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Tear film breakup time and Schirmer tear test in normal dogs: Effects of age, sex, reproductive status, skull type, and nasolacrimal duct patency

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Abstract

Objective: The present study aimed to determine the effects of age, sex, reproductive status, skull type, and nasolacrimal duct (NLD) patency on tear production and tear film breakup time (TBUT) in normal dogs.

Animals studied: The ophthalmic data of 82 healthy adult dogs were evaluated in this study.

Procedures: Age, sex, breed, and reproductive status were recorded. Schirmer tear test (STT) and TBUT were assessed in all dogs, and interferometry was available for the selected dogs. The Jones test was used to evaluate NLD patency. The cephalic index (CI) was calculated for each dog (skull width/skull length ×100).

Results: Mean (SD) values for the STT results for the right (OD) and left (OS) eyes were 20.6 (2.7) and 20.2 (2.7) mm/min, respectively. Mean (SD) TBUT values for OD and OS were 6.5 (2.5) and 6.1 (2.3) mm/min in all dogs, respectively. Sex and reproductive status had no significant effect on STT and TBUT ($P_{[OU]} > 0.05$). Skull type significantly affected TBUT in both eyes ($P_{(OD)} = 0.01$, $P_{(OS)} = 0.003$), but had no effect on STT ($P_{[OU]} > 0.3$). Age had no correlation with STT and TBUT in either eye ($P_{[OU]} > 0.05$). STT and TBUT had no correlation in either eye ($P_{[OU]} > 0.2$). NLD patency had no significant effect on STT or TBUT ($P_{[OU]} > 0.1$).

Conclusions: The results of this study showed lower TBUT values in brachycephalic breeds than in non-brachycephalic breeds. A compensatory increase in STT values was observed in dogs with low TBUT values.

KEYWORDS
brachycephalic dogs, interferometry, ocular surface disease, STT, TBUT, tear film

1 | INTRODUCTION

Tear film (TF) is vital for corneal health and clear vision. It lubricates the corneal surface and supplies oxygen to the cornea.[1] The conventional description of TF is the superimposition of three layers (lipid, aqueous, and mucin).[1] These layers are intermingled, and there is no distinct separation between them.[2]

The main portion of TF is the aqueous part, which is produced by orbital lacrimal glands and the gland of the nictitans. Lipid and mucin layers are produced by meibomian glands and goblet cells, respectively.[1] The lipid layer enhances the stability of TF by reducing the evaporation of its aqueous portion, and mucin binds TF to the epithelium layer of the cornea.[1,2]

TF has several functions such as lubricating the corneal surface, supplying oxygen to the corneal epithelium, flushing the debris, and acting as a barrier to opportunistic microorganisms. It is also the first refractive surface of the eye.[1–3]

TF abnormalities are divided into quantitative and qualitative deficiencies.[1] Quantitative TF deficiency or keratoconjunctivitis sicca (KCS) is deficiency in the aqueous portion of the TF and is common in dogs.[4] It has a negative impact on ocular surface health and causes desiccation and inflammation in the cornea and conjunctiva.[4] Abnormalities in the mucin or lipid tear layers cause qualitative TF deficiency.[1]

Evaluation of the TF is the main part of the ophthalmic examination in dogs. The Schirmer tear test (STT) is a conventional method for evaluating the aqueous portion of TF. Typical clinical signs such as copious mucopurulent ocular discharge, conjunctival hyperemia, and blepharospasm along with STT values below 15 mm/min are diagnostic criteria for KCS.[1,3]

Qualitative TF deficiency is a common disorder among dogs.[1] Several methods have been recommended to evaluate the quality of TF such as rose-bengal staining, non-invasive TF breakup time, and TF breakup time (TBUT/TFBUT).[1] TBUT is a valuable diagnostic test to assess the quality of TF in dogs.[1,3] It examines the capability of the cornea to maintain a homogenous TF layer.[1] It is defined as the time taken for the first dry spot of TF to appear on the cornea after a complete blink.[1,3] To evaluate the lipid layer and meibomian glands, interferometry and meibometry are recommended, respectively.[3,5]

Currently, more than 197 breeds are registered by the American Kennel Club. Many of these breeds are brachycephalic dogs, such as Shih Tzu and Pekingese. The cephalic index (CI) is commonly used to classify dogs into brachycephalic, mesocephalic, and dolichocephalic breeds. Brachycephalic dogs have a cephalic index over 60.[6,7] There are many concerns regarding health issues in these breeds, one of which is eye diseases.[8,9]

Brachycephalic breeds are prone to a wide variety of ocular problems, which mainly occur due to macroblepharon, lagophthalmos, skin folds, and low corneal sensitivity.[5,9]

The purpose of this study was to compare TBUT and STT in brachycephalic and non-brachycephalic breeds and determine the effects of age, sex, reproductive status, skull type, and nasolacrimal duct (NLD) patency on STT and TBUT in dogs.

2 | MATERIALS AND METHODS

This research had a retrospective design. Medical records of 82 dogs (164 eyes) were used in this study. All animals were referred to Negah veterinary centre from May 2020 to August 2020 for an ophthalmic examination.

Dogs without any ophthalmic diseases at the time of examination met the inclusion criteria. Demographic data (age, sex, breed, reproductive status, and CI) and the results of complete ophthalmic examination were documented, and any ocular abnormalities were recorded. All dogs underwent physical examination by an internist as well.

Ophthalmic examinations included STT (Intervet Inc., Merck Animal Health, Summit, NJ, USA), slit-lamp biomicroscopy (Kowa SL-15; Kowa, Tokyo, Japan), tonometry (TonoVet®, icare, Tiolat, Helsinki, Finland), fluorescein staining (Fluorescein Glostrips™, Nomax Inc., St. Louis, USA), Jones test, TBUT assessment, and indirect ophthalmoscopy (Binocular Indirect Ophthalmoscope, Welch Allyn Inc., NY, USA). All ophthalmic examinations were performed by a single investigator and the same assistant.

STT was evaluated by sterile strips from same manufacturer, and the wetness of STT strips was measured in mm/min after placement of the strip in the lateral part of the lower eyelid for 1 min.

TBUT was assessed using fluorescein sodium strips diluted with 0.3 ml of sterile saline (diluted in a syringe). One drop of diluted fluorescein was instilled in the eye, and the eyelids were subsequently closed. After opening the eyelids, the dorsotemporal part of the cornea was observed using the cobalt blue filter of a slit-lamp biomicroscope with 16X magnification. TBUT was measured using a stopwatch as the time between the opening of the eyelid and the development of the first dry spot on the cornea observed by the investigator. The measurement was performed in standard room conditions, with the air conditioner turned off.

Nasolacrimal duct patency was evaluated using the Jones test and normograde flushing of the upper and lower puncta. NLD flushing was performed with 2 ml of sterile saline using single-use cannula after the instillation of two drops of topical anesthetic (tetracaine 0.5%). Both nares and mouth/pharynx of the animals were examined using the cobalt blue filter of a slit-lamp biomicroscope for the presence of fluorescein stain every 5 min up to 20 min after fluorescein instillation in the eyes. Retrograde flushing was not performed because it required anesthesia.

CIs were calculated by measuring the skull's length and width using the standard method (multiplying the skull's width by 100 and dividing the result by the skull's length).[6,7] Interferometry (TEARVET-A Tear Film

Analyzer, SBM Sistemi, Italy) data were available for some dogs (several owners denied alternative ocular examination such as interferometry for their dogs). The authors decided to include the data in the study but did not perform statistical analysis on the data.

Statistical analysis was carried out using SPSS software ver. 26.0 for Mac (SPSS Inc., Chicago, IL, USA). Normality was assessed using the Kolmogorov–Smirnov test. Mean STT and TBUT were calculated for each eye and compared between the left (OS) and right (OD) eyes by paired-sample *t*-test. Pearson correlation was utilized to evaluate any relationship between TBUT and STT, age and TBUT, and age and STT. An independent sample *t*-test was applied to determine the effects of skull type, NLD patency, and reproductive status on TBUT and STT. Descriptive statistics were employed to describe the demographic data.

3 | RESULTS

3.1 | Demographic data

This study was conducted on 19 different dog breeds including Pomeranian (7), English Cocker Spaniel (7), Shih Tzu (24), Yorkshire Terrier (1), West Highland White Terrier (12), Maltese (1), Japanese Spitz (3), English Bulldog (2), Dachshund (1), Miniature Poodle (1), Chihuahua (3), Pekingese (9), Chow chow (1), Japanese Akita (1), Pug (3), King Charles Cavalier Spaniel (1), Golden Retriever (3), French Bulldog (1), and German Shepherd (1).

The mean (SD) age of dogs was 60.7 (44.0) months, with a minimum of 14 and a maximum of 193 months. Forty-seven dogs were female and 35 were male. Fifty-four dogs were neutered/castrated and 38 were intact. Mean (SD) CIs of brachycephalic and non-brachycephalic dogs were 98.8 (8.3) and 67.8 (15.9), respectively. Forty-five dogs were brachycephalic, and 37 dogs were non-brachycephalic. Right eyes had 65 NLDs with a negative Jones and flushing test and 17 NLDs with a positive Jones test, while in the left eyes, 70 NLDs were not patent and 12 NLDs were patent.

3.2 | Ophthalmic findings

Forty-seven eyes out of 164 had ophthalmic findings. Trichiasis (10 eyes), pigmentary keratitis (10 eyes), vortex keratopathy (5 eyes), corneal dystrophy (3 eyes), iris atrophy (4 eyes), peripheral anterior synechiae (1 eye), cataract (4 eyes), nuclear sclerosis (6 eyes), persistent hyperplastic primary vitreous (1 eye), and progressive retinal atrophy (1 eye) were recorded in the examined dogs.

TABLE 1 Mean and Standard deviation (SD) of Schirmer tear test (STT) in the examined dogs

Parameters	Eyes	Mean	SD
Brachiocephalic breeds	OD	20.4	2.7
	OS	20.1	2.7
Non-brachiocephalic breeds	OD	21.0	2.8
	OS	20.6	2.9
Female dogs	OD	20.7	2.7
	OS	20.5	2.9
Male dogs	OD	20.4	2.8
	OS	20.1	2.7
Neutered/Castrated dogs	OD	20.7	2.9
	OS	20.1	3.0
Intact dogs	OD	20.5	2.4
	OS	20.9	2.3
Eyes with positive Jones test	OD	20.6	2.8
	OS	20.3	2.5
Eyes with negative Jones test	OD	20.6	2.6
	OS	20.7	4.1

3.3 | STT

Mean (SD) values for the STT results for OD and OS were 20.6 (2.7) and 20.2 (2.7) mm/min, respectively. There was no statistical difference between OD and OS ($p = 0.5$). Minimum and maximum STT values in the study population were 15 and 30 mm/min, respectively.

Sex, reproductive status, NLD patency, and skull type had no significant effects on the STT results ($p = 0.1$, $p = 0.4$, $p = 0.5$, and $p = 0.2$, respectively). Mean values of the STT results based on sex, reproductive status, and NLD patency are summarized in Table 1.

3.4 | TBUT

Mean (SD) TBUT values for OD and OS were 6.5 (2.5) and 6.1 (2.3) mm/min in all dogs, respectively. The highest and lowest recorded TBUT for all the eyes were 2.14 and 16.12 s, respectively. Statistical analysis revealed no significant differences in mean TBUT between OD and OS ($p = 0.3$). Skull type had a significant effect on TBUT ($p = 0.02$; $p = 0.01$). The difference in mean TBUT for OD and OS between brachycephalic and non-brachycephalic breeds was 1.3 s. Mean TBUT values based on sex, reproductive status, and NLD patency are summarized in Table 2.

TABLE 2 Mean and Standard deviation (SD) of tear film breakup time (TBUT) in the examined dogs

Parameters	Eyes	Mean	SD
Brachycephalic breeds	OD	6.0	2.0
	OS	5.4[b]	1.9
Non-brachycephalic breeds	OD	7.6	2.9
	OS	7.1[b]	2.7
Female dogs	OD	7.0	2.4
	OS	6.3	2.3
Male dogs	OD	6.3	2.6
	OS	5.8	2.5
Neutered/Castrated dogs	OD	6.5	2.3
	OS	5.9	2.3
Intact dogs	OD	7.1	2.9
	OS	6.6	2.6
Eyes with positive Jones test	OD	7.6	3.3
	OS	5.7	2.4
Eyes with negative Jones test	OD	6.4	2.2
	OS	6.2	2.4

Note: Superscript a and b: significant statistical difference.

3.5 | Inter-test correlation

Pearson correlation analysis revealed no correlation between STT and TBUT in either eye (OD: $r^2 = -0.133$, $p = 0.2$; OS: $r^2 = -0.01$, $p = 0.9$). Figures 1 and 2 depict the correlation between STT and TBUT in OD and OS, respectively. Age had no correlation with STT and TBUT in the dogs ($r^2 = -0.07$, $p = 0.5$; $r^2 = -0.3$, $p = 0.1$; $r^2 = -0.008$, $p = 0.9$; $r^2 = -0.05$, $p = 0.6$). The scatter plot of STT and TBUT data is presented in Figure 1.

CI had a weak negative correlation with TBUT in both eyes ($r^2 = -0.27$, $p = 0.01$; $r^2 = -0.24$, $p = 0.02$) (Figure 2). No significant correlation was found between STT and CI in either eye ($r^2 = -0.01$, $p = 0.9$; $r^2 = 0.03$, $p = 0.7$) (Figure 3).

3.6 | Interferometry

Interferometric data were available for 82 out of 168 eyes. Fifty-eight eyes had an open meshwork pattern (grade 1; ~15 nm of the lipid layer thickness), and 24 eyes had a close meshwork pattern (grade 2; 15–30 nm of the lipid layer thickness). All 52 brachycephalic eyes were classified as grade 1, six non-brachycephalic eyes were grade 1, and 24 eyes were grade 2 in interferometry. Interferometric data of the eye of an examined dog are presented in Figure 4.

4 | DISCUSSION

There are three basic skull types in dogs: Dolichocephalic, mesaticephalic, and brachycephalic.[6] Brachycephalic dogs have a wide head, short muzzle, and unique anatomical features,[6] which makes them prone to develop various disorders, such as respiratory (brachycephalic airway syndrome), dental, skin, and ophthalmic diseases.[10]

The results of this study showed unstable TF in brachycephalic dogs compared to non-brachycephalic ones. TBUT of brachycephalic breeds in this study was 18% more accelerated than non-brachycephalic breeds. Mean normal TBUT for adult healthy dogs has been reported to be 14.7–28.9 s.[11] Mean TBUT of both brachycephalic and non-brachycephalic breeds in the present study was at least 50% more accelerated than the normal reference values. However, some dogs showed TBUT values within the normal reference interval. For instance, dog no. 52 had a TBUT value equal to 16.12 s in OD and 15.01 s in OS. This difference might be due to various factors such as the environment, fluorescein volume and/or concentration (which might differ from previous studies), and the poor reliability of TBUT. The animals in the present study have been living in a city with dry and hot weather, especially in the summer. This hot and dry weather can be the possible cause of short TBUT in the dogs, but further research is necessary to evaluate the effects of season and environmental factors (such as temperature, humidity, and altitude) on TBUT in dogs. In the current study, the authors used diluted fluorescein sodium. A recently published study reported no difference in mean TBUT between different methods of fluorescein sodium application.[12] By reviewing the literature, mean TBUT values for normal dogs have also been reported to be 5.9–8.6 s,[13] which was similar to the findings of the present study.

A number of TBUT-affecting factors have been reported in several investigations on cats and horses. Topical anesthetics, such as tetracaine, significantly decrease TBUT in horses.[14] In cats experimentally infected with feline herpesvirus 1 (FHV-1), TBUT was shorter in comparison with normal cats.[15] TBUT is shown to be accelerated in cats with ulcerative keratitis,[16] conjunctivitis,[17] and corneal sequestrum.[18] Anesthesia is also a reducing factor for TBUT in cats.[19]

In humans, altitude has been reported as a factor influencing TBUT.[20] One study conducted on humans indicated that high altitude caused a reduction in TBUT because of air flow (wind), low humidity, and ultraviolet radiation (UV).[20] Another investigation on humans showed the effects of climate and environmental factors on dry eye disease.[21] In humans, TBUT has been reported to be positively correlated with weather temperature and humidity.[21]

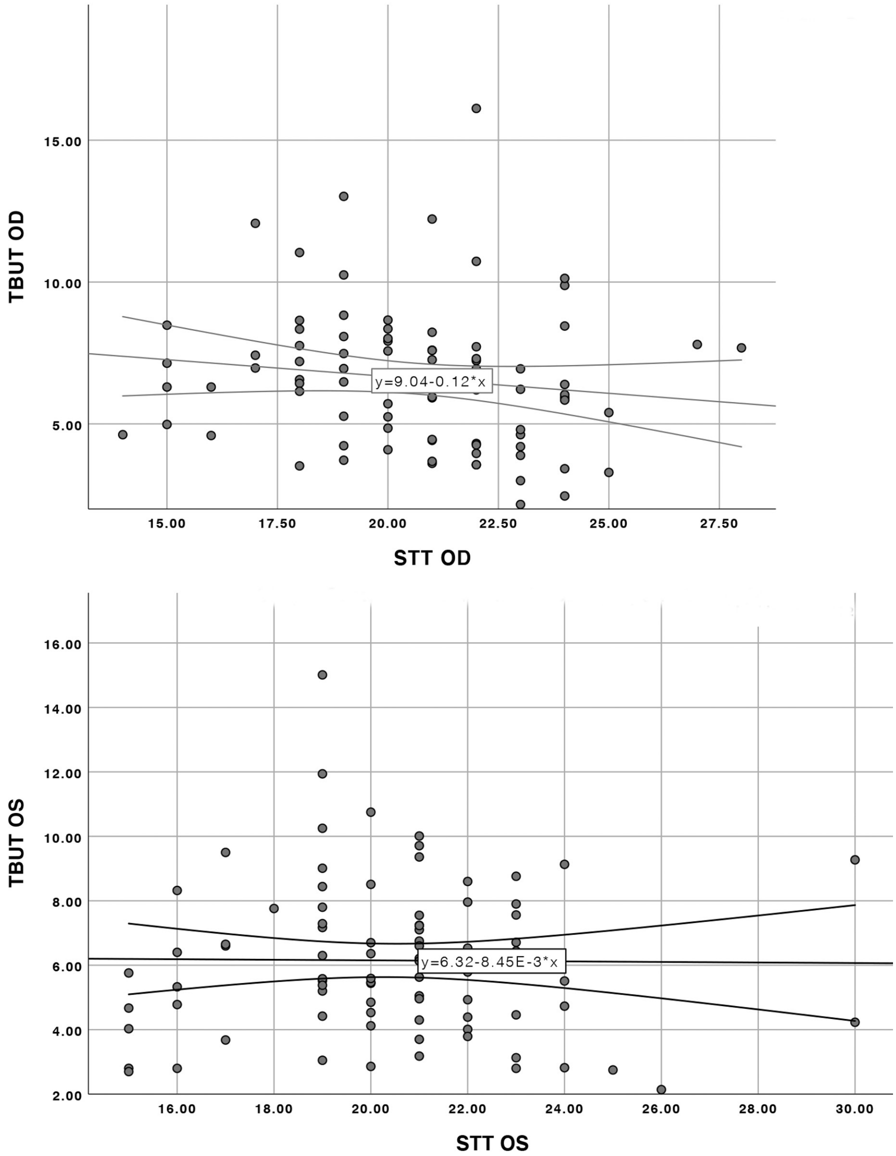

FIGURE 1 Scatter plot of TBUT by STT in right (OD) and left (OS) eyes

An additional factor affecting TBUT is the observer. In a study on TBUT in dogs, poor to moderate reliability was reported in 21 dogs and a difference of up to 1.7 s was seen between the values recorded by two observers.[13]

Schirmer tear test showed no statistically or clinically significant differences between brachycephalic and non-brachycephalic breeds. The STT values obtained in this study were within the normal reference interval of STTs reported for dogs (15–28 mm/min).[11] In a study comparing tear production between brachycephalic and non-brachycephalic dogs, the STT-1 values were reported to be significantly higher in non-brachycephalic dogs.[7] In cats, a brachycephalic breed (Persian) has been reported to have higher tear production compared to domestic short-haired cats.[22]

In the present study, no significant correlation was observed between STT and TBUT in dogs. This finding was in agreement with the results of a study in which no correlation was found between TBUT and STT in cats experimentally infected with FHV-1.[15] Sex and reproductive status had no effect on STT and TBUT in the studied dogs. This finding was in line with a previous report on dogs.[23] In cats, sex is reported to have no effect on STT, whereas neuter status has been suggested to have an impact on STT.[22]

Even age had no significant correlation with TBUT and STT, but a statistically insignificant increase in age was associated with a decrease in TBUT and STT in the dogs examined in this study. This finding was consistent with the significant reduction in STT in dogs with the increase of age.[23] Hartley et al. revealed a 0.4-mm decrease in STT for each year of increase in age.[23] In cats, age is reported to show a weak correlation with TBUT.[24] Kittens are shown to have higher STT values than adult cats[22]; however, age has not been reported as an effective factor on STT among adult cats.[24]

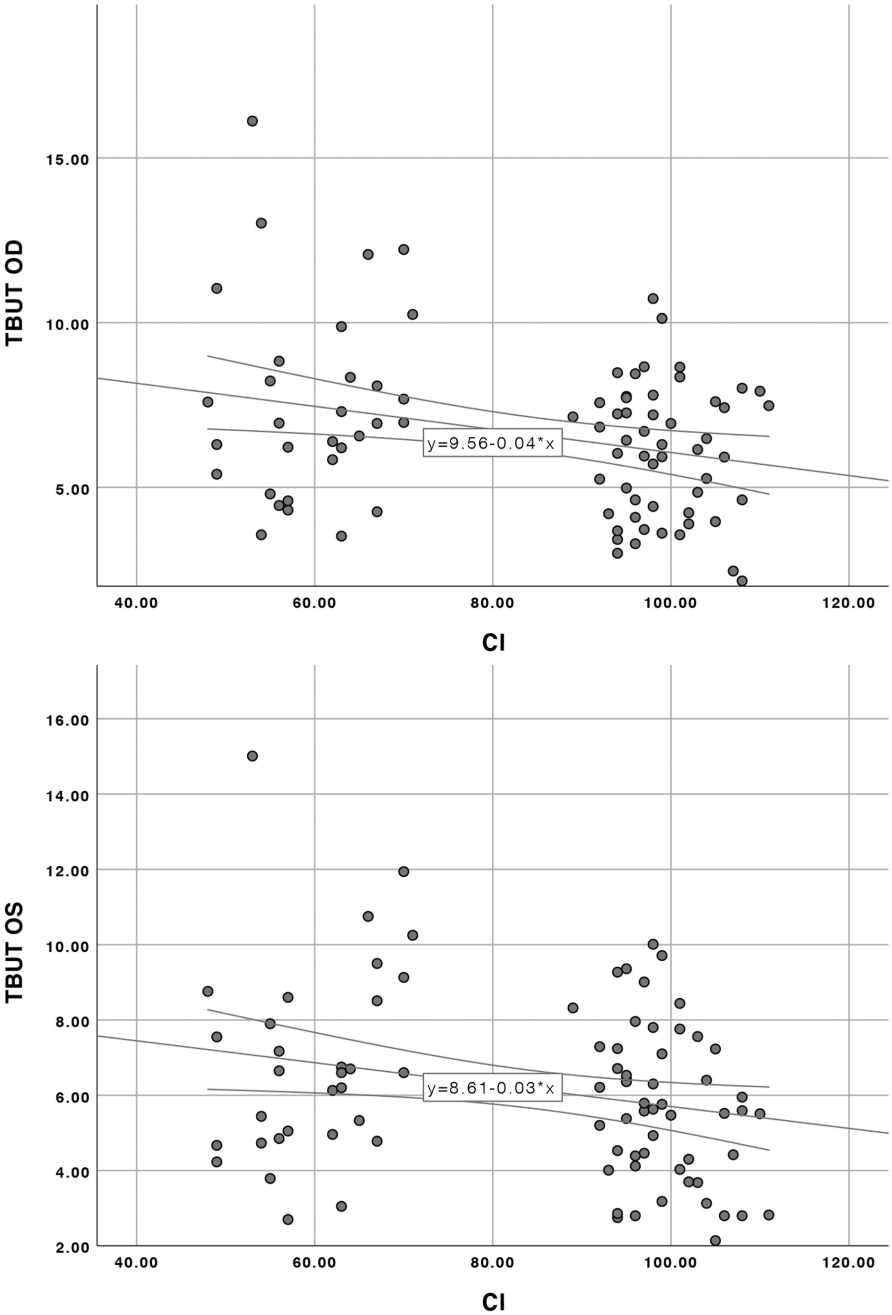

FIGURE 2 Scatter plot of TBUT by cephalic indices (CI) in right (OD) and left (OS) eyes

In this study, CI had a negative correlation with TBUT, but there was no correlation between CI and STT. Bolzanno et al. indicated a significant negative correlation between CI and phenol red thread as well as CI and STT-2, but not between CI and STT-1.[7]

Nasolacrimal transit time depends on the skull type, muzzle length, age, and reproductive status in dogs.[25] In humans, the applied fluorescein volume has been reported to have a significant influence on the nasolacrimal transit time.[26] One drop of fluorescein had a transit time of 8 min while multiple drops led to a 1.4-min transit time.[26] Normal transit time of fluorescein in dogs has been reported to be from 30s to 10 min.[11] Fluorescein solution has been reported to cause a faster transit time of fluorescein through NLD compared to the fluorescein strip.[25]

A comparative computed tomographic analysis of brachycephalic breeds has demonstrated malformation, U- to V-shaped appearance, and reduction in the length of the nasolacrimal system.[27] In brachycephalic cats, steeper alignment of NLD along with a lack of accessory orifices was reported in an anatomical study.[28]

Interferometry is a useful method for the assessment of the lipid layer thickness.[11] All the studied dogs in the present study were classified as grade 1 and 2 in interferometry. It means that the maximum lipid layer thickness was 30nm based on the pattern presented by the manufacturer of the interferometry device (Figure 4). In a study by Viñas et al.[5]

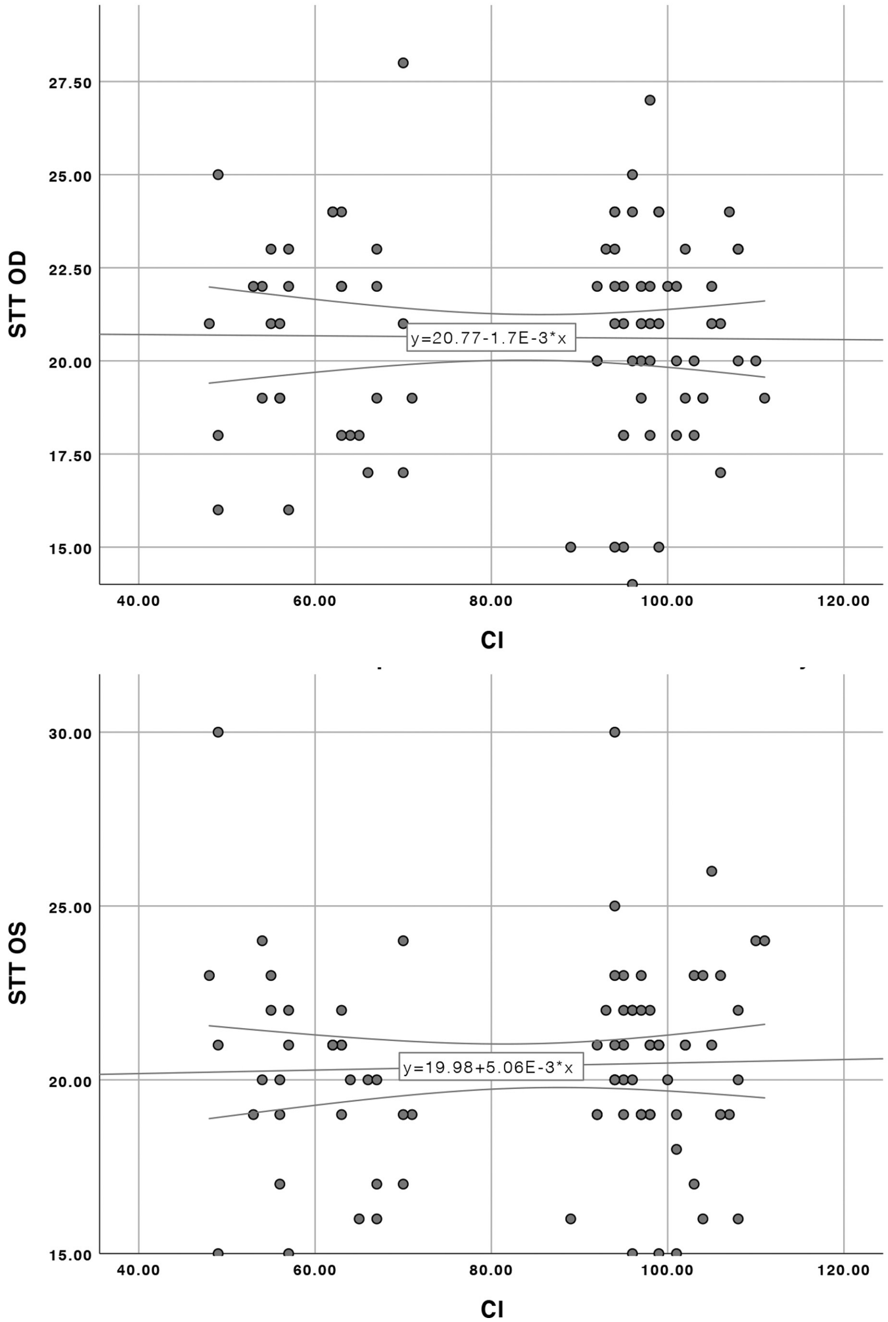

FIGURE 3 Scatter plot of STT by cephalic indices (CI) in right (OD) and left (OS) eyes

on dogs with ocular surface disorders, 23 eyes of brachycephalic dogs had <15 nm lipid layer thickness, 21 eyes had 15–30 nm lipid layer thickness, 9 had 31–60 nm lipid layer thickness, 3 had 61–100 nm lipid layer thickness, and the remaining 3 eyes had more than 100 nm lipid layer thickness. In the present study, none of the examined dogs had any ocular surface disorders, but all the brachycephalic dogs had a maximum of 15 nm lipid layer thickness.

In normal beagle dogs, the results of interferometry were remarkably different from this study. All the studied beagles had a lipid layer thickness between 80 and 140 nm.[29]

Despite the fact that there was no significant correlation between TBUT and STT in the dogs, most of the individual eyes with accelerated TBUT had higher STT values. This finding could be attributed to the compensatory increase in the aqueous portion of TF in case of lipid layer deficiency due to meibomian gland dysfunction, which was previously reported in humans.[30,31]

There were some limitations in this study. The transit time of fluorescein was not available in the recorded data. Moreover, meibography data were not available for most of the dogs; therefore, they were omitted from the datasheet.

The authors of this paper presented ocular findings that may raise concerns regarding the ocular health of the examined dogs. Deep corneal ulceration[32] and persistent corneal irritation are common findings in brachycephalic

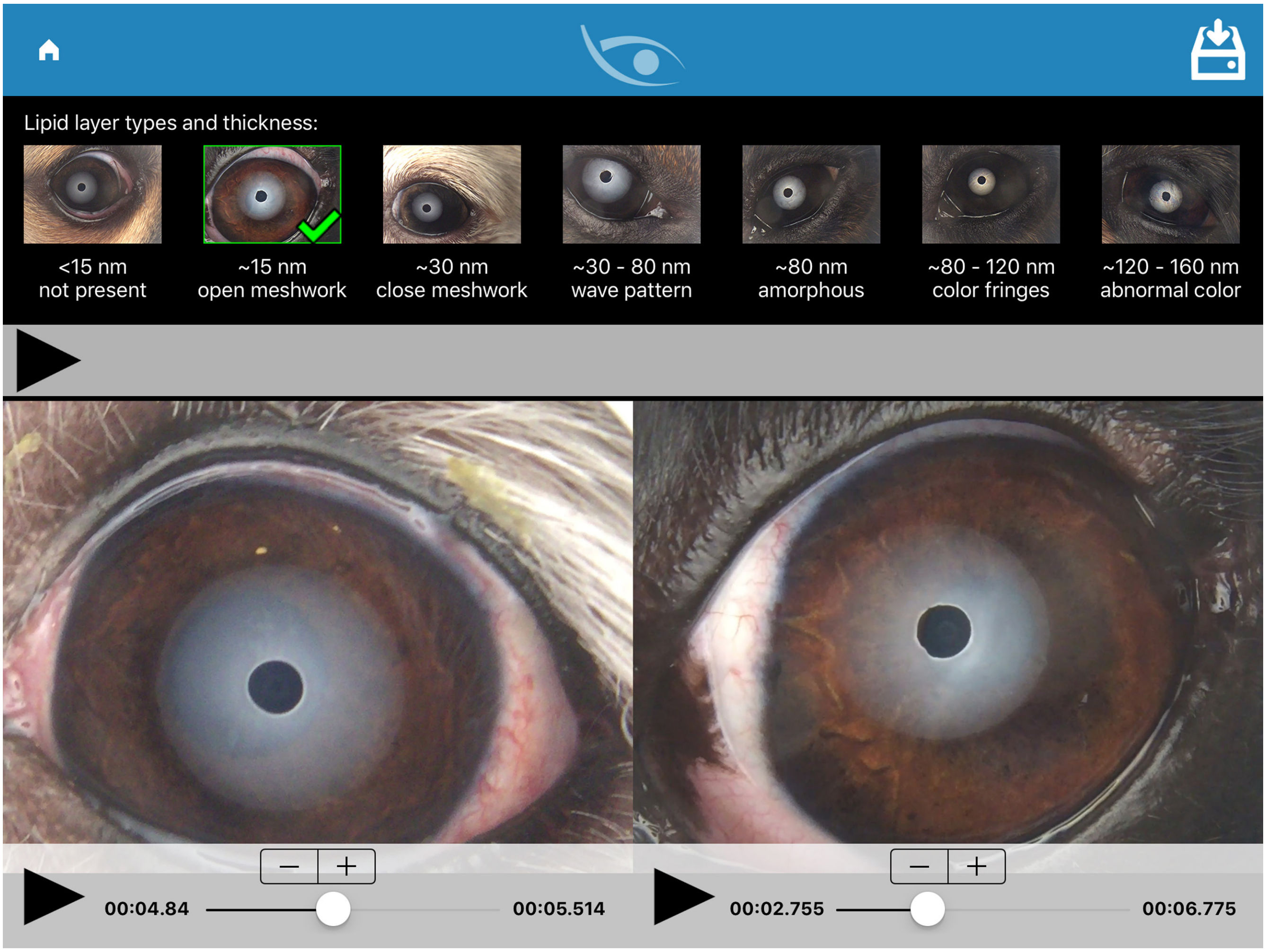

FIGURE 4 Software environment (SBM Sistemi, Italy) and the results of interferometry in a dog examined in this study. The left image belongs to an examined dog while the right image is the default pattern

dogs due to the unique anatomic features (such as facial folds and entropion) of these breeds.[11,32] Furthermore, long-term consequences (vortex keratopathy and pigmentary keratitis) of the ocular anomalies have been reported in Shihtzu dogs.[33] Trichiasis and NLD blockage are common anatomical variations in these dogs.[7,10,11] However, dense pigmentary keratitis could likely influence the results of TBUT, and this possibility should not be ignored.

In conclusion, the results of this study demonstrated accelerated TBUT in brachycephalic breeds compared to non-brachycephalic breeds; however, this difference seemed clinically insignificant. Age, sex, reproductive status, and NLD patency had no effects on mean TBUT and STT. A compensatory increase in STT values was observed in dogs with accelerated TBUTs.

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REFERENCES

1. Giuliano EA. Diseases and surgery of the canine lacrimal secretory system. In: Gelatt KA, ed. *Veterinary Ophthalmology*. 6th ed. John Wiley & Sons, Inc; 2021:1008-1044 chap 17.
2. Tiffany JM. The normal tear film. *Dev Ophthalmol*. 2008;41:1-20. doi:10.1159/000131066
3. Leiva M, Peña Giménez T. Diseases of the lacrimal system. In: Maggs DJ, Miller PE, Ofri R, eds. *Slatter's Fundamentals of Veterinary Ophthalmology*. 6th ed. Elsevier; 2018:186-212: chap 9.
4. Williams DL. Immunopathogenesis of keratoconjunctivitis sicca in the dog. *Vet Clin North Am Small Anim Pract*. 2008;38(2):251-268, vi. doi:10.1016/j.cvsm.2007.12.002
5. Viñas M, Maggio F, D'Anna N, Rabozzi R, Peruccio C. Meibomian gland dysfunction (MGD), as diagnosed by non-contact infrared Meibography, in dogs with ocular surface disorders (OSD): a retrospective study. *BMC Vet Res*. 2019;15(1):443. doi:10.1186/s12917-019-2203-3
6. Evans HE, de Lahunta A. The skeleton. In: Evans HE, de Lahunta A, eds. *Miller's Anatomy of the Dog*. 4th ed. Saunders; 2013:80-156 chap 4.

7. Bolzanni H, Oriá AP, Raposo ACS, Sebbag L. Aqueous tear assessment in dogs: impact of cephalic conformation, inter-test correlations, and test-retest repeatability. *Vet Ophthalmol*. 2020;23(3):534-543. doi:10.1111/vop.12751
8. O'Neill DG, Pegram C, Crocker P, Brodbelt DC, Church DB, Packer RMA. Unravelling the health status of brachycephalic dogs in the UK using multivariable analysis. *Sci Rep*. 2020;10(1):17251. doi:10.1038/s41598-020-73088-y
9. Ambrósio CE, Packer RMA, Hendricks A, Burn CC. Impact of facial conformation on canine health: corneal ulceration. *Plos One*. 2015;10(5):e0123827. doi:10.1371/journal.pone.0123827
10. Gyles C. Brachycephalic dogs – time for action. *Can Vet J*. 2017;58(8):777-780.
11. Featherstone HJ, Heinrich CL. Ophthalmic examination and diagnostics. In: Gelatt KA, ed. *Veterinary Ophthalmology*. 6th ed. John Wiley & Sons, Inc; 2021:564-777 chap 10.
12. Palmer S, Ramos RV, Rodriguez Galarza RM. Clinical comparison of tear film breakup time measurements in normal dogs using three different methods of fluorescein solution administration. *Vet Ophthalmol*. 2021;24:503-508. doi:10.1111/vop.12933
13. Seyer LD, Wills RW, Betbeze CM. Investigation of fluorescein stain–based tear film breakup time test reliability in dogs in a clinical setting. *Am J Vet Res*. 2021;82(12):1019-1025. doi:10.2460/ajvr.21.01.0002
14. Monclin SJ, Farnir F, Grauwels M. Determination of tear break-up time reference values and ocular tolerance of tetracaine hydrochloride eyedops in healthy horses. *Equine Vet J*. 2011;43(1):74-77. doi:10.1111/j.2042-3306.2010.00119.x
15. Lim CC, Reilly CM, Thomasy SM, Kass PH, Maggs DJ. Effects of feline herpesvirus type 1 on tear film break-up time, Schirmer tear test results, and conjunctival goblet cell density in experimentally infected cats. *Am J Vet Res*. 2009;70(3):394-403. doi:10.2460/ajvr.70.3.394
16. Cullen CL, Njaa BL, Grahn BH. Ulcerative keratitis associated with qualitative tear film abnormalities in cats. *Vet Ophthalmol*. 1999;2(3):197-204. doi:10.1046/j.1463-5224.1999.00082.x
17. Davis K, Townsend W. Tear-film osmolarity in normal cats and cats with conjunctivitis. *Vet Ophthalmol*. 2011;14:54-59. doi:10.1111/j.1463-5224.2011.00916.x
18. Grahn BH, Sisler S, Storey E. Qualitative tear film and conjunctival goblet cell assessment of cats with corneal sequestra. *Vet Ophthalmol*. 2005;8(3):167-170. doi:10.1111/j.1463-5224.2005.00362.x
19. Cullen CL, Lim C, Sykes J. Tear film breakup times in young healthy cats before and after anesthesia. *Vet Ophthalmol*. 2005;8(3):159-165. doi:10.1111/j.1463-5224.2005.00347.x
20. Jha KN. Tear break-up time in high altitude areas. *Med J Armed Forces India*. 2009;65(1):2-3. doi:10.1016/s0377-1237(09)80042-7
21. Berg EJ, Ying G-s, Maguire MG, et al. Climatic and environmental correlates of dry eye disease severity: a report from the dry eye assessment and management (DREAM) study. *Transl Vis Sci Technol*. 2020;9(5):1-11. doi:10.1167/tvst.9.5.25
22. Rajaei SM, Faghihi H, Williams DL, Aftab G. Evaluation of tear production using the Schirmer tear test I in healthy cats; effect of age, life stage, sex, breed and neuter status. *Vet Rec*. 2019;184(26):799. doi:10.1136/vr.104974
23. Hartley C, Williams DL, Adams VJ. Effect of age, gender, weight, and time of day on tear production in normal dogs. *Vet Ophthalmol*. 2006;9(1):53-57. doi:10.1111/j.1463-5224.2005.00437.x
24. Sebbag L, Kass PH, Maggs DJ. Reference values, intertest correlations, and test-retest repeatability of selected tear film tests in healthy cats. *J Am Vet Med Assoc*. 2015;246(4):426-435. doi:10.2460/javma.246.4.426
25. Binder DR, Herring IP. Evaluation of nasolacrimal fluorescein transit time in ophthalmically normal dogs and nonbrachycephalic cats. *Am J Vet Res*. 2010;71(5):570-574. doi:10.2460/ajvr.71.5.570
26. Tucker NA, Codère F. The effect of fluorescein volume on lacrimal outflow transit time. *Ophthal Plast Reconstr Surg*. 1994;10(4):256-259. doi:10.1097/00002341-199412000-00006
27. Ambrósio CE, Sahr S, Dietrich A, Oechtering G. Evaluating malformations of the lacrimal drainage system in brachycephalic dog breeds: a comparative computed tomography analysis. *Plos One*. 2021;16(9):e0257020. doi:10.1371/journal.pone.0257020
28. Breit S, Kunzel W, Oppel M. The course of the nasolacrimal duct in brachycephalic cats. *Anat Histol Embryol*. 2003;32(4):224-227. doi:10.1046/j.1439-0264.2003.00464.x
29. Kim Y, Kang S, Kim S, Shim J, Go S, Seo K. Reference values for selected dry eye tests in normal beagle dogs: a pilot study. *J Vet Sci*. 2022;23(1):e10. doi:10.4142/jvs.21169
30. Arita R, Morishige N, Koh S, et al. Increased tear fluid production as a compensatory response to meibomian gland loss. *Ophthalmology*. 2015;122(5):925-933. doi:10.1016/j.ophtha.2014.12.018
31. Inaba T, Tanaka Y, Tamaki S, Ito T, Ntambi JM, Tsubota K. Compensatory increases in tear volume and mucin levels associated with meibomian gland dysfunction caused by stearoyl-CoA desaturase-1 deficiency. *Sci Rep*. 2018;8(1):3358. doi:10.1038/s41598-018-21542-3
32. Palmer SV, Espinheira Gomes F, McArt JAA. Ophthalmic disorders in a referral population of seven breeds of brachycephalic dogs: 970 cases (2008–2017). *J Am Vet Med Assoc*. 2021;259(11):1318-1324. doi:10.2460/javma.20.07.0388
33. Kim S, Thomasy SM, Ramsey D, Zhao M, Mannis MJ, Murphy CJ. Whorl pattern keratopathies in veterinary and human patients. *Vet Ophthalmol*. 2018;21(6):661-667. doi:10.1111/vop.12552

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