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Keratoconjunctival effects of diabetes mellitus in dogs

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Abstract

Objectives To compare Schirmer tear test (STT) values, corneal sensitivity, tear film break up times (TFBUTs), and tear glucose concentrations in relation to conjunctival microflora, and conjunctival cytologic and histologic findings among diabetic cataractous, nondiabetic cataractous, and nondiabetic noncataractous dogs.

Procedures Fifteen dogs in each category underwent neuro-ophthalmic examination; aerobic, anaerobic and fungal conjunctival cultures; assessment of corneal touch threshold (CTT), STT, tear glucose, TFBUT; and conjunctival cytology and histology (in certain cases only). Degree of cataract and uveitis were critically graded. Glycemic control was estimated using serum fructosamine and glycosylated hemoglobin.

Results STT values were significantly lower in diabetic cataractous than nondiabetic noncataractous dogs. CTT of diabetic cataractous dogs was significantly lower than that of nondiabetic noncataractous dogs. Mean TFBUTs were significantly less in diabetic cataractous dogs than nondiabetic cataractous and nondiabetic noncataractous dogs. Tear glucose concentrations were significantly higher in diabetic cataractous dogs than nondiabetic cataractous and nondiabetic noncataractous dogs. Conjunctival microbial isolates did not differ among groups. There were no significant differences in degree of cataract or uveitis between diabetic cataractous and nondiabetic cataractous groups. There was mild submucosal inflammatory infiltrate in conjunctival specimens from diabetic dogs. Conjunctival epithelial dysplasia and/or squamous metaplasia was/were detected in conjunctival biopsies of 5/7 diabetic dogs. Reductions in conjunctival goblet cell (GC) densities were noted in 4/7 diabetic dogs; there were no significant differences in mean GC densities among the three groups.

Conclusions Diabetic cataractous dogs have significantly altered keratoconjunctival characteristics compared to nondiabetic cataractous and nondiabetic noncataractous dogs.

Key Words: conjunctival microflora, corneal sensitivity, glycemic control, goblet cells, tear film break up time, tear glucose

INTRODUCTION

Diabetes mellitus is a common endocrinopathy of dogs, affecting 1 in 500 to 1 in 100 dogs.[1] Ocular manifestations of diabetes mellitus in dogs include cataract formation,[2] corneal endothelial cell loss,[3,4] corneal endothelial pleomorphism and polymegathism,[3] reduced corneal sensitivity,[5] and retinal vascular damage such as formation of microaneurysms.[6] The most common ocular manifestation of canine diabetes is cataract formation.[7] One study reported that 75% of the canine population diagnosed with diabetes mellitus developed cataracts by approximately 12 months while 80% of the population developed cataracts by approximately 16 months.[8] Consequently, diabetic dogs with cataracts are frequently referred for surgical cataract extraction. One report documented that ulcerative keratitis developed postoperatively more commonly in diabetic than in nondiabetic dogs undergoing cataract surgery.[5] Authors of this same study concluded that corneal sensation is reduced in diabetic dogs compared to normal dogs.[5] Subsequently, they inferred that axonal degeneration of corneal sensory nerves, as reported in human diabetics, was associated with altered corneal wound healing.[5]

In humans, 47 to 67% of diabetic patients experience primary corneal lesions during their lifetime.[9] As in diabetic dogs, reduced corneal sensitivity has been reported in human diabetic patients.[10] In addition, abnormalities in other ocular parameters in patients with diabetes have been reported, which could contribute to these primary corneal lesions.[10] Tear film break up times (TFBUTs) and Schirmer tear test (STT) values were significantly lower in human diabetics than in healthy control individuals, and were related to peripheral neuropathy, poor diabetic control, and decreased corneal sensitivity.[10] Analysis of impression cytology of the conjunctiva of affected patients revealed goblet cell loss and epithelial squamous metaplasia, both of which were again related to peripheral neuropathy, poor diabetic control, and decreased corneal sensitivity.[10] In that study, none of the ocular parameters evaluated in affected patients was related to duration of diabetes.[10]

Elevated tear glucose concentrations have been reported in human diabetic patients.[11] Glucose is used by bacteria and other micro-organisms to support their metabolism.[12] Bacteria and fungi have been cultured from conjunctival sacs of both clinically normal[13–15] and diseased[16] canine eyes. In addition, human patients with certain systemic risk factors, including diabetes, have been reported to be more likely to harbor conjunctival bacterial flora that are resistant to multiple antibiotics.[17] In particular, 32 of 71 patients (45%) with systemic risk factors harbored multiresistant organisms, compared to 32 of 136 patients (24%) without systemic risk factors.[17] As such, it is conceivable that conjunctival microflora of diabetic dogs may differ from those of nondiabetic dogs, assuming that tear glucose concentration, tear film quantity, or tear film quality is reduced in diabetic dogs. If there is a clinically relevant difference between the conjunctival microflora of diabetic and nondiabetic dogs, this information may help direct topical prophylactic antibiotic therapy prior to and following cataract extraction in diabetic dogs.

The purpose of the present study was to compare STT values, corneal sensitivity, TFBUTs, tear glucose concentrations in relation to conjunctival microflora, and conjunctival cytologic and histologic findings among diabetic cataractous, nondiabetic cataractous, and nondiabetic noncataractous dogs, and to investigate the correlation among these ocular parameters and status of glycemic control in diabetic cataractous dogs. The authors hypothesized that STT values, corneal sensitivity and TFBUTs in diabetic cataractous dogs would be low compared to nondiabetic cataractous and nondiabetic noncataractous dogs. As well, it was hypothesized that diabetic cataractous dogs would have increased conjunctival goblet cell loss and epithelial squamous metaplasia compared to conjunctival specimens from nondiabetic cataractous and nondiabetic noncataractous dogs. Furthermore, the authors theorized that elevated tear glucose concentrations in diabetic dogs would alter conjunctival microflora in affected dogs when compared with nondiabetic cataractous and nondiabetic noncataractous dogs.

MATERIALS AND METHODS

Animal selection and inclusion criteria

Fifteen diabetic cataractous, 15 nondiabetic cataractous, and 15 nondiabetic noncataractous dogs were examined for this study. A complete blood count, serum biochemical profile, and urinalysis were performed on all dogs. Dogs were considered diabetic if blood and urine tests revealed persistent hyperglycemia (> 11 mmol/L (200 mg/dL)) and glucosuria, respectively, and if the dog had a history of clinical signs consistent with diabetes (i.e. polyuria, polydipsia, weight loss, and/or polyphagia). Only diabetic dogs with bilateral cataracts were included in this study. Dogs were included in the nondiabetic cataractous group if they had no clinical signs indicative of diabetes, blood glucose concentrations were within the normal reference range (3.5–5.5 mmol/L (84–124 mg/dL)), no glucose was detected on urinalysis, and no evidence of other systemic disease was found on physical examination, or blood and urine test results. Dogs were included in the nondiabetic noncataractous group if they had physical examination and blood and urine parameters as described for nondiabetic cataractous dogs, and if cataracts were not detected during a complete ophthalmic examination. Dogs with recent or current ocular conditions (other than cataracts for diabetic cataractous and nondiabetic cataractous dogs), previously diagnosed keratoconjunctivitis sicca, systemic abnormalities (other than diabetes for diabetic cataractous dogs), or dogs receiving topical ocular medications were excluded.

For diabetic dogs, glycemic control was estimated using serum fructosamine concentration (μmol/L) and glycosylated hemoglobin values (%). Diabetic dogs were assigned, for statistical purposes, to one of four categories of glycemic control based on serum fructosamine concentrations (< 400 μmol/L = excellent; 400–500 μmol/L = good; 501–650 μmol/L = fair; and > 650 μmol/L = poor), and based on serum glycosylated hemoglobin values (< 5% = excellent; 5–6% = good; > 6–7% = fair; and > 7% = poor).

Ophthalmic examination

A complete ophthalmic examination was performed on all study participants by a board-certified veterinary ophthalmologist (CLC). Ophthalmic examinations included, in the following order, a neuro-ophthalmic examination; aerobic, anaerobic, and fungal cultures from the ventral conjunctival sacs; assessment of corneal touch threshold (CTT), STT, tear glucose concentrations, and TFBUT; and applanation tonometry. Slit-lamp biomicroscopy (Kowa SL-14; Kowa, Tokyo, Japan); and indirect ophthalmoscopy (Keeler All Pupil Indirect; Keeler Instruments, Inc., Broomall, PA, USA) were then performed following pharmacologic pupillary dilation using topical 1% tropicamide. In addition, specimens for cytologic or histologic assessment, respectively, were collected using conjunctival swabs (all dogs) or palpebral conjunctival biopsy from certain dogs (seven diabetic cataractous eyes; three nondiabetic cataractous eyes; five clinically healthy control

eyes from dogs euthanized for other reasons). For statistical purposes, critical evaluation and ranking on an ordinal scale were carried out for cataract (0 = none, 1 = incipient, 2 = immature, 3 = mature, and 4 = hypermature) and uveitis (0 = none, 1 = trace aqueous flare, 2 = mild aqueous flare, 3 = moderate aqueous flare, 4 = marked aqueous flare).

Culture specimen collection and methods

Two culture specimens were obtained from the ventral conjunctival sac of both eyes of all dogs for aerobic and anaerobic bacterial and fungal identification. The ventral eyelid was everted and specimens were collected by rotating a sterile culturette swab (BBL Culture Swab Collection and Transport, Becton Dickinson; VWR/CANLAB, Mississauga, Ontario, Canada for aerobic bacterial and fungal isolation; Anaerobic Culturette for Collection and Transport of Microorganisms, Becton Dickinson for anaerobic bacterial isolation) within the ventral conjunctival sac, and removing it carefully to avoid contact with eyelid hairs or skin. Conjunctival swab specimens were submitted to the Bacteriology Laboratory at the Atlantic Veterinary College where they were inoculated onto trypticase soy agar Petri plates (Oxoid, Inc., Nepean, Ontario) supplemented with 5% bovine blood, and Sabouraud's dextrose agar (BD Biosciences, Oakville, Ontario). Blood agar plates were incubated at 35 °C in an atmosphere of 5% CO_2, and examined for growth after 24 and 48 hours. Anaerobic specimens were incubated in anaerobic jars using an anaerobic atmosphere generating system (AnaeroGen; Oxoid, Inc.) and were examined for growth for 7 days. Sabouraud's plates were incubated at 25 °C and examined periodically for up to 3 weeks before being considered negative.

Corneal sensitivity

A Cochet–Bonnet aesthesiometer was used to measure corneal touch threshold (CTT) in all dogs while in a quiet examination room, with dogs minimally restrained and either standing or in sternal recumbency. The same investigator (CLC) took all CTT measurements to decrease variability in technique. The nylon monofilament of the aesthesiometer was extended to its full length of 60 mm to commence testing. Stimulations were delivered to the central cornea of each dog. Monofilament length was decreased by 5 mm increments until the dog demonstrated a blink reflex in response to at least 3 of 5 stimulations.[5] Monofilament length was then recorded without conversion to CTT (g/mm^2).

Schirmer tear test (STT)

The STT was performed by placing a standardized test strip (Schirmer tear test strips; Alcon Canada, Mississauga, Ontario) from the same lot number within the ventral conjunctival sac of each dog. Tear production was recorded in mm/min for each eye.

Tear glucose determination

Tear glucose concentration was measured semiquantitatively using a urine dipstick (Multistix® 8 SG (Lot. No. 2304A); Bayer Inc., Toronto, Ontario). Specifically, the glucose portion of the test stick was placed in the meniscus of tear film at the medial canthus of each eye of all dogs. The strip was immediately removed from the medial canthus and following 30 s, glucose concentration was determined according to the manufacturer's color chart (light green to dark olive green = negative to 18 mmol/L).

Tear film break up time (TFBUT)

A fluorescein dye strip (Fluor-I-Strip AT; Ayerst Laboratories, St. Laurent, Quebec, Canada) was wetted with eyewash (Eyestream; Alcon Canada), touched once to the dorsal bulbar conjunctiva of each eye, and the eyelids held closed. Timing began when the eyelids were opened and the dorsolateral corneal surface was observed using the slit-lamp biomicroscope at ×16 magnification with the cobalt blue filter. Timing ended when the first sign of tear break up (i.e. – a 'dry spot'), represented by a dark area in the yellow-green fluorescent tear film, was noted. A stopwatch was used to ensure accurate timing and TFBUTs were measured to the nearest one hundredth of a second on three occasions for each eye. These three TFBUTs were averaged for each eye of each dog; mean TFBUTs were recorded to the nearest one tenth of a second. The fluorescein dye was then rinsed from the eye using eyewash.

Conjunctival cytology

Following anesthesia of the ocular surface with topical 0.5% proparacaine (Alcaine; Alcon Canada), a sterile cotton swab was rotated numerous times within the ventral conjunctival sac of each eye. The swab then was gently rolled back-and-forth over the surface of four glass slides, which underwent cytologic assessment following application of Wright's Giemsa stain (three slides) or periodic acid-Schiff (PAS) stain (one slide).

Conjunctival histology

When permitted by owners, a conjunctival biopsy, approximately 3 mm in diameter, was harvested from the ventromedial fornix of one eye chosen at random of each of seven diabetic cataractous dogs and three nondiabetic cataractous dogs. The ocular surface was anesthetized with topical 0.5% proparacaine. The conjunctival specimen was placed, epithelial-side up, in a small tissue cassette, fixed in 10% neutral-buffered formalin, routinely processed, paraffin-embedded, and sectioned. One section was stained with hematoxylin and eosin (H&E) and one with PAS to facilitate goblet cell (GC) assessment. Specifically, GC densities were determined by counting 50 epithelial cells (ECs) along the palpebral conjunctival surface. Number of GCs within this region was expressed relative to total number of ECs counted (i.e. GC : 50 EC).[18] This was repeated two times to provide an average GC : 50 EC. Conjunctival biopsies were not taken from nondiabetic noncataractous dogs. Instead, control specimens were obtained from the ventromedial palpebral conjunctiva of one eye from each of five clinically healthy dogs free of ocular disease

immediately following their humane euthanasia for other reasons.

Statistical analyses

Data analyses were performed using SigmaStat® statistical software version 2.0 for Windows® (SPSS Inc., Chicago, IL, USA). All data are reported throughout the text as mean ± standard deviation (SD). Figures depict data as mean ± standard error (SE) bars. A one-way analysis of variance (ANOVA) test was used to detect significant differences in mean ages, and STT values, intraocular pressures (IOPs), corneal sensitivity, tear glucose concentrations, and GC densities (certain cases only) among groups. The one-way ANOVA also was used to detect significant differences in these ocular parameters among diabetic cataractous dogs based upon glycemic control. The Kruskal–Wallis one-way ANOVA on ranks test was used to determine whether degree of cataract or uveitis varied significantly between diabetic cataractous and nondiabetic cataractous dogs. The Spearman rank order correlation test was used to determine whether subject age was correlated with degree of cataract or uveitis. Results were considered statistically significant when $P < 0.05$.

RESULTS

Study population

Many breeds of dogs were evaluated in each group; however, for all groups, skull conformation was primarily mesaticephalic (14/15 nondiabetic noncataractous; 14/15 nondiabetic cataractous dogs; 13/15 diabetic cataractous dogs). One nondiabetic cataractous dog was brachycephalic; the remaining three dogs were dolichocephalic. Mean (± SD) age of diabetic cataractous (10.4 ± 2.5 years) and nondiabetic cataractous (9.5 ± 3.5 years) dogs was significantly greater than nondiabetic noncataractous dogs (4.6 ± 2.7 years; $F_{2,42} = 17.2$, $P < 0.001$). Significant differences were not detected between mean ages of diabetic cataractous and nondiabetic cataractous dogs. There were seven male dogs (all neutered) and eight female dogs (all spayed) in the diabetic cataractous group. The nondiabetic cataractous group consisted of 10 male dogs (eight neutered and two intact) and five female dogs (all spayed), while the nondiabetic noncataractous group was comprised of four male dogs (all neutered) and 11 female dogs (eight spayed and three intact).

Degree of cataract and uveitis

Median cataract stage scores for nondiabetic cataractous dogs were 2 (immature) for both eyes, while those for diabetic cataractous dogs were 3 (mature) and 2 (immature) for OD and OS, respectively. Median uveitis score for nondiabetic cataractous dogs was 0 (none clinically detected) for both eyes, while that for diabetic cataractous dogs was 1 (trace aqueous flare) for both eyes. Anterior chamber cells were not detected in any patients. There were no significant differences in degrees of cataract or uveitis between diabetic cataractous and nondiabetic cataractous groups. Subject age was significantly ($P < 0.01$) positively correlated with degree of cataract but not with degree of uveitis ($P > 0.05$). Mean IOPs of each eye were not statistically different between diabetic cataractous (9.6 ± 3.6 mmHg OD; 8.9 ± 3.7 mmHg OS) and nondiabetic cataractous (10.7 ± 3.6 mmHg OD; 11.9 ± 3.1 mmHg OS) groups. Mean IOPs of nondiabetic noncataractous dogs (14.5 ± 3.4 mmHg OD; 14.2 ± 3.5 mmHg OS) were significantly ($F_{2,42} = 8.1$, $P < 0.001$ OD; $F_{2,40} = 8.9$, $P < 0.001$ OS) higher than those of both the diabetic cataractous and nondiabetic cataractous groups.

Table 1. Frequency of isolation of bacterial and fungal agents from both ventral conjunctival sacs of diabetic cataractous (D), nondiabetic cataractous (C), and nondiabetic noncataractous (N) dogs ($n = 15$ of each)

Micro-organism	Total positive eyes (D/C/N groups)	Total number of dogs (D/C/N groups)
Gram-positive bacteria		
Staphylococcus intermedius	1/4/1	1/2/1
Gram-positive bacilli	0/5/0	0/3/0
Coagulase negative *Staphylococcus* spp.	0/1/3	0/1/3
Aerobic diphtheroids	3/0/1	2/0/1
Micrococcus spp.	1/1/0	1/1/0
Alpha-hemolytic *Streptococcus* spp.	0/2/0	0/1/0
Gram-negative bacteria		
Enterobacter cloacae	0/0/1	0/0/1
Gram-negative bacilli	1/0/0	1/0/0
Anaerobic bacteria		
Clostridium perfringens	0/1/0	0/1/0
Total number of bacterial isolates	6/14/6	
Fungi	0/0/0	0/0/0

Conjunctival microflora

Conjunctival cultures from eyes of four diabetic cataractous dogs, seven nondiabetic cataractous dogs, and four nondiabetic noncataractous dogs yielded one or more isolates of aerobic bacteria (Table 1). The most common isolates were Gram-positive bacteria including *Staphylococcus intermedius*, Gram-positive bacilli, and coagulase-negative *Staphylococcus* spp. Gram-negative bacteria were rarely isolated. *Clostridium perfringens* (one nondiabetic cataractous eye) was the sole anaerobic species isolated. All conjunctival cultures were negative for growth of fungi.

Corneal sensitivity

Mean (± SD) length of filament required to elicit a blink reflex in diabetic cataractous dogs (29.1 ± 15.8 mm OD; 26.2 ± 15.1 mm OS) was significantly shorter than that required for nondiabetic noncataractous dogs (43.1 ± 9.5 mm OD, $F_{2,41} = 3.8$, $P < 0.05$; 44.3 ± 9.2 mm OS, $F_{2,39} = 6.2$, $P < 0.01$; Fig. 1). A significant difference in mean filament lengths was not detected between diabetic cataractous and nondiabetic cataractous (40.9 ± 18.3 mm OD; 37.1 ± 17.6 mm OS; $P > 0.05$) dogs, or between nondiabetic noncataractous and nondiabetic cataractous dogs for either eye ($P > 0.05$). There

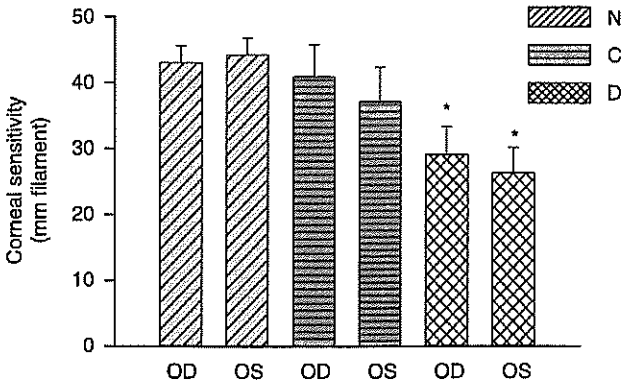

Figure 1. Histogram depicting mean (+ SE) corneal sensitivity (filament length in mm) as assessed using the Cochet–Bonnet aesthesiometer in diabetic cataractous (D), nondiabetic cataractous (C), and nondiabetic noncataractous (N) dogs. The asterisk (*) indicates a significant difference ($P < 0.05$) from the N dogs.

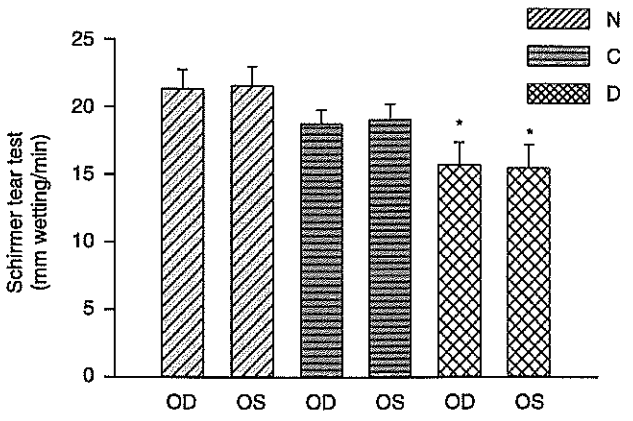

Figure 2. Histogram depicting mean (+ SE) Schirmer tear test (STT) values (mm/min) in diabetic cataractous (D), nondiabetic cataractous (C), and nondiabetic noncataractous (N) dogs. The asterisk (*) indicates a significant difference ($P < 0.05$) from the N dogs.

were no significant differences in mean lengths of filament required to elicit a blink reflex between OD and OS within any group of dogs.

Schirmer tear test (STT)

Mean (± SD) STT values were significantly lower in diabetic cataractous dogs (15.7 ± 6.5 mm/min OD; 15.5 ± 6.4 mm/min OS) than in nondiabetic noncataractous dogs (21.3 ± 5.9 mm/min OD, $F_{2,42} = 3.9$, $P < 0.05$; 21.6 ± 5.5 mm/min OS, $F_{2,42} = 4.8$, $P < 0.05$; Fig. 2). There were no significant differences in mean STT values between nondiabetic cataractous dogs (18.8 ± 3.8 mm/min OD; 19.1 ± 4.0 mm/min OS), and either of the other two groups for either eye. There were no significant differences in mean STT values between OD and OS within any group of dogs.

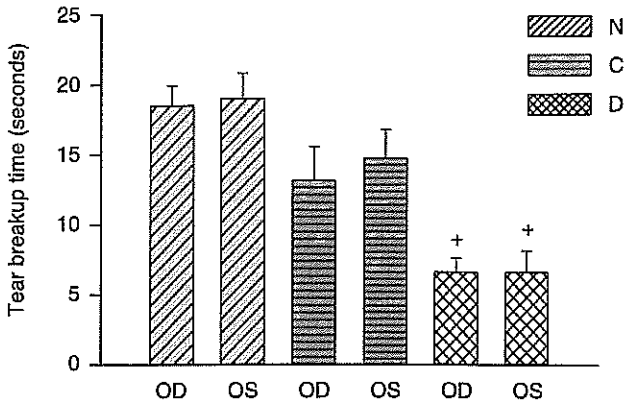

Figure 3. Histogram depicting mean (+ SE) tear break up times (seconds) in diabetic cataractous (D), nondiabetic cataractous (C), and nondiabetic noncataractous (N) dogs. The plus (+) indicates a significant difference ($P < 0.05$) from the N and C dogs.

Tear glucose

Median tear glucose concentrations were significantly higher in diabetic cataractous dogs (6 mmol/L OU) than nondiabetic noncataractous and nondiabetic cataractous dogs (0 mmol/L OU, $P < 0.001$). There were no significant differences in tear glucose concentrations between nondiabetic noncataractous and nondiabetic cataractous dogs for either eye.

Tear film break up time (TFBUT)

Mean (± SD) TFBUTs were significantly shorter in diabetic cataractous dogs (6.6 ± 3.8 s OD, $F_{2,42} = 13.9$, $P < 0.001$; 6.5 ± 6.4 s OS, $F_{2,40} = 12.1$, $P < 0.001$) than in nondiabetic noncataractous (18.4 ± 5.2 s OD; 18.9 ± 7.1 s OS) and nondiabetic cataractous dogs (13.2 ± 8.4 s OD; 14.7 ± 7.5 s OS; Fig. 3). There were no significant differences between mean TFBUTs in nondiabetic noncataractous and nondiabetic cataractous dogs for either eye. There were no significant differences in mean TFBUTs between OD and OS within any group of dogs.

Conjunctival cytology

Bilateral conjunctival cytologic assessment of nondiabetic noncataractous dogs revealed mild to moderate numbers of epithelial cells with variable numbers of squamous, columnar, intermediate and/or superficial epithelial cells with or without melanin granules. All nondiabetic noncataractous samples contained mild to moderate amounts of mucus. Six of 30 conjunctival specimens from four nondiabetic noncataractous dogs had rare neutrophils. When assessed using PAS stain, only one conjunctival GC was detected from a single nondiabetic noncataractous eye. Low to marked numbers of epithelial cells (squamous to columnar in nature) were detected in all conjunctival specimens from diabetic cataractous and nondiabetic cataractous dogs. Six eyes of four diabetic cataractous dogs and eight eyes of four nondiabetic cataractous dogs had mild neutrophilic inflammation

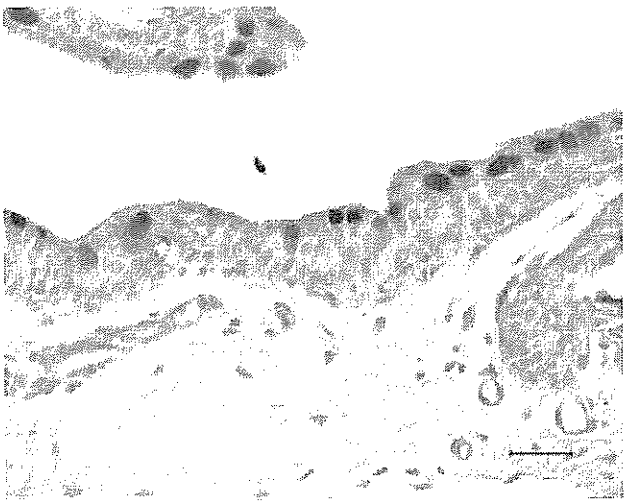

Figure 4. Photomicrograph of a section of ventromedial palpebral conjunctiva from a control dog. Note the abundance of periodic acid Schiff (PAS)-positive goblet cells and normal epithelial architecture (PAS and hematoxylin counterstain; scale bar = 35 μm).

Figure 6. Photomicrograph of a section of ventromedial palpebral conjunctiva from a diabetic cataractous dog. Note the epithelial thinning and absence of goblet cells (PAS; scale bar = 35 μm).

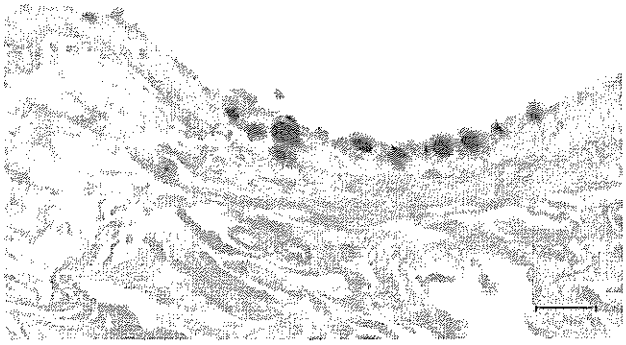

Figure 5. Photomicrograph of a section of ventromedial palpebral conjunctiva from a diabetic cataractous dog. Note epithelial thinning and squamous metaplasia (PAS; scale bar = 35 μm).

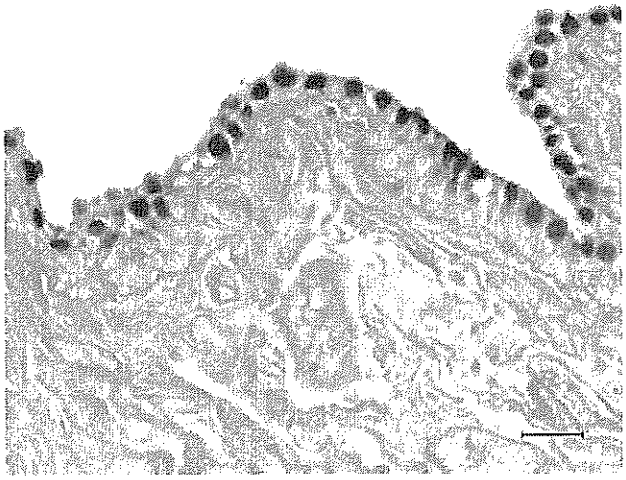

Figure 7. Photomicrograph of a section of ventromedial palpebral conjunctiva from a nondiabetic cataractous dog. Note the abundance of periodic acid Schiff (PAS)-positive goblet cells and normal epithelial architecture (PAS; scale bar = 35 μm).

detected on conjunctival cytology. Twenty of 30 conjunctival smears from both diabetic cataractous and nondiabetic cataractous dogs had mild to moderate mucus. Conjunctival GCs were detected using PAS stain in 6/15 diabetic cataractous dogs (nine eyes) and in 4 /15 nondiabetic cataractous dogs (six eyes).

Conjunctival histology

Specimens from control dogs had a mean GC : EC density of 28 : 50, an orderly arrangement of epithelial cells from basal to superficial layers, and minimal mononuclear submucosal inflammatory infiltrate (Fig. 4). Conjunctival specimens from diabetic cataractous dogs had mild to moderate mononuclear and/or neutrophilic submucosal infiltration. Those from nondiabetic cataractous dogs contained minimal mononuclear submucosal inflammatory infiltrates similar to control specimens. Specimens from 5/7 diabetic cataractous dogs also exhibited varying degrees of conjunctival epithelial dysplasia with or without squamous metaplasia (Fig. 5), while one specimen from a nondiabetic cataractous dog had multifocal areas of mild epithelial squamous metaplasia. Mean GC density for all seven diabetic cataractous dogs was 20 : 50; however, GC densities varied widely. Three diabetic cataractous dogs had normal (27 : 50) mean GC densities (Fig. 5), while four had moderately or markedly diminished (11.5 : 50) mean GC densities (Fig. 6). Mean GC : EC

density for nondiabetic cataractous dogs (27 : 50) was comparable to that of control specimens (28 : 50; Fig. 7). There were no statistically significant differences in mean GC : EC densities among the three groups of dogs.

Glycemic control of diabetic dogs

Based on serum fructosamine concentrations, the majority (11/15) of diabetic cataractous dogs had fair ($n = 7$) or good ($n = 4$) glycemic control. Glycemic control in the remaining dogs was assessed as excellent ($n = 3$) or poor ($n = 1$). When assessed using glycosylated hemoglobin values 7/15 dogs had fair ($n = 5$) to good ($n = 2$) glycemic control, while a larger proportion of dogs (6/15) were poorly controlled. Glycemic control of one diabetic cataractous dog was classified as excellent using glycosylated hemoglobin values. Significant differences in mean STT values, TFBUTs, corneal sensitivities, IOPs, tear glucose concentrations, or degrees of cataract and uveitis were not detected among diabetic cataractous dogs with excellent, good, fair, or poor glycemic control; however, it should be noted that statistical power was lacking (< 0.8) for these comparisons.

DISCUSSION

Diabetes mellitus has significant effects on several keratoconjunctival parameters of dogs. In particular, diabetic cataractous dogs have altered precorneal tear film compared to nondiabetic noncataractous dogs. The aqueous portion of the tear film, as measured by the STT, was significantly decreased for diabetic cataractous dogs compared to nondiabetic noncataractous dogs. Similarly, a 37% reduction in reflex tearing has been demonstrated in humans with insulin dependent diabetes mellitus compared to tearing in nondiabetic individuals.[19] In addition, concurrent keratoconjunctivitis sicca and diabetes mellitus in dogs have previously been documented.[20,21] Although the STT values for diabetic cataractous dogs in our study remained greater than 10 mm/min, reduction in aqueous tear volume may be clinically relevant especially in diabetic dogs undergoing cataract surgery. General anesthesia,[22] and topical[23] and systemic[24] atropine have previously been reported to cause temporary reductions in aqueous tear production in dogs. General anesthesia, with or without topical atropine therapy (used by some veterinary ophthalmologists pre- and/or postcataract surgery), may further compromise aqueous tear production in these diabetic dogs thereby potentially increasing postoperative risk of these dogs developing ulcerative keratitis.

The TFBUT is a measure of precorneal tear film stability and may be used to support a presumptive diagnosis of qualitative tear film dysfunction (e.g. mucin deficiency).[25,26] Mean TFBUTs were significantly lower in diabetic cataractous than both nondiabetic noncataractous and nondiabetic cataractous dogs. Mean TFBUTs in our nondiabetic noncataractous dogs (18.4 s OD; 18.9 s OS) are similar to those previously established in healthy dogs (19.7 s).[27] Rapid TFBUTs (2–5 s) have previously been documented in dogs with mucin deficiency and associated ulcerative and nonulcerative keratoconjunctivitides.[25] Similarly, diabetic cataractous dogs in our study were diagnosed, cytologically (6/15 dogs) and histologically (7/7 dogs), with mild to moderate suppurative and/or lymphoplasmacytic conjunctivitis and concurrent rapid TFBUTs (6.6 s OD and 6.5 s OS). Clinically, the only overt sign of conjunctivitis in these diabetic cataractous dogs was mild to moderate conjunctival hyperemia. In contrast, none of the nondiabetic noncataractous or control dogs had evidence of conjunctivitis clinically, cytologically, or histologically (samples from control dogs only). Additionally, only 4/15 nondiabetic cataractous dogs had mild neutrophilic inflammation noted solely on cytologic assessment of conjunctival samples.

The TFBUT test is a clinically useful diagnostic tool. However, rapid TFBUTs, as noted in our diabetic cataractous dogs, may arise as a result of factors other than a suspected qualitative tear film deficiency including: (1) irregularities in corneal surface (2) corneal anesthesia (3) corneal exposure (4) ocular surface frictional irritants, and (5) preservatives in ophthalmic medications, irrigating solutions or fluorescein dye preparations, among others.[26] As such, a confirmatory test is warranted in cases of suspected qualitative tear film deficiency. Quantifying conjunctival epithelial GCs using palpebral conjunctival biopsies of dogs and cats provides an indirect measure of mucin production.[18,26,27] In humans, conjunctival goblet cell content has been reported to be a more sensitive indicator of primary ocular surface disease than is tear mucin.[28] Conjunctival brush cytology[29] or impression cytology[30] has also been used to assess GC density and squamous metaplasia in human patients, including diabetic individuals.[29] In the present study, histologic evaluation of conjunctival biopsies was the only diagnostic technique that permitted consistent evaluation of both conjunctival epithelial architecture and GCs. It is possible that using a different technique to obtain samples from the conjunctiva[29,30] rather than a sterile cotton swab, as used in this study, may have resulted in increased cells for cytologic assessment.

In the current study, consent from the owner of diabetic or nondiabetic cataractous dogs was required in order to permit harvesting a palpebral conjunctival biopsy. As such, not every dog underwent biopsy. Considering that client consent for conjunctival biopsy was not knowingly influenced by the authors, histologic findings regarding conjunctival biopsies should be unbiased. Conjunctival biopsy specimens from 5/7 diabetic cataractous dogs exhibited varying degrees of conjunctival epithelial dysplasia with or without squamous metaplasia. This finding is similar to that in human diabetic patients, in whom pronounced signs of conjunctival surface disease, including squamous metaplasia, have been reported.[19] Conjunctival metaplasia in these diabetic humans was considered secondary to a reduction in reflex tearing, altered trophic function of the tear film, and/or a primary surface disorder or metabolic alteration of conjunctival epithelial cells. It is likely that similar factors may be playing a role in the conjunctival metaplasia noted in diabetic cataractous dogs in our study.

In addition to conjunctival inflammation and metaplasia noted in conjunctival specimens of diabetic cataractous dogs, four of these seven dogs also exhibited moderately to markedly diminished mean GC densities (11.5 : 50) compared with those of control specimens (28 : 50). However, we were unable to detect statistically significant differences in mean GC : EC densities among the three groups of dogs. This could be due to insufficient statistical power as a result of small sample size, leading to increased likelihood of a type-2 statistical error. Nevertheless, documenting reductions in GC densities in 4/7 diabetic cataractous dogs and altered conjunctival epithelial architecture in 5/7 diabetic cataractous dogs indicates that diabetic cataractous dogs have qualitative tear film (mucin) abnormalities and/or conjunctival surface disease, respectively. Qualitative tear film abnormalities may either predispose these animals to further ocular surface disease or may be the primary cause of the ocular condition.[25,26] Clinical implications of this ocular surface disorder for diabetic dogs may include an increased tendency toward nonulcerative and/or ulcerative keratoconjunctivitides, especially following ocular surgery such as cataract removal.

A direct relationship between degree of ocular surface hydration and conjunctival GC density in dogs has been proposed.[27] Results of our study indicate that diabetic cataractous dogs have both quantitative and qualitative tear film alterations. Consequently, our findings indicate that therapy including topical artificial tear supplementation with or without a topical lacromimetic[31] and mucinomimetic[32] drug such as cyclosporine may be warranted in diabetic dogs. Additional or alternate ocular therapeutic strategies may also be advisable in diabetic dogs. Increased oxidative stress has been implicated in complications seen with diabetes mellitus.[33] Supplementation with antioxidants such as vitamins C and E has been reported to significantly decrease nitrite levels and improve STT values, TFBUTs, and conjunctival GC density and squamous metaplasia in human diabetic patients. Future studies are warranted to investigate whether or not antioxidant supplementation is also warranted in diabetic dogs.

There were no significant differences in degree of cataract or uveitis between diabetic cataractous and nondiabetic cataractous groups. In addition, mean IOPs for each eye were not statistically different between diabetic cataractous and nondiabetic cataractous groups. As such, rapid TFBUTs, trend toward lower conjunctival GC densities, and alterations in conjunctival epithelial architecture noted in diabetic cataractous compared to nondiabetic cataractous dogs were not deemed to be secondary to cataracts or uveitis.

Corneal aesthesiometry, a means of evaluating corneal sensitivity, is an indirect measure of corneal innervation.[5] Similar to findings in a previous study,[5] diabetic cataractous dogs in the current study had significantly reduced corneal sensitivity compared to nondiabetic noncataractous dogs. In the previous study, corneal touch threshold (CTT; g/mm^2) was recorded with CTT being inversely proportional to corneal sensitivity. In our study, mean filament lengths (mm) were not converted to CTT values for clinically practical purposes, thus filament lengths were directly proportional to corneal sensitivity. Diabetic cataractous dogs in our study population had slightly higher central corneal sensitivity for both eyes (median filament length = 25 mm; corresponding CTT = 1.8 g/mm^2) than that previously reported for the central corneal region of diabetic dogs (median CTT = 2.8 g/mm^2; corresponding filament length = 20 mm).[5] Interestingly, there were no significant differences between corneal sensitivities for diabetic cataractous compared to nondiabetic cataractous dogs, although there was a trend toward lower corneal sensation in the diabetic cataractous group. Besides possible clinical implications of this corneal hyposensitivity in diabetic dogs,[5] use of topical nonsteroidal anti-inflammatory agents, shown to significantly decrease corneal sensation in normal human volunteers,[34] may further increase the risk of corneal disease in diabetic dogs, especially if these agents are used long-term following cataract surgery.

Human diabetic patients have also been reported to have reduced corneal sensation compared with nondiabetic controls.[10,35] This decrease in corneal sensation, a manifestation of diabetic neuropathy, has been correlated with stage of diabetic retinopathy, lending support for the notion that both diabetic neuropathy and retinopathy may result from a basement membrane abnormality.[35] Additionally, diabetic neuropathy is clinically the most well recognized long-term complication of diabetes mellitus in small animals when compared to diabetic retinopathy and nephropathy.[36] However, until the recent description of reduced corneal sensation in diabetic dogs,[5] this ocular alteration had not been documented as a potential manifestation of canine diabetic neuropathy. Several theories exist regarding the pathogenesis of late complications of diabetes mellitus, including diabetic neuropathy. Alterations in the polyol metabolic pathway with metabolic imbalances in nervous tissues, vascular changes contributing to neural hypoxia, and impaired nerve conduction have been proposed mechanisms contributing to diabetic neuropathy.[36] Targeting these areas has resulted in development of novel therapeutic strategies for certain aspects of diabetic neuropathy including corneal hyposensitivity. In particular, aldose reductase inhibitors have been shown to increase nerve conduction velocities in diabetic humans.[37] Reports have documented that treatment with topical or oral aldose reductase inhibitors results in improved corneal sensation in diabetic rats[38] and humans.[39,40] Perhaps similar therapeutic strategies may be helpful in diabetic dogs.

A previous report documented that dogs with dolichocephalic skull types have the most sensitive corneas while corneal sensitivity was lowest in brachycephalic skull-type dogs.[41] In our study, the majority of dogs assessed in all groups had mesaticephalic skull conformation, and no brachycephalic dogs were evaluated in the diabetic cataractous or nondiabetic noncataractous groups. Consequently, reduced corneal sensitivity in diabetic cataractous dogs compared to nondiabetic noncataractous dogs was not a result of our having evaluated breeds predisposed to lower corneal sensitivity. In addition, the central cornea, the region of canine cornea we

assessed, is the most sensitive of all corneal regions regardless of skull conformation[41] or glycemic status.[5]

Alterations in ocular surface parameters including aqueous tear production,[42,43] and corneal sensitivity[44] have been associated with normal aging in humans. In our study, nondiabetic noncataractous dogs were significantly younger than both diabetic cataractous and nondiabetic cataractous groups of dogs. Ocular parameters for which we detected statistically significant differences between only diabetic cataractous dogs and nondiabetic noncataractous dogs included only STT values and corneal sensitivity. It is possible that age may partially explain differences observed in these ocular parameters between these two groups of dogs. However, a previous study documented no effects of age on STT values measured both pre- and postanesthetically in dogs ranging in age from 4 months to 11 years.[22] In addition, reductions in corneal sensitivity in diabetic dogs have been reported in comparison to age-matched, normoglycemic control dogs.[5]

Tear glucose concentrations were significantly higher in diabetic cataractous dogs than both nondiabetic noncataractous and nondiabetic cataractous dogs. Elevated tear glucose levels have been reported to contribute to altered precorneal tear film stability in human diabetic patients,[45] and may account, in part, for the rapid TFBUTs we noted in our diabetic cataractous dogs. Despite elevated tear glucose concentrations and the findings of conjunctivitis, and quantitative and qualitative tear film alterations in diabetic cataractous dogs, there did not appear to be any alteration in micro-organisms isolated from diabetic cataractous dogs compared to other groups. Our findings are in contrast to a study conducted in human patients in which patients with similar local and/or systemic risk factors were nearly twice as likely to harbor antibiotic-resistant bacteria on their conjunctiva than were individuals with no such risk factors.[17] Bacterial species found in dogs in our study were compatible with previous reports documenting conjunctival microflora in healthy dogs.[13,15] However, unlike a previous report documenting fungal isolation from 22% of dogs free of ocular disease,[14] none of the canine eyes sampled in our study demonstrated fungal growth. Lack of fungal isolation from our canine globes may have been a result of differences in geographic location of these dogs, seasons in which eyes were sampled, and/or laboratory techniques.

Serum fructosamine and glycosylated hemoglobin concentration are increasingly used to complement plasma glucose concentrations to diagnose diabetes mellitus and to monitor diabetic animals' response to treatment.[46,47] In our study, neither serum fructosamine nor glycosylated hemoglobin concentration was significantly correlated with ocular parameters evaluated. Lack of correlation between glycemic control and reduced corneal sensation in diabetic dogs corresponds with findings from a previous report.[5] However, in our study, statistical power was lacking for these comparisons. Future studies may be warranted to further assess impact, if any, of glycemic control on these ocular parameters in diabetic dogs.

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