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Evaluating the aqueous portion of tears in shih tzu dogs: comparing Schirmer tear test 1 with tear meniscus height

[Avaliação da porção aquosa da lágrima em Shih-Tzu: comparação do teste lacrimal de Schirmer 1 com a altura do menisco lacrimal]

P.H.S. *Silva*[1], L.K.S. *Cruz*[2], B.M. *Ferreira*[2], M.S.A. *Falcão*[2], A.R.C. *Barreto-Vianna*[3], E.M.M. *Lima*[1*]

[1]Universidade de Brasília, Brasília, DF, Brasil
[2]Practictioner, Brasília, DF, Brasil
[3]Universidade Federal do Paraná, Curitiba, PR, Brasil

ABSTRACT

The aqueous portion of the tear film has been characterized as an essential structure for ocular surface health. Among various assessment tests, some measure the aqueous portion, such as Schirmer Tear Test 1, Meniscometry using a strip, and the test to determine Tear Meniscus Height (TMH). Conversely, few studies have specifically evaluated tear meniscus height, especially those utilizing ocular surface analyzers. Therefore, the aim of this study was to comparatively evaluate the results obtained from employing Tear Meniscus Height (TMH) using an Ocular Surface Analyzer and Schirmer Tear Test 1 (STT-1) in Shih Tzu dogs. To achieve this, the eyes of 18 brachycephalic Shih Tzu dogs of both sexes and varying ages were assessed and compared. The results revealed that the TMH for the right eye was 0.59±0.29mm, and for the left eye was 0.88±0.27mm; p=0.0135. For STT-1, it was found that the right eye had a value of 19.06±4.87mm/min, and the left eye had a value of 23.11±4.80mm/min; p=0.0238. Furthermore, the Pearson correlation test applied between TMH and STT-1 yielded the following results for the right eye (r=0.601; p=0.008) and the left eye (r=-0.032; p=0.8979). The TMH shows potential as an alternative to STT-1 for evaluating the aqueous component of tear film in a similar manner. However, further research is warranted, particularly for establishing reference values for TMH and conducting a comprehensive comparison between STT-1 and TMH. This research is especially important for gathering information that can contribute to the understanding of ophthalmic and systemic conditions that directly influence the physiological composition of tears. Critical for establishing the values obtained when measuring the canine lacrimal meniscus using different techniques is the consideration of associated functional factors.

Keywords: cornea, eye, brachycephalic, ophthalmology

RESUMO

A porção aquosa do filme lacrimal tem sido caracterizada como uma estrutura essencial para a saúde da superfície ocular. Entre vários testes de avaliação, alguns medem a porção aquosa, como o teste lacrimal de Schirmer 1, a meniscometria usando uma tira, e o teste para determinar a altura do menisco lacrimal (TMH). Por outro lado, poucos estudos avaliaram especificamente a altura do menisco lacrimal, especialmente aqueles que utilizam analisadores de superfície ocular. Portanto, o objetivo deste estudo foi avaliar comparativamente os resultados obtidos ao empregar a altura do menisco lacrimal (TMH) usando um analisador de superfície ocular, e o teste lacrimal de Schirmer 1 (STT-1), em cães Shih-Tzu. Para isso, os olhos de 18 cães Shih-Tzu braquiocefálicos, de ambos os sexos e de idades variadas, foram avaliados e comparados. Os resultados revelaram que a TMH, para o olho direito, foi de 0,59±0,29 mm e, para o olho esquerdo, foi de 0,88±0,27mm; P=0,0135. Para o STT-1, verificou-se, no olho direito, 19,06±4,87mm/min e, no olho esquerdo, 23,11±4,80 mm/min; P=0,0238. Além disso, o teste de correlação de Pearson aplicado entre TMH e STT-1 produziu os seguintes resultados para o olho

*Corresponding author: limaemm@unb.br
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direito (r=0,601; P=0,008) e para o olho esquerdo (r=-0,032; P=0,8979). A TMH mostra potencial como um teste alternativo ao STT-1 para avaliar a porção aquosa do filme lacrimal de maneira semelhante. No entanto, mais pesquisas serão necessárias, especialmente para estabelecer valores de referência para a TMH e realizar uma comparação abrangente entre o STT-1 e a TMH. Esta pesquisa reuniu informações que contribuíram para a compreensão de condições oftálmicas e sistêmicas que afetam diretamente a composição fisiológica das lágrimas. Determinante para estabelecimento, dos valores obtidos ao medir o menisco lacrimal em cães por diferentes técnicas, além de considerar fatores funcionais associados.

Palavras-chave: córnea, olho, braquiocefálico, oftalmologia

INTRODUCTION

The preocular tear film is a complex structure that is essential for ocular surface health once it has functions such as being a refracting surface for the entrance of light, moisturizing the cornea, protecting the eye from external agents, and giving nutrients to the cornea (Maggio, 2019). The preocular film comprises the outer lipid layer and the inner aqueous-mucinous layer. The aqueous portion of the aqueous-mucinous layer is the thicker part of the precorneal tear film and comprises 98% water and 2% solids (Featherstone and Heinrich, 2021). It is produced by the lacrimal and nictitating glands (Zwingenberger *et al.*, 2014; Maggio, 2019).

The aqueous portion of the aqueous-mucinous layer has essential functions, such as providing glucose and oxygen to the metabolic processes of the cornea, and has important components such as immunoglobulins and glycoproteins, giving protection to the eye (Hartley *et al.*, 2006; Chandler *et al.*, 2013; Samuelson, 2014; Maggio, 2019). The aqueous part of the tear film (quantitative measure) can be evaluated using the Schirmer tear test 1 (STT-1). The test measures the basal tear secretion and reflex tearing by inserting a folded absorbent strip in the lateral conjunctival fornix for one minute (Samuelson, 2014).

However, it is known that STT-1 is considered an invasive test, and reflex tears can influence it (Hartley *et al.*, 2006; Featherstone and Heinrich, 2021). Another way to estimate the aqueous portion is by evaluating the lacrimal meniscus. The Strip Meniscometry Test (SMT) uses a strip that absorbs tears by capillary action (Miyasaka *et al.*, 2019; Bolzanni *et al.*, 2020).

Recent studies showed that SMT has high sensitivity and specificity compared to STT-1 (Nascimento *et al.*, 2023). However, another way of examining the meniscus is by measuring the Tear Meniscus Height (TMH) using an Ocular Surface Analyzer (Viñas *et al.*, 2019). This is a minor invasive way of assessing TMH.

Nevertheless, there are few works regarding the use of ocular surface analyzers, especially related to the standardization of the TMH. Thus, the aim of this study was to evaluate the TMH using an Ocular Surface Analyzer (OSA), and to compare and analyze if the STT-1.

MATERIAL AND METHODS

For comparison of metric data obtained from the use of Tear Meniscus Height (TMH) using an Ocular Surface Analyzer and Schirmer Tear Test 1 (STT-1), 18 Shih Tzu dogs were evaluated. All were healthy, without ophthalmic disorders or any other systemic conditions. The dogs included both sexes, with 12 females and six males, with an average age of 4.72±3.86 years.

All of them were examined and considered clinically healthy before the evaluation. Before the analysis of TMH and STT-1, the dogs were subjected to complete ophthalmic evaluation (Viñas *et al.*, 2019):

Schimer tear test (Ophthalmos®, São Paulo, BR);
Rebound tonometry (Tonovet, Icare ® Finland, FI; Reichert Techonologies® Tonovera® Vet, New York Depew, USA);

Biomicroscopy by slit lamp (Keeler®; PSL Classic, Windsor, Berkshire, UK), posterior segment evaluation of lens, vitreous and retina (Hillrom®, WelchAllyn® PanOptic ®, Amsterdam, NE);

Fluorescein dye (Ophthalmos®, São Paulo, BR).

Schirmer tear test values were computed in mm/min (Featherstone and Heinrich, 2021). Since the test measures the aqueous portion of the tear, the tests were conducted 10 minutes apart, to allow the lacrimal turnover (Fetaherstone and Heinrich, 2021), in the same environment, with controlled temperature (25°C) and control of humidity.

The same veterinarian analyzed the TMH to complete the ophthalmic examination (Viñas *et al*., 2019; Kim *et al*., 2021). To access the TMH, an ocular surface analyzer was used (OSA-VET®, SBM Sistemi, Torino, Italy), connected to a notebook (Lenovo® Ideapad 10210U) with ICP Medical System for Windows (SBM Sistemi, Medical System® For Windows, Torino, Italy). The probe of the ocular surface analyzer was positioned at a safe distance from the cornea of the patient. Thus, images from the inferior tear meniscus were taken (Kim *et al.*, 2021). The measures were taken following the guidelines from the manufacturer (Peruccio *et al.*, 2021), with a previous calibration serving as a reference for the measure of TMH. This calibration consists of measuring a known circle, the circle of the probe projected on the ocular surface, and using its dimension to calibrate de program (Figure 1). After that, two anchor points were positioned in the inferior and superior delimitation of the TMH (Peruccio *et al.*, 2021). Thus, the value of the TMH was computed in millimeters (mm).

The data underwent a Shapiro-Wilk normality test. The results were presented as mean ± standard deviation for TMH (mm) and STT-1 (mm/min). The outcomes obtained from the application of the paired t-test, regarding the measurements obtained through TMH and STT-1 (Figure 2), were significant at $p<0.05$, comparing the right and left eyes. The data were also subjected to Pearson correlation analysis, assessing the measurements obtained between STT-1 (mm/min) and TMH (mm) in Shih tzu dogs for the right and left eyes (Figure 3). Data were analyzed using Prism (Prism®, GraphPad Software, version 6.01 for Windows, 2012, La Jolla, CA, USA).

RESULTS

All the patients were clinically and ophthalmologically healthy, after the clinical and ophthalmological exam.

The results obtained from the application of the paired t-test, regarding the measurements obtained through TMH and STT-1 (Figure 2), showed that for the right eye, TMH was 0.59±0.29mm, and for the left eye, it was 0.88±0.27mm, with p=0.0135. For STT-1, the values observed for the right eye were 19.06 ± 4.87 mm/min, and for the left eye, they were 23.11±4.80mm/min, with p=0.0238.

The Pearson correlation test (Figure 3) was applied between TMH and STT-1. The values obtained for the right eye were (r=0.601; p=0.008), and for the left eye, they were (r=-0.032; p=0.8979).

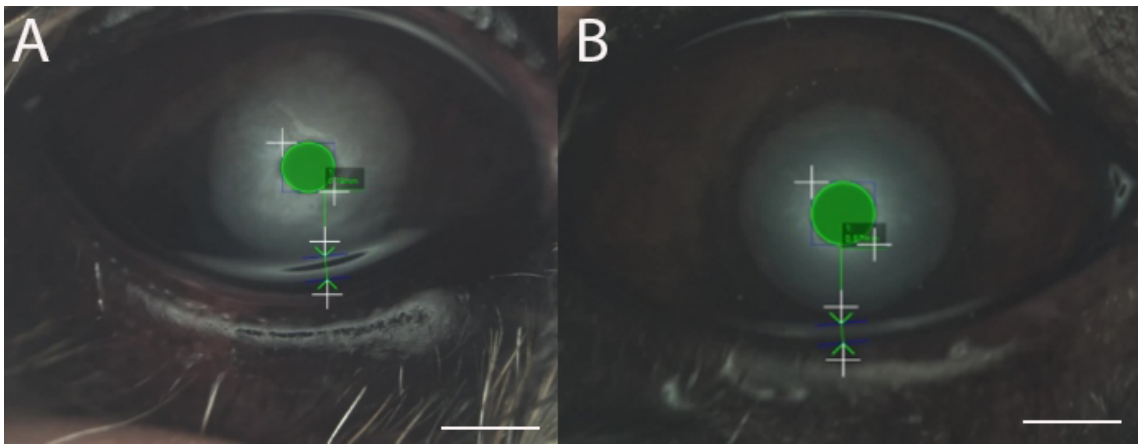

Figure 1. Measurement of TMH in Shih tzu dogs, right eye (A) and left eye (B). Two points are positioned, one at the superior, and another on the inferior meniscus limit. Then, a calibration is made in the center of the disk, and the value of the tear meniscus height is calculated.

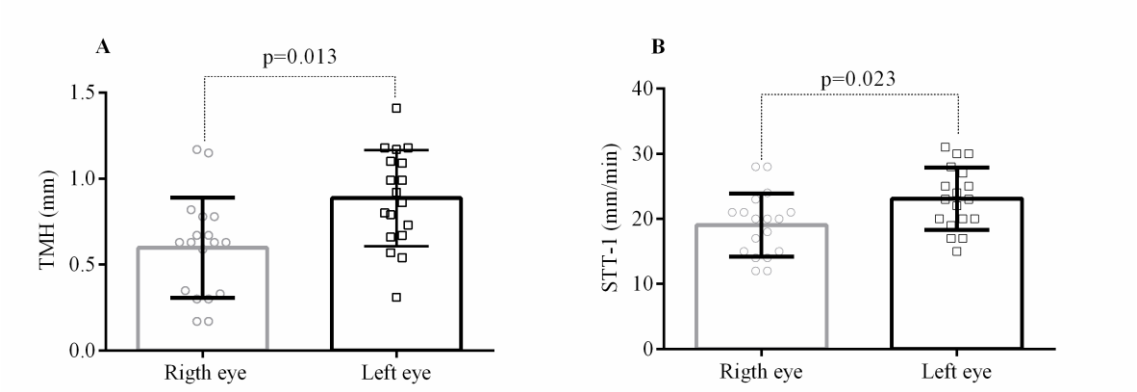

Figure 2. Graphical representations of the values obtained in Shih Tzu dogs, corresponding to the values obtained and submitted when applying the paired t-test, $p<0.05$. (A) TMH values (mm) for the right and left eyes, $p=0.013$. (B) STT-1 values (mm/min) for the right and left eyes, $p=0.023$.

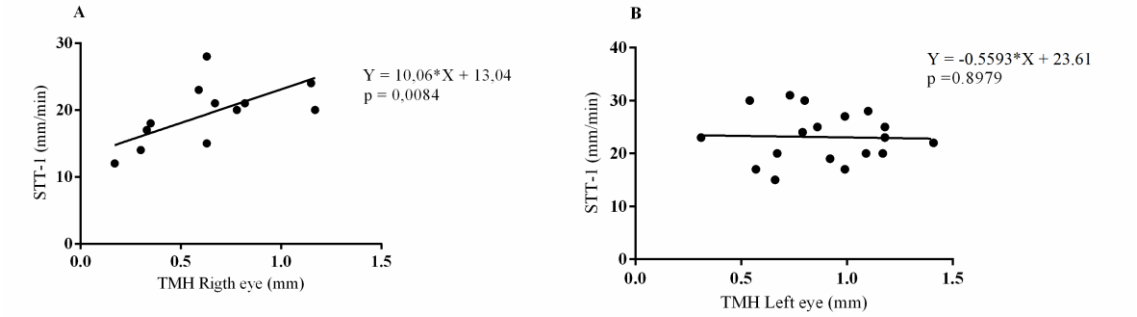

Figure 3. Graphical representations of the values obtained when applying the Pearson correlation test between STT-1 (mm/min) and TMH (mm) in Shih Tzu dogs for the right eye (A) and left eye (B).

DISCUSSION

The tear meniscus has been reported as a source of 75-90% of the aqueous tear volume, and has been positively correlated with the lacrimal secretory rate (Rajaei *et al.*, 2017). To this statement, tests using this source for evaluation could work as an alternative source for the STT-1 tests. Similarly, substantial efforts should be undertaken to identify potential correlations between them, to gain a deeper understanding of the lacrimal meniscus dynamics and its relationships with ocular conditions. Furthermore, this will facilitate advancements in comprehending the dynamics of the meniscus and its variations. It has been referred that STT-1 can cause more irritation, due to its long duration (60 seconds), with activation of the afferent and efferent ways that stimulate the lacrimal glands (Bolzanni *et al.*, 2020).

The strip meniscometry (SM) is one form of accessing this lacrimal volume of the meniscus, in a five second test, by touching a strip in the lower tear meniscus (Dogru *et al.* 2006; Bolzanni *et al.*, 2020; Featherstone and Heinrich, 2021). The SM has been correlated with the STT-1 in dogs (brachycephalic and non-brachycephalic), with and without keratoconjunctivitis sicca (KCS) (Rajaei *et al.*, 2017; Miyasaka *et al.*, 2019; Bolzanni *et al.*, 2020; Nascimento *et al.*, 2023; Sebbag *et al.*, 2023). However, the SM still needs the touching of the lower meniscus, also being invasive in a certain way. Also, the works regarding the correlations between SM and STT-1 are far away from conclusions, with positive and negative correlations being found, no linear regressions being performed, and doubts about significance (Bolzanni *et al.*, 2020; Nascimento *et al.*, 2023; Sebbag *et al.*, 2023).

Thus, another form for access should be considered since there are conflicting results regarding the SM way of accessing the tear meniscus. The evaluation of the lacrimal meniscus using an ocular surface analyzer gives the opportunity to obtain images from the inferior tear meniscus and measure its height directly, in a less invasive form (Peruccio *et al.*, 2021). This kind of access also includes the opportunity to show the owner what we are investigating properly. Another advantage of the OSA, is that the TMH is measured manually, making it a more accurate test (Sánchez-González *et al.*, 2022).

Regarding the TMH in this work, a positive and significant correlation was found between STT-1 and TMH, a result that was not attested by Kim *et al.* (2021) in healthy beagle dogs. It's important to consider this kind of correlation, in order to attest if the tests can walk in the same direction and evaluate if one can predict another. However, in the present study, the mathematical model of linear regression was weak in explaining the inter-test behavior, with the TMH explaining only 36% of de STT-1, and more studies regarding the relationship between the tests must be conducted. Alternatively, when brachycephalic, mesocephalic, and dolichocephalic breeds were grouped together, there was no statistically significant effect of breed (Chandler *et al.*, 2013).

A mean value of 0.53±0.11mm was proposed for TMH in dogs, but there was no distinction between breeds or skull conformation in this attempt to set a mean interval of reference (Chandler *et al.*, 2013; Peruccio *et al.*, 2021). Then, no comparisons were made between our results for TMH and the above-proposed reference value, once it was not specified the breed, if the dogs were healthy, craniofacial conformation, or the number of dogs. We observed a significant decrease in lacrimal production values in canine patients hospitalized in an intensive care unit (Chandler *et al.*, 2013). However, no significant differences were identified in lacrimal production associated with specific pathological processes (Chandler *et al.*, 2013). These values tend to decrease over the years in dogs (Hartley *et al.*, 2006).

In healthy beagle dogs, the TMH showed mean values of 0.41±0.21mm (Kim *et al.*, 2021). The values of TMH for Shih tzu dogs in our work were higher than beagle dogs (Kim *et al.*, 2021). However, because beagles are different from Shih tzu dogs, regarding their cranial conformation, for purposes of comparison, this characteristic must be considered (Featherstone and Heinrich, 2021).

The brachycephalic characteristics, present in Shih tzu breeds, like the exophthalmos condition, associated with blinking abnormalities (lagophthalmos), can result in tear film abnormalities in the cornea, and can lead to the incorrect distribution of the aqueous layer in it (Bolzanni *et al.*, 2020; Featherstone and Heinrich, 2021; Nutbrown-Hughes, 2021). Associated with the other layers-mucinous and lipids- the aqueous layer can accumulate or evaporate in the wrong way and give the false sensation of a higher or lower tear meniscus, due to the non-adherence to the cornea or the early evaporation of the aqueous layer (Featherstone and Heinrich, 2021). Also, it is known that Shih tzu dogs present a diminished quality of tear film (Sebbag *et al.*, 2023). Thus, works using OSA also must consider these particularities regarding conformational characteristics. Above all, changes resulting from cranial conformation have continued to generate speculations. Therefore, further investigations, particularly those focused on different canine breeds, will be required. These efforts aim to elucidate the factors responsible for the reduction in tear production, whether of primary or secondary origin.

We found that the TMH can be a good alternative way to access the lacrimal volume of the aqueous layer, being correlated with STT-1 and with a good prediction of the behavior of the last one. Although, other studies must be conducted to evaluate the correlations between the tests, and to investigate if there are differences between skull conformations and breeds.

CONCLUSIONS

The TMH could serve as an alternative test to STT-1, as it appears to assess the aqueous component of tears in a similar manner. However, further research is needed, particularly in terms of establishing reference values for TMH and conducting a comprehensive

comparison between STT-1 and TMH. This is especially important for gathering information that can contribute to the understanding of ophthalmic and systemic conditions that directly affect the physiological composition of tears. Such conditions can have a direct impact on the values obtained when measuring the lacrimal meniscus in dogs using different techniques, while also considering the associated functional factors.

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