Measurement of tear osmolarity in the canine eye: a new diagnostic tool for canine keratoconjunctivitis sicca?

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Research Article

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ABSTRACT

In this study the osmolarity of the canine tear film as measured by the TearLab micro-osmometer was compared with the Schirmer Tear Test I (STT) in 100 dogs from the patient population at the Queen's Veterinary School hospital, University of Cambridge and a UK animal rehoming facility. 153 eyes were ophthalmoscopically normal and 47 had varying degrees of keratoconjunctivitis sicca (KCS) as determined by STT values below 15mm/min.

Animals underwent a full ophthalmic examination and then were sampled using the TearLab device to measure tear osmolarity followed by a standard Schirmer tear test I. Tear osmolality in eyes with normal tear production was compared to that in eyes with KCS using the Student's T test. Correlation between STT and osmolarity data was investigated using the Pearson rank correlation coefficient. Tear osmolality was significantly higher in eyes with KCS (350 ± 27 mOsm) than in normal eyes (339 ± 23 mOsm) this difference significant at p<0.02. As high tear omolarity is considered to be a key factor in the pathogenesis of ocular surface damage in eyes with aqueous tear film deficiency in human patients, measuring tear film osmolarity in dogs with suspected KCS may be an important diagnostic step in these animals.

INTRODUCTION

Canine Dry Eye (Keratoconjunctivitis Sicca, or KCS) is a frequently encountered ophthalmological disease in veterinary practice [1]. It is normally diagnosed by a Schirmer tear test reading of less than 10 mm tear absorption by the test strip in one minute in the affected eye, together with suggestive clinical signs [2]. In acute cases these may include pain, a mucopurulent conjunctivitis, blepharospasm and conjunctival hyperaemia. In more chronic cases there may also be conjunctival hyperplasia, a pigmented or vascular keratitis and an irregular appearance to the corneal surface. Corneal ulceration is a frequent sequela, particularly in exophthalmic breeds, such as the Pug or Cavalier King Charles Spaniel, where an imperfect lid closure may exacerbate the problem. There are recognized breed dispositions, particularly in the West Highland White Terrier, Spaniel breeds, Dachshund and Yorkshire Terrier [1,3]. The most frequent cause of the syndrome in dogs is believed to be autoimmune destruction of the lacrimal gland, and as such the disease is often treated with topical immunosuppressive drugs e.g. cyclosporine (Optimumune, Schering Plough) [4].

In order for the health and function of the ocular surface to be maintained, it is necessary for a complete tear film to be produced. Thus any imbalance in production, retention or elimination of the tear film will alter the tear dynamics, producing dry eye disease [5]. A single measurement that shows the balance of inputs i.e. production and retention, and outputs i.e. drainage and evaporative loss in tear film dynamics is tear film osmolarity (Tomlinson et al., 2006). It has been suggested that tear hyperosmolarity is the main cause of discomfort, ocular surface damage and inflammation in dry eye disease [6]. This may be due to both direct effects on the surface corneal epithelial cells and the activation of an inflammatory cascade in corneal and conjunctival epithelial cells [7]. Until recently, measurement of tear osmolarity required a sample of 0.2 mL and the use of a freezing point depression osmometer [8]. However, the TearLab™ osmolarity system is a hand held device **(Figure 1)**, requiring only a minute tear sample of less than 50nl [9]. Using a disposable sampling tip with microchip it analyses the osmolarity of tears, providing an almost instantaneous reading in mOsm/l. A referent value of 316 mOsm/l has been suggested in Tomlinson and colleagues as a useful predictive value for dry eye in humans, following meta-analysis of primary literature [10].

The Schirmer tear test is the backbone of dry eye disease diagnosis in veterinary medicine. The method was first introduced in humans in 1903 and was standardized in 1941 with the use of the Whatman 41 filter paper strip [11]. Previously quoted Schirmer tear test values without anaesthesia in the dog are between 18.6 and 20.4 mm/min [12]. In human ophthalmology the

test is considered inaccurate and unrepeatable due to the production of reflex tearing, and because of the low volume of tears produced in one minute, necessitating the test to be extended for 5 minutes. It is claimed that it is not a sensitive test for dry eye as it measures tear production only, overlooking the evaporative aspect of dry eye disease and thus a battery of tests are generally used in investigating human dry eye patients [13].

The tear film has traditionally been considered as consisting of three discrete layers; an innermost mucin layer, an intermediate aqueous layer produced by the lacrimal glands and an outer lipid layer produced by the meibomian glands. This three-layered structure was first proposed by Wolff in 1946 [14], however, the more current model is that of a tear film consisting of an aqueous gel, with a gradient of mucin content decreasing from the ocular surface to the lipid layer [15]. There is thought to be some interaction between this lipid layer and the underlying aqueous to decrease the evaporative loss of tears and increase tear film stability [16].

A recent study sought to assess tear film osmolarity using the TearLab osmometer in six beagles with normal tear production and 5 with spontaneously arising KCS. In that small sample the researchers found, counter to results in human patients, that dogs with KCS had a lower tear osmolarity than that of tears from eyes with normal tear production. We had previously conducted research in a larger sample with contradictory results, first presented at the British Small Animal Veterinary Association meeting in April 2008 which we report herein. The aims of this study were to determine a reference value for normal canine tear osmolarity and to examine the degree of correlation between Schirmer tear test results and tear osmolarity. It would be expected that as the Schirmer tear test value increases, the tear osmolarity would decrease due to a dilution effect. Current diagnosis of canine dry eye relies on the use of the Schirmer tear test, which, while it documents aqueous tear volumes in the eye, do not take account of evaporative tear loss, nor evaluate pathological changes in tear composition, which may be more accurately documented by measurement of tear film osmolarity.

MATERIALS AND METHODS

100 dogs were examined at the Queen's Veterinary School Hospital, Cambridge and Wood Green Animal Shelter, Huntingdon, UK between February and June 2010. Tear osmolarity was measured in each eye from the lateral lower conjunctival sac, using the TearLab™ osmolarity system (TearLab Corp.) **(Figure 1)**. The device is placed gently in the lower conjunctival sac, takes up the required 50nL of tears by capillary action, and defines the osmolarity by measurement of electrical conductivity across the sample. The device was calibrated at the start of each clinic, using a monodose saline with an osmolarity of 300 mOsm/L and the system test card. A Schirmer tear test I was subsequently performed in each eye without topical anaesthesia for a time of 1 minute, using test strips from one batch (Eaglevision, Memphis TN, USA), as is standard in veterinary ophthalmology. The first eye tested was randomised for each patient. Supplementary data collected from subjects included age, gender, and breed. Results were analysed using a standard statistical analysis computer program (SPSS, IBM). Normality of data was assessed using the Kolmogorov–Smirnov test. A Pearson Correlation was used to analyse the relationship between Schirmer tear test and tear osmolarity, as well as investigating variation between left and right eye results for each subject.

RESULTS

Schirmer tear test (STT) results for the 153 eyes with an STT of equal or more than 15mm/min and thus considered to have a normal tear film were normally distributed with a mean value of 18.8±2.3mm/min. Values for tear osmolarity in these eyes were normally distributed, with a range from 227-380 mOsm/l, a mean value of 339.2 ± 22.7 mOsm/l and median of 340 mOsm/l. Mean Schirmer tear test readings for the 47 eyes with ophthalmic signs of KCS (ocular discharge, lack of corneal lustre, corneal vascularisation and pigmentation) and STT values of less than 15 mm/min were also normally distributed with the median of 10 and mean of 9.7 mm/min. Values for tear osmolarity in eyes with STT readings below 15 mm/min were normally distributed, with a range from 275 to 400 mOsm/l, a mean value of 350.5 ± 26.7 mOsm/l and a median of 349 mOsm/l. Mean values of tear osmolarity varied slightly between the eyes, with the right eye measuring 338 ± 24.9 mOsm/l and the left 342 ± 23.3 mOsm/l, these two values not being statistically different and being strongly positively correlated (r=0.433, p<0.001). Tear osmolarity was weakly negatively correlated r=-0.103 (n=201) with Schirmer tear test results, this correlation being statistically significant (r=-0.263, p=0.008).

Given that evaporative tear dynamics may be crucial in changing tear osmolality, we compared the tear omsolarity of brachycephalic dogs with protruberant globes with those of doliocephalic or mesaticephalic skull shapes with more normally positioned eyes. When animals with tear deficiency had been removed from the sample, doliocephalic and mesaticephalic breeds, of which in our sample there were 70 examples, had a mean STT of 18.5 ± 2.8 mm/min and tear osmolarity of 331 ± 17.6 mOsm/l while the 8 brachycephalic dogs comprising in our sample pug, French bulldog (2), Cavalier King Charles spaniel (2), boxer (1) and Lhasa Apso (3) had a mean STT of 18.6 ± 2.0 mm/min, not significantly different from that of the doliocephalic dogs but a tear osmolarity of 343 ± 14.8, significantly higher than that of doliocephalic breeds at p=0.03.

Figure 1. Use of the TearLab device.

DISCUSSION

Tear osmolarity in humans is considered to be the best single test for the diagnosis of dry eye disease (Khanal et al., 2008). When defined by a referant of 316 mOsm/l it gave a sensitivity of 59% and specificity of 94% and a predictive accuracy for dry eye disease of 89% (Tomlinson et al., 2006). However, the difficulty of using this single parameter to diagnose dry eye is the overlap of values in normal and diseased eyes (Tomlinson et al., 2006). A battery of tests including tear osmolarity measurements, were found to be most sensitive and specific for the diagnosis of dry eye disease.

In this study we determined that the mean tear osmolarity in the normal canine eye was higher than that of humans, at 339.2 ± 22.7 mOsm/l. A possible reason for this higher mean osmolarity may be the decreased normal blink rate in dogs compared to that of humans with subsequent higher evaporative tear loss. In humans, the interblink time in normal individuals is 4 ± 2s, equating to a blink rate of 10-30 per minute. However, in dogs the blink rate is merely 3-5 times per minute [17]. This low blink rate may result in greater evaporative loss of the tear film from the surface of the eye, increasing the mean osmolarity of tears in clinically normal dogs. Blinking is highly important in appropriate tear film dynamics, as it allows both the spread of tears across the surface of the eye in a controlled fashion, and the drainage of excess tear fluid though the lacrimal pucta. Hence a low blink rate in dogs may prevent drainage of the tear lake, explaining the much increased Schirmer tear test values in dogs compared with humans, the normal range of Schirmer tear test values in humans being 10-30 mm in five minutes [18], whereas in dogs a normal Schirmer tear test value is above 15 mm in only one minute. Another factor may be exposure of the ocular surface in several breeds of dog. Here we show that brachycephalic breeds with a normal STT value have a statistically significantly higher tear osmolarity than doliocephalic or mesaticephalic breeds.

The standard deviation of tear osmolarity was found to be high in this study, despite the large sample size, suggesting that large variation amongst dogs or the osmometer itself are to blame. The strongly positive and statistically significant correlation found when tear osmolarity values for both eyes were compared (r=0.433, p<0.001, n=100) suggests that the large standard deviation may be due to inter- rather than intra-subject variation. Unlike humans, who have a largely conserved palpebral aperture size and conformation, that of the dog is highly variable between breeds. For example, breeds with a particularly exophthalmic conformation may show greater evaporative loss of tears from the corneal surface, which may lead to higher tear osmolarity

readings compared to more enophthalmic breeds. It would be interesting to look further at a number of breeds and determine whether ocular conformation plays a role in tear osmolarity.

Tear hyperosmolarity is considered to be the central mechanism causing ocular inflammation and damage and the symptoms of dry eye caused, at least in part by the activation of the inflammatory NFkB and MAP kinase pathways in cells of the ocular surface. It is, then unsurprising that tear osmolarity is correlated with dry eye disease severity as defined by the DEWS classification system [19].

The TearLab™ osmometer is still very new technology, having become commercially available in Europe only in 2008. Some authors feel that there may still be problems with sample collection, particularly with reflex tearing. This may cause inaccuracies in the data, as reflex tearing is known to have a differing composition to basal tears and may have led to altered values for tear osmolarity in this study. Having said that, we did not recognize excessive blinking during tear sampling in the animals involved in the current study, so reflex tearing may not be a problem in dogs being tested with the TearLab™.

Further testing focussing on intra-subject variation in tear osmolarity readings taking repeated readings from several subjects would be useful, to help to explain the high standard deviation seen in tear osmolarity readings. Other possible causes of the high standard deviation seen in tear osmolarity of dogs may include a difference in tear film composition compared with that of humans or their high conformational variation, as discussed above. Repeating the study using a population of cats would also be interesting as Domestic Shorthair cats tend to have fairly well conserved ocular conformation which would help to eliminate some of the problems encountered in this study.

The multifactorial aetiology of dry eye disease in humans causes great difficulty in diagnosis. Equally, poor correlation of clinical symptoms and clinical signs is noted, with only 57% of symptomatic patients shown to have objective measures of dry eye. To address these diagnostic difficulties, a matrix combining diagnostic tests and clinical signs has been developed known as the DEWS definition and classification scheme for dry eye disease [20]. This enables practitioners to grade the severity of dry eye disease in patients, as well as allowing effective diagnosis of the disease. It would be both interesting and clinically useful to create a similar matrix for dry eye disease in dogs.

CONCLUSION

This study showed the potential value of measuring tear omolarity in gaining a better understanding of the tear film in normal and KCS-affected dogs. The use of the TearLab™ system in canine patients is more rapid and easily employed than the Schirmer tear test, with less patient irritation. The minimally invasive nature and rapidity of sample taking is highly desirable. Further testing needs to be performed to discover whether the TearLab™ osmometer would be truly useful as an aid to diagnosis of dry eye disease in veterinary patients. Ultimately, a more effective diagnosis of dry eye disease, such as is used in humans, is important in veterinary ophthalmology both for the welfare of patients under our care and the diagnostic satisfaction of veterinary surgeons.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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