Pen Vet[®]; Reichert, Inc., USA) (Fig. 2). All measurements were performed between 4 pm and 8 pm to avoid circadian changes. To minimize individual deviations, the restraining of animals (ID) and measurements of IOP (KK) were performed by the same investigators. Moreover, to minimize the stress of displacement, all measurements were carried out in the animals' own boxes. Great care was taken to ensure that the dogs were in a standing posture and that their head was above the heart level during the measurements. Twenty-eight Kangal dogs were divided into 2 equal groups: group 1: < 1 year old (n = 14, 7 males, 7 females) and group $2: \ge 1$ year old (n = 14, 7 males, 7 females). The investigators avoided applying any excessive pressure on the neck or the eyelids while performing IOP measurement. Animals and eye sides were randomly selected, and data were recorded on the basis of the animals' ear tags. Measurements were initially taken with TonoVet[®] set to the ,d' mode, and no topical anesthetic agent was used prior to measurement. During measurement, special attention was paid to keep the angle between the probe and the center of the cornea at 90° and the distance between them at about 4-8 mm. In addition, the investigators took care to hold the device firmly enough to avoid measurement errors due to vibration.

Each time TonoVet[®] takes six measurements, but records an average of four, which are displayed on the led screen, after the largest and smallest values have been rejected. Ten minutes after the completion of measurement with TonoVet[®], the investigators administered one drop of topical anesthetic (Alcaine[®], 0.5% proparacaine hydrochloride, Alcon, Puurs, Belgium) to both eyes of the animal and waited for one minute. Then Tono-Pen Vet[®] was applied gently to the center of the cornea until 4 consecutive measurements had been recorded. In Tono-Pen Vet[®] measurements, deviations greater than 5% were rejected, and the latex cap was changed after each measurement.

Student's t-test (independent-samples t-test) was performed for pairwise comparisons between groups by IBM

Tab. 1. Mean (Mean ± SD) IOPs measured with TonoVet[®] and Tono-Pen Vet[®] in dogs of group 1 (< 1 year old) according to age, sex, and eye side variables

Age	Sex	Eye Side	TonoVet® (mmHg)	Tono-pen Vet® (mmHg)	Р
< 1 years (Group 1)	Male	Right (n = 7)	17.00 ± 0.82	15.00 ± 1.29	0.005
		Left (n = 7)	16.00 ± 0.82	15.00 ± 1.00	0.063
	Р		0.052	0.999	
	Male Right + Left Eyes (n = 14)		16.50 ± 0.94	15.00 ± 1.11	0.001
	Female	Right (n = 7)	18.57 ± 0.53	15.71 ± 1.11	0.0001
		Left (n = 7)	17.71 ± 0.76	15.00 ± 1.63	0.002
	Р		0.051	0.358	
	Female Right + Left Eyes (n = 14)		18.14 ± 0.77	15.36 ± 1.39	0.0001
	Total	Right (n = 14)	17.79 ± 1.05	15.36 ± 1.22	0.0001
		Left (n = 14)	16.86 ± 1.17	15.00 ± 1.30	0.0001
	Р		0.051	0.460	
	Total (n = 28)		17.32 ± 1.19	15.18 ± 1.25	0.0001

Explanation: The difference between means in the same line is significant (P < 0.05, P < 0.001 and P < 0.0001)

Tab. 2. Mean IOP (Mean ± SD) values obtained with TonoVet [®] and Tono-Pen
Vet [®] in dogs of group 2 (\geq 1 year old) according to age, sex, and eye side variables

Age	Sex	Eye Side	TonoVet® (mmHg)	Tono-pen Vet® (mmHg)	Р
≥ 1 years (Group 2)	Male	Right (n = 7)	19.14 ± 5.14	17.14 ± 4.67	0.461
		Left (n = 7)	19.00 ± 4.00	16.00 ± 3.46	0.159
	Р		0.955	0.613	
	Male Right + Left Eyes (n = 14)		19.07 ± 4.43	16.57 ± 3.99	0.129
	Female	Right (n = 7)	16.14 ± 5.37	13.29 ± 5.15	0.330
		Left (n = 7)	17.57 ± 7.63	12.57 ± 4.12	0.153
	Р		0.693	0.779	
	Female Right + Left Eyes (n = 14)		16.86 ± 6.38	12.93 ± 4.50	0.071
	Male + Female	Right (n = 14)	17.64 ± 5.29	15.21 ± 5.13	0.228
		Left (n = 14)	18.29 ± 5.90	14.29 ± 4.07	0.048
	Р		0.764	0.600	
	Total (n = 28)		17.96 ± 5.59	14.75 ± 4.58	0.021

Explanation: The difference between data in the same line is significant (P < 0.05)

SPSS 21.0 version (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: USA). P values of less than or equal to 0.05 were considered as statistically significant.

Results and discussion

The difference between the mean IOPs measured by TonoVet[®] and Tono-Pen Vet[®] for both male and female dogs in group 1 was statistically significant (P = 0.0001, Tab. 1). All dogs in this group showed a statistically significant difference between the mean IOP obtained with TonoVet[®] (17.32 ± 1.19 mmHg) and that measured with Tono-Pen Vet[®] (15.18 ± 1.25 mmHg) when cumulative data were considered regardless of the sex and eye side variables (P = 0.0001, Fig. 3).

The mean IOPs for male and female dogs in group 1

were 16.50 ± 0.94 and 18.14 ± 0.77 mmHg, respectively, according to TonoVet[®], and 15.00 ± 1.11 and 15.36 ± 1.39 mmHg, respectively, according to Tono-Pen Vet[®]. There was no significant difference in average IOPs in both sexes in term of the eye side (P > 0.05, Tab. 1).

The mean IOPs obtained with TonoVet[®] and Tono-Pen Vet[®] in group 2 were 19.07 ± 4.43 and 16.57 ± 3.99 mmHg, respectively, for male dogs, and 16.86 ± 6.38 and 12.93 ± 4.50 mmHg, respectively, for female dogs. The difference between these two means in both sexes was statistically nonsignificant (P > 0.05, Tab. 2). When data for this group were evaluated cumulatively, regardless of sex and eye side, the mean IOPs obtained with TonoVet[®] and Tono-Pen Vet[®] were 17.96 ± 5.59 and 14.75 ± 4.58 mmHg, respectively, with a statistically significant (P < 0.05, Fig. 4) difference between them.

The mean IOPs in group 2 were determined by TonoVet[®] as 19.07 ± 4.43 mmHg for males and 16.86 ± 6.38 mmHg for females. According to Tono-Pen Vet[®], the mean IOPs were 16.57 ± 3.99 mmHg for males and 12.93 ± 4.50 mmHg for females. With regard to the eye side, the difference was statistically non-significant for both TonoVet[®] and Tono-Pen Vet[®] (P > 0.05, Tab. 2).

Regardless of age, sex and eye side, the cumulative mean IOPs obtained with TonoVet[®] and Tono-Pen Vet[®] in both groups were 17.63 ± 3.34 and 14.95 ± 2.92 mmHg, respectively, showing a statistically significant difference (P < 0.0001, Fig. 5).

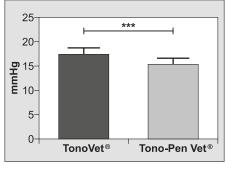


Fig. 3. Comparison of mean IOP values (Mean \pm SD) obtained with TonoVet[®] and Tono-Pen Vet[®] in dogs of group 1 (< 1 year old) (*** *P* < 0.0001)

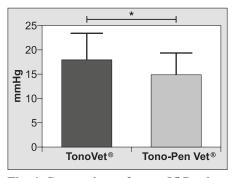


Fig. 4. Comparison of mean IOP values (Mean \pm SD) obtained with TonoVet[®] and Tono-Pen Vet[®] in dogs of group 2 (\geq 1 year old) (* P < 0.05)

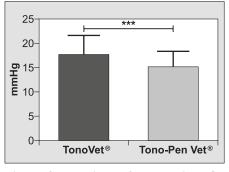


Fig. 5. Comparison of cumulative IOP means (Mean \pm SD) obtained with TonoVet[®] and Tono-Pen Vet[®] in all dogs (*** P < 0.0001)

Early diagnosis of high IOP in pets is important for the management of ocular diseases such as glaucoma and chronic uveitis, which are likely to cause permanent blindness (2). High IOP can be determined by manometric or tonometric techniques. Manometry, a measurement performed by direct cannulation of the eyeball, is accepted as the gold standard for IOP measurement. However, due to its invasiveness, it is used extensively in experimental studies rather than in clinical practice (6). Tonometry is a non-invasive IOP measurement technique performed with a device called a tonometer. The tonometer is used frequently in both clinical practice and experimental studies, as it is easy to use, portable and provides fast readings (2). Current tonometers operate according to 3 different principles, namely, identification, applanation and rebound. Applanation and rebound tonometers have been preferred in recent years (29). In veterinary ophthalmology, applanation tonometers (Tono-Pen XL[®], Tono-Pen AVIA[®], Tono-Pen Vet[®]) preceded rebound tonometers (TonoVet[®]) (11). TonoVet[®], offered as an alternative to Tono-Pen tonometers, has become especially popular among small-animal practitioners. TonoVet[®] has the advantages of competitive prices and faster and more reliable measurements and does not require topical anesthesia before use. In addition, the corneal contact with Tono-Pen tonometers is controlled manually, whereas that with TonoVet[®] is achieved automatically by means of its small and light probe (32). There are a large number of studies using both tonometric methods in domestic animals and dogs in which increased IOP tends to produce significant risk (2, 29, 32). In the current study, we tried to determine a reference IOP value for Kangal dogs with both applanation (Tono-Pen Vet[®]) and rebound tonometers (TonoVet[®]). We also investigated the relationship between these values and such variables as age, sex and eye side.

The IOP value in domestic animals may vary with age, sex, measurement technique used, surgeon's experience, time of day, stress and anesthetic applied (30). The effect of the breed variable on IOP values has also been investigated. IOP measurements with a Tono-Pen XL[®] applanation tonometer, following topical tropicamide administration, revealed that the mean IOP was significantly higher in Siberian Huskies (17.2) \pm 3.7 mmHg) than in Spaniels (14.2 \pm 2.8 mmHg) or Retrievers $(14 \pm 1.9 \text{ mmHg})$ (34). A similar result was obtained in one of our studies (unpublished results) on Awassi sheep when compared to that of Ghaffari et al. (2011) on Sanjabi sheep. These results suggest that mean IOP values may vary between different breeds within the same species. In order to accurately and reliably demonstrate the effect of breed on IOP, it is necessary to use the same tonometer. As a matter of fact, an accurate and standard assessment cannot be obtained by tonometers operating according to different principles. In three different studies using TonoVet[®], the mean IOP in Beagles was determined as 14.3 ± 2.4 mmHg (4), 15.2 ± 1.0 mmHg (18), and 15.6 ± 2.0 and 17.1 ± 2.4 mmHg (20). In studies in which measurements were made with TonoVet[®] and many mix breeds were used as study material, various IOP values were recorded: 15.9 ± 2.1 mmHg and 16.9 $\pm 3.4 \text{ mmHg}(3)$; 16.0 $\pm 3.2 \text{ to } 18.3 \pm 1.8 \text{ mmHg}(17)$; 14.2 ± 3.6 to 17.7 ± 3.1 mmHg (16) and 15.5 ± 2.7 to 15.7 ± 2.8 mmHg (9). In the present study, regardless of age, sex, and eye side, the mean IOP value for Kangal dogs measured with TonoVet[®] was 17.63 ± 3.34 mmHg, which was higher than mean IOPs obtained in three studies on Beagles and similar to mean IOPs in other studies in which different breeds were used (3, 9, 16, 17). With regard to studies using applanation tonometers, mean IOPs obtained with Tono-Pen XL \mathbb{R} for Beagles ranged from 12.7 ± 0.8 to 15.3 ± 1.7 mmHg in one study (10) and was found to be 15.2 ± 2.2 mmHg in another (19). Another study using Tono-Pen XL[®] in Labrador Retrievers determined their mean IOP as 11.93 ± 1.59 mmHg and 12.38 ± 2.10 mmHg (12). In the present study, the mean IOP measured with the Tono-Pen Vet[®] applanation tonometer was 14.95 ± 2.92 mmHg. All these results support the hypothesis that IOP may vary according to breed, even when the same tonometric device is used for the same species.

The IOP value varies according to the measurement technique used, and the difference may amount to as

much as 3-4 mmHg in manometrically controlled IOP studies. For this reason, it is necessary to determine an individual IOP value for each animal with each tonometer operating by a different principle (21). For example, in a study on healthy dogs, the IOP value obtained with TonoVet[®] was 16.9 ± 3.7 mmHg, whereas the IOP value obtained with Tono-Pen XL® was 11.6 ± 2.7 mmHg, and the difference between these means was statistically significant (29). Similarly, in glaucomatous cases, IOP measured by TonoVet[®] was higher than that measured by Tono-Pen Vet[®], and the difference between their mean values was also significant (32). In a study on rabbits, the mean IOP was 9.51 ± 2.62 mmHg when measured by TonoVet[®] and 15.44 ± 2.16 mmHg when measured by Tono-Pen Avia[®], and the difference was also significant (30). In the current study, regardless of age, sex, and eye side variables, the cumulative mean IOPs obtained with TonoVet[®] and Tono-Pen Vet[®] were 17.63 ± 3.34 mmHg and 14.95 ± 2.92 mmHg, respectively, and this difference was statistically significant, as well. These results show that the mean values of IOP obtained for the same animals with tonometers operating by different principles may differ.

IOP is essentially a physiological parameter and it is closely influenced by local and systemic changes (especially those associated with the respiratory and cardiovascular systems). IOP values may be high in excited, nervous and mobile dogs, especially if they are under severe stress (21). Similarly, IOP may be greatly influenced by excessive pressure exerted on the neck and eyelids during the restriction of the animal for measurement (31). The dogs used in the present study were subjected to through systemic and ophthalmologic examinations. Extremely nervous animals and those with a history of ocular or systemic diseases were excluded from the study. During measurements, great care was taken not to exert abnormal pressure on the animal. Also, to avoid excitement, nervousness, stress, and movement, all measurements were taken in the dogs' own boxes. In addition, all measurements were taken by the same investigator in order to avoid individual deviations and bias.

Different studies report different results regarding the relationship between IOP and age in dogs. Former clinical trials using two different applanation tonometers in dogs determined that IOP tended to decrease with age (7, 13). Similar results were obtained in a study on Anatolian buffaloes (28). On the other hand, in a study using applanation tonometry in Capuchin monkeys no statistically significant difference was found between IOP values of different age groups (23); similar results have also been reported in ferrets (22) and three different herbivorous wildlife species (25).

In the present study, the mean IOPs measured by TonoVet[®] were 17.32 ± 1.19 mmHg in dogs under 1 year of age and 17.96 ± 5.59 mmHg in older dogs, whereas according to Tono-Pen Vet[®] these means were

 15.18 ± 1.25 mmHg and 14.75 ± 4.58 mmHg, respectively. The statistical evaluation showed no difference between the means for the two age groups obtained by both devices. The present study, along with others (22-24), does not confirm the reports that IOP significantly changes with age (7, 13, 28).

In our literature review, except for a study on humans (37) and a study on lions (24), none of recent studies (22, 23, 25, 30, 36) has reported a significant relationship between the sex variable and IOP. The present study confirms these findings, since no significant difference was found between the mean IOPs obtained with TonoVet[®] and Tono-Pen Vet[®] for male and female dogs of all ages.

In animals, IOP parameters may vary depending on species, sex, and age. To our knowledge, no study has measured the mean IOP value in Kangal shepherd dogs raised in our region and frequently brought to our clinics. In the current study, mean IOP values in healthy Kangal dogs was determined by two different tonometric methods, and the relationship between this parameter and such variables as sex, age, and eye side was investigated. The mean IOP measured by the rebound tonometer was higher than that measured by the applanation tonometer, and this difference was statistically significant. In addition, IOP parameters in Kangal dogs were not significantly affected by gender, age, or eye side. The present findings may remedy data deficiency with regard to IOP in this breed and may prove useful for veterinary ophthalmologists.

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