Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
27 (3): 331-337, 2021
DOI: 10.9775/kvfd.2021.25375

RESEARCH ARTICLE

Evaluation of Feline-Specific Serum Sulphated Glycosaminoglycan and Dermatan Sulphate Levels in Cats with Non-Obstructive Lower Urinary Tract Dysfunction [1]

Didem PEKMEZCI [1,a (*)] Gulay CIFTCI [2,b] Umit OZCAN [1,c] Yucel MERAL [1,d] Duygu DALGIN [1,e]

[1] *This study was funded by The Scientific Research Council of Ondokuz Mayis University in Samsun, Turkey (Project number PYO.VET.1901.17.002)*

[1] Ondokuz Mayis University, Faculty of Veterinary Medicine, Internal Medicine Department, TR-55200 Samsun - TURKEY
[2] Ondokuz Mayis University, Faculty of Veterinary Medicine, Biochemistry Department, TR-55200 Samsun - TURKEY
ORCIDs: [a] 0000-0003-2072-8165; [b] 0000-0001-5384-2381; [c] 0000-0002-0868-6399; [d] 0000-0001-9099-5061; [e] 0000-0001-5253-5232

Article ID: KVFD-2021-25375 Received: 05.01.2021 Accepted: 19.05.2021 Published Online: 19.05.2021

Abstract

Lower urinary tract dysfunction (LUTD) is a commonly seen problem in cats. This chronic condition with no specific underlying cause remains a challenge for achieving effective treatment. Glycosaminoglycans (GAGs) are linear polysaccharides possessing characteristic repeated disaccharide sequences, thought to be involved in the pathogenesis of feline LUTD. The aim of the present study is to evaluate feline-specific serum sulphated glycosaminoglycan (S-GAG) and serum dermatan sulphate (DS) levels in cats with non-obstructive LUTD versus healthy controls. Eighteen client-owned cats suffering non-obstructive LUTD and 16 client-owned healthy cats were enrolled in this case-control study. Pre-treatment serum samples from cats in both the study (non-obstructive LUTD cats) and control (healthy cats) groups were analysed with "Quantitative Sandwich ELISA method" using feline-specific S-GAG and DS kits. The mean serum S-GAG and DS levels of the study group were measured against the control group. Measurements for study males were compared to the control males, and the neutered cats in the study group were compared to the intact ones in control group. Cats in study group had lower serum S-GAG concentrations (3.52±0.26 ng/mL) than the control ones (3.93±0.27 ng/mL). Cats in study group had higher serum DS levels (27.20±6.62 ng/mL) than control cats (16.79±5.21 ng/mL). This study reports serum S-GAG and DS data in cats with non-obstructed LUTD and in healthy cats for the first time.

Keywords: *Biomarker, Cat, Dermatan sulphate, Lower urinary tract dysfunction, Sulphated glycosaminoglycan*

Tıkanıklık Olmayan Aşağı Üriner Sistem Disfonksiyonu Olan Kedilerde Felin Spesifik Serum Sulfürlü Glikozaminoglikan ve Dermatan Sülfat Seviyelerinin Değerlendirilmesi

Öz

Alt üriner sistem disfonksiyonu (AÜSD) kedilerde yaygın olarak görülen bir sorundur. Altta yatan belirli bir nedeni olmayan bu kronik durum, etkili tedaviye ulaşmak için bir zorluk olmaya devam etmektedir. Glikozaminoglikanlar (GAG'lar), AÜSD'nun patogenezinde rol oynadığı düşünülen karakteristik tekrarlanan disakkarit sekanslarına sahip doğrusal polisakkaritlerdir. Bu çalışmanın amacını, tıkanıklık olmayan AÜSD gösteren kedilerde, kedilere özgü serum sülfatlanmış glikozaminoglikan (S-GAG) ve dermatan sülfat (DS) düzeylerinin sağlıklı kontrollere göre karşılaştırılması oluşturmuştur. Bu vaka kontrol çalışmasında, tıkanıklık olmayan AÜSD'dan mustarip, sahipli on sekiz kedi ile sahipli 16 adet sağlıklı kedi yer almıştır. Hem çalışma (tıkanıklık olmayan AÜSD kediler) hem de kontrol (sağlıklı kediler) grubundaki kedilerden alınan tedavi öncesi serum örnekleri, kedilere özgü S-GAG ve DS kitleri kullanılarak "Kantitatif Sandviç ELISA yöntemi" ile analiz edilmiştir. Çalışma ve kontrol gruplarına ait tüm kediler ile, çalışma ve kontrol grubundaki erkek kediler ve her iki gruptaki sterilize edilmiş kedilerin ortalama serum S-GAG ve DS seviyeleri karşılaştırılmıştır. Çalışma grubunun serum S-GAG konsantrasyonları (3.52±0.26 ng/mL) kontrol grubuna kıyasla daha düşük (3.93±0.27 ng/mL) bulunmuştur. Tersine çalışma grubu serum DS seviyeleri ise (27.20±6.62 ng/mL) kontrol grubuna (16.79±5.21 ng/mL) oranla yüksek bulunmuştur. Bu çalışma, tıkanma olmayan AÜSD'si kedilerde ve sağlıklı kedilerde ilk kez serum S-GAG ve DS verilerini bildirmektedir.

Anahtar sözcükler: *Alt üriner sistem disfonksiyonu, Biyobelirteç, Kedi, Dermatan sülfat, Sülfürlenmiş glikozaminoglikan*

How to cite this article?

Pekmezci D, Ciftci G, Ozcan U, Meral Y, Dalgin D: Evaluation of feline-specific serum sulphated glycosaminoglycan and dermatan sulphate levels in cats with non-obstructive lower urinary tract dysfunction. *Kafkas Univ Vet Fak Derg,* 27 (3): 331-337, 2021.
DOI: 10.9775/kvfd.2021.25375

(*) Corresponding Author

Tel: +90 362 312 1919, Cellular phone: +90 505 477 9524
E-mail: dkazanci@omu.edu.tr (D. Pekmezci)

Introduction

Feline lower urinary tract dysfunction (FLUTD) with a subset of problems, such as haematuria, periuria, pollakiuria, and stranguria, is a commonly seen problem in cats. It may occur as acute or chronic and can result from various abnormalities within the lumen of the lower urinary tract (local, external abnormalities) or other organ systems (internal abnormalities) that lead to dysfunction [1]. Various known aetiologies for FLUTD include bladder stones, bacterial and viral infections, urethral plugs, and neoplasia. However, bacterial infections associated with urinary system rarely seen in cats [2]. Urethral plugs associated with mucous-based sludge within the bladder are commonly seen as causative agents [3,4].

The terms "feline interstitial cystitis" or "feline idiopathic cystitis (FIC)" have been described for referring to the chronic conditions with no specific underlying cause of FLUTD as a naturally occurring model of interstitial cystitis (IC) in women [5,6]. Recently, the term "Pandora syndrome" proposed for describing chronic, recurrent FLUTD signs in the presence of comorbid disorders, such as behavioural, dermatological, endocrine, or gastrointestinal disorders, until a more biologically appropriate term is accepted [1]. Moreover, many treatment models for the aforementioned challenging situations in cats still seek understanding of their aetiologies and new treatment options [7-9].

To date, research has been focussed on creating a successful FIC or non-obstructive FLUTD treatment protocol, especially models of treatment protocols for IC in women, while cats, llamas, and dogs present similar signs of IC [10]. Urothelial ulceration with mural inflammation and fibrosis without bacterial cystitis in cats are accepted as the most similar findings to human IC [11]. Male, middle-aged (≈2-7 years), overweight cats are found to be at risk for having FIC [12]. FIC and IC share many similarities; the only major difference between the two seems to be the gender distribution. However, recent data shows that males suffering from some forms of chronic prostatitis could also have IC [13]. Findings in the last decade suggest that damage to the urothelial GAG layer might be played role in IC pathophysiology [14,15]. Moreover, it is now well-known that normal bladder urothelium in humans is lined with a specific GAG, defined as GP-51 [16], that inhibits bacterial adherence and protects the urothelium from noxious urine constituents. Alterations in urine GAGs have been reported in urolithiasis [17], renal injury [18], and IC [19]. However, decreased amounts of urine GAGs have also been reported in cats with FIC [10] and Feline Urologic Syndrome [20].

Glycosaminoglycans (GAGs) are linear polysaccharides possessing characteristic repeated disaccharide sequences, thought to be involved in several immune, cancer, inflammatory, and degenerative diseases [21,22]. Chondroitin sulphate (CH), DS, heparin (H), heparan sulphate (HS), keratan sulphate (KS), and hyaluronan are the most common GAG structures that are important biological response modifiers by acting as stabilisers, cofactors, or coreceptors for growth factors, cytokines, and chemokines [23].

New discoveries of the biological properties of GAGs-such as signalling molecules in response to cellular damage, including wounding, infection, and tumorigenesis-make these mucopolysaccharides have been the subject of more studies [23]. Semi-synthetic GAGs, such as *N*-acetyl glucosamine [24-26] and Pentosan polysulfide sodium [2,9], are the most commonly used agents in treating cats with FIC. It is still unknown whether GAG deficiency (when it occurs) is the primary reason for IC in people or whether it is secondary to other bladder processes, such as inflammation [27]. Another important issue to be clarified is how urine GAG levels reflect the state of the urothelium [27]. Pereira et al.[20] affirmed that cats with FUS might also have a decreased concentration of circulating GAGs.

Therefore, the main purpose of this study is to elucidate this unsolved issue by comparing serum feline S-GAG and DS levels in cats with non-obstructive LUTD to healthy cats.

Material and Methods

Ethical Statement

The present study was also approved by the Ondokuz Mayis University Animal Experiments Local Ethics Committee (Approval no: 2017/05).

Patients

Thirty-four client-owned cats between 5 months and 13 years of age, any breed and either sex, brought to the Veterinary Teaching Hospital between June and December 2017 were enrolled in the current case-control study. Eighteen cats diagnosed with non-obstructive LUTD formed the study group. None of the 16 healthy cats that formed the control group had any clinical signs of non-obstructive LUTD or other diseases prior to the sampling. Cats having urinary tract infections, azotaemia, diabetes mellitus, or hyperthyroidism with any recently administered GAG were excluded from the study. Client consent forms were provided to owners for all cats enrolled in this study. Appropriate treatments for the cats diagnosed with non-obstructive LUTD were performed, according to their clinical symptoms and the aetiologies of their LUTDs.

Procedures

All cats enrolled in the present study underwent a standard physical examination by the same veterinarian at their first visit. An FLUTD evaluation chart modified from Meyer and

Bečvářová [8] was completed for the study group. At the time of admission to the Veterinary Teaching Hospital, venous blood was taken from the cephalic vein of all searched cats, with 2 mL evacuated into a plain additive tube with K_3 EDTA (7.5% 0.040 mL) and 5 mL into a vacutainer without anticoagulant, for biochemical analysis. The 5 mL sample was centrifuged at 3000 g for 10 min at room temperature. The serum samples were separated and stored at -80ºC until analysis. Complete Blood Count analysis was performed by a BC-5000 Vet Auto Hematology Analyzer Mindray, and results were recorded. Ultrasonographic examinations of the extended urine bladders of the study group cats was performed with a micro convex high-frequency 3.5-7.0 MHz transducer (MyLab30; Esaote Pie Medical). The existence of a urolith was investigated by presenting the twinkling artefact (TA) during the colour Doppler ultrasonography examinations [28]. Urine samples were collected by voiding the midstream or catheterisation of the study group at the time of initial presentation. Cybow Urine reagent strips were used for analysis of pH, blood, leukocyte, nitrite, protein, ascorbic acid, ketone, glucose, and specific gravity levels. Microscopic examination of the urine sediment was also done, looking for existing RBC, WBC, casts, and crystals. While detection of nitrite in urine is routinely used for bacterial cystitis [29], any of the collected urine samples were submitted for quantitative urine culture because of their nitrite-negative status in the urine reagent strip analyses.

Serum S-GAG and DS Determination

The thirty-four stored serum samples from the cats in the present study were measured by the Quantitative Sandwich ELISA method using Cat S-GAG and Cat DS ELISA kits (MyBioSource©, San Diego, CA, USA, Cat. No.: MBS9348264, MBS077381), following the manufacturer's instructions. All samples were later measured on a spectrophotometer (Digital and Analog Systems S.R.L.).

Statistical Analysis

Feline serum-specific S-GAG and DS levels were the analysed parameters. The datasets were analysed for normality using the Kolmogorov-Smirnov test. The quantitative data on feline serum S-GAG and DS levels was found to be normally distributed. The differences between the means of feline serum S-GAG and DS levels in the study group versus the control group, between the two sexes, and between neutered cats versus non-neutered cats were compared using the Student-t test. The Mann-Whitney U test was used for the variables that did not show normality. The significance level of all comparisons was considered when P values <0.05. Sample size was calculated using an additional software (Gpower 3.0.10). The main outcome of the study sample size was the incidence of non-obstructed LUTD cases. Eighteen cats in the study group are required to demonstrate a difference with 95% statistical confidence and a power of 80%.

Results

Thirty-four cats were enrolled in this case-control study. Eighteen of these cats were clinically diagnosed with non-obstructed LUTD. Sixteen were clinically healthy controls. The mean ages of the non-obstructed LUTD and the clinically healthy cats were 5.3 and 4 years, respectively. The mean weight of the study and control cats were 3.9 and 3.3 kg, respectively. Ten of the 34 (29.4%) cats were domestic, short-haired cats. Eight (23.5%) were mackerel tabby, 7 (20.5%) were orange tabby, 5 (14.7%) were Persians, and there was one Chinchilla (2.9%), Van (2.9%), British short-haired (2.9%), and Scottish Fold (2.9%) cat. Sixteen (88.8%) cats in the study group were neutered, and four cats in the control group were neutered (25%). The CBC results of all the searched cats were within the reference ranges [30]. All cats in the study group demonstrated perianal grooming behaviour to varying degrees, at their clinical scoring. None of the study cats showed a TA during the colour Doppler ultrasonography examination. Urobilinogen, glucose, bilirubin, ketone, and nitrite were all negative in the urine dipsticks of the cats with non-obstructed LUTD. Most of the cats with non-obstructed LUTD had a urine specific gravity (SG) >1035. There were only four study cats that had a urine specific gravity <1035. However, their serum creatinine and urea concentrations were within the reference intervals. The urine pH of the study group cats measured 5.5-8.0. Seven of the study cats (38.8%) had a 2+ or 3+ dipstick protein reaction. Twelve (66.6%) of the cats with non-obstructed LUTD had more than three white blood cells in their microscopic urine sediment examination. Only four (22.2%) cats in the study group also had more than three red blood cells per high-power field. Struvite crystals (ST) were the most prevalent type crystals found in the urine sediment of the study cats (44.4%). However, four of the study cats (22.2%) had Ca oxalate monohydrate (CaOXM) crystals in their urine sediment. Two cats (11.1%) had both ST and CaOXM crystals in their urine sediment. Only two cats (11.1%) in the study group had more than three transitional epithels (TE) per high-power field in their urine sediment. Renal epithels (RE) were not seen in any of the study group cats *(Table 1)*.

The mean serum S-GAG level in cats with non-obstructed LUTD (3.52±0.26 ng/mL) was lower than the healthy cats' level (3.93±0.27 ng/mL) *(Table 2)*, however were not significantly different between the groups (P>0.05) *(Fig. 1)*. The cats with non-obstructed LUTD had higher serum DS (27.20±6.62 ng/mL) levels than the control group (16.79±5.21 ng/mL) *(Table 2)*. This difference was also not statistically significant (P>0.05) *(Fig. 1)*. The mean S-GAG levels in the male cats with non-obstructed LUTD (3.69±0.26 ng/mL) were not statistically different (P>0.05) *(Table 2)* from the healthy male cats' levels (3.98±0.45 ng/mL) *(Fig 2)*. The mean DS levels in the male cats with non-obstructed LUTD (28.67±7.79 ng/mL) were also not statistically different (P>0.05) *(Table 2)* from the healthy male cats' levels

Table 1. *Results of the urinalysis of the cats with non-obstructed LUTD*

Parameter	Value	Non-obstructed LUTD Cats		Parameter	Value	Non-obstructed LUTD Cats	
		n (18)	Percentage (%)			n (18)	Percentage (%)
Urine colour	Normal Concentrated Haematuria	13 1 4	72.2 5.5 22.2	Ascorbic acid	Negative + ++ +++	9 3 6 0	50 16.6 33.3 0
Urobilinogen	Negative	18	100	sWBC	0-3/Hpf >3/Hpf	6 12	33.3 66.6
Glucose	Negative	18	100	sRBC	0-3/Hpf >3/Hpf	16 4	88.8 22.2
Bilirubin	Negative	18	100	sTE	0-3/Hpf >3/Hpf	15 3	83.3 16.6
Ketone	Negative	18	100	sRE	Negative	18	100
SG	>1.035 <1.035	14 4	77.7 22.2	sST	Negative + ++ +++	10 4 3 1	55.5 22.2 16.6 5.5
Blood	Negative + ++ +++	11 3 1 3	61.1 16.6 5.5 16.6	sCaOXM	Negative + ++ +++	14 3 0 1	77.7 16.6 0 5.5
pH	5.0-6.0 6.0-7.0 7.0-8.0	3 12 3	16.6 66.6 16.6	TA	Negative Positive	18 0	100 0
Protein	Negative + ++ +++	11 0 4 3	61.1 0 22.2 16.6	Bladder Uroliths	Negative	18	100
Leukocyte	Negative + ++ +++	0 5 2 11	0 27.7 11.1 61.1	Perianal Grooming	Negative + ++ +++	0 12 4 2	0 66.6 22.2 11.1
Nitrite	Negative	18	100				

sWBC: *White Blood Cells in the high-power field,* ***sRBC:*** *Red Blood Cells in the high-power field,* ***sTE:*** *Transitional epithels in the high-power field,* ***sRE:*** *Renal epithels in the high-power field,* ***sST:*** *Struvite crystals in the high-power field,* ***sCaOXM:*** *Ca oxalate monohydrate crystals in the high-power field*

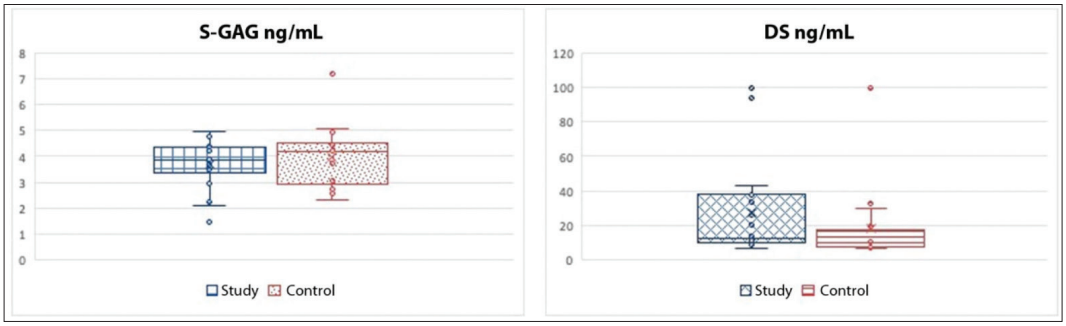

Fig 1. Box-and-whiskers plot of serum S-GAG and DS values in study (blue boxes) or control (red boxes) groups. The box incorporates the middle 50% of observation; the bottom of the box is the first quartile (25th percentile) and the top of the box is the third quartile (75th percentile). The horizontal line in the middle of the box is the median (50th percentile). The cross within each box represents the mean value. The whiskers extend to the smallest and largest observations that are 1.5 times removed from the interquartile range are plotted separately as dots

(23.14±9.07 ng/mL) *(Fig. 2)*. The mean serum S-GAG levels in neutered cats with non-obstructed LUTD (3.56±0.28 ng/mL) and in neutered control cats (3.53±0.23 ng/mL) *(Table 2)* were not significantly different (P>0.05) *(Fig. 3)*. Neutered cats with non-obstructed LUTD had higher serum DS levels (23.66±5.65 ng/mL) than the neutered control group (13.29±2.63 ng/mL) *(Table 2)*. However, this difference was not statistically significant (P>0.05) *(Fig. 3)*.

Discussion

The pioneer study conducted by Pereira et al.[20] provided the inspiration for the study. Pereira et al.[20] hypothesised that cats with low GAG levels in urologic syndrome might have damaged bladder surfaces and might also have a decreased concentration of circulating GAGs. We aimed to investigate Pereira et al.'s [20] results by comparing serum

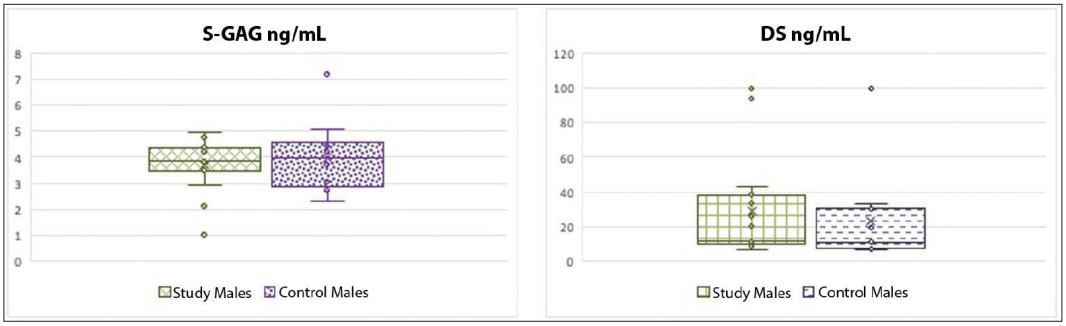

Fig 2. Box-and-whiskers plot of serum S-GAG and DS values in study male cats (green) or control male cats (purple). The box incorporates the middle 50% of observation; the bottom of the box is the first quartile (25th percentile) and the top of the box is the third quartile (75th percentile). The horizontal line in the middle of the box is the median (50th percentile). The cross within each box represents the mean value. The whiskers extend to the smallest and largest observations that are 1.5 times removed from the interquartile range are plotted separately as dots

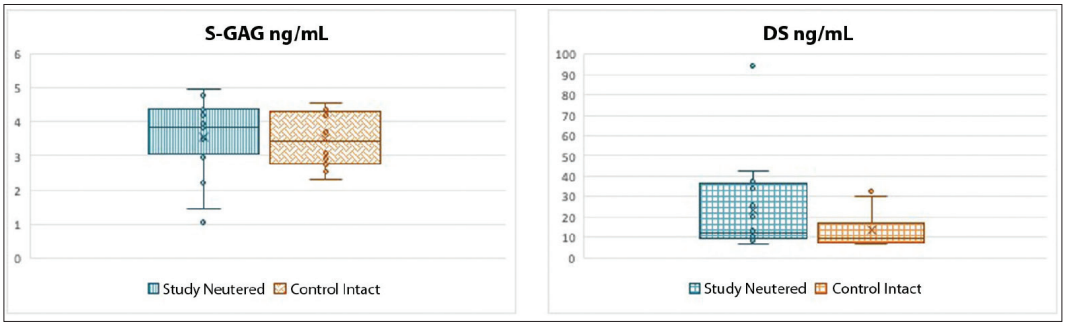

Fig 3. Box-and-whiskers plot of serum S-GAG and DS values in neutered cats in study group (turquoise) or intact control cats (orange). The box incorporates the middle 50% of observation; the bottom of the box is the first quartile (25th percentile) and the top of the box is the third quartile (75th percentile). The horizontal line in the middle of the box is the median (50th percentile). The cross within each box represents the mean value. The whiskers extend to the smallest and largest observations that are 1.5 times removed from the interquartile range are plotted separately as dots

Table 2. *Mean S-GAG and DS levels (ng/mL) of the Study and Control cats*

Parameters	Groups		P Value
	Study Cats	Control Cats	
Mean±S.E. S-GAG levels (ng/mL)	3.52±0.26	3.93±0.27	0.293
Mean ± S.E. DS levels (ng/mL)	27.20±6.62	16.79±5.21	0.226
	Study Male Cats	**Control Male Cats**	
Mean ± S.E. S-GAG levels (ng/mL)	3.69±0.26	3.98±0.45	0.554
Mean ± S.E. DS levels (ng/mL)	28.67±7.79	23.14±9.07	0.652
	Study Neutered Cats	**Control Neutered Cats**	
Mean ± S.E. S-GAG levels (ng/mL)	3.56±0.28	3.53±0.23	0.930
Mean ± S.E. DS levels (ng/mL)	23.66±5.65	13.29±2.63	0.148

feline S-GAGs and DS levels in cats with non-obstructive LUTD against healthy controls.

Interestingly, the main finding of the study presented here was an unexpected result. Contrary to the results found by Pereira et al.[20] our results showed that feline pre-treatment serum S-GAG and DS levels in cats with non-obstructed LUTD versus did not differ from the levels in the healthy controls. Research about FIC treatments showed that these cats have decreased levels of urine GAGs [20,31],

and treatment options are mainly aimed at replacing this deficiency by using semi-synthetic GAGs with various routes [2,7,9,24,26]. However, all these study results were not able to show the beneficial result of decreasing the recurrence rate, with amelioration of the clinical signs, by using semi-synthetic GAGs in FIC patients. Therefore, circulating GAG levels in FIC, both in cats with LUTD and their relations among the disease processes, has to be investigated, to determine an accurate treatment protocol.

To our knowledge after Pereira et al.[20], there was only one study that presents plasma GAG concentrations in FIC patients [25].

In the study presented here, we used feline-specific ELISA kits, rather than human kits. Therefore, our results first demonstrate the pre-treatment serum S-GAGs and DS levels in cats with non-obstructed LUTD against healthy controls. Our mean serum S-GAG level (3.52±0.26 ng/mL) in cats with non-obstructed LUTD was found to be lower than in healthy controls (3.93±0.27 ng/mL), but a statistically significant difference between groups was not found (P>0.05).

The DS level was the other investigated parameter in our study. Pereira et al.[20] showed that the main GAGs found in a cat's kidney and urinary tract was HS and DS. They also found that, in contrast to other GAGs, CS was the only GAG detected in the plasma of cats having urologic syndrome. Contrary to Pereira et al.[20], DS was measured in the cats' serum in the present study. Moreover, in the present study, cats with non-obstructed LUTD had higher serum DS levels (27.20±6.62 ng/mL) than the control group (16.79±5.21 ng/mL), but again this difference was not found to be statistically significant (P>0.05).

The trend of increased mean DS levels in the study group may be explained by the relationship between DS, chemokines, and cytokines. Recently, Parys et al.[32] investigated serum cytokine profiling in cats with acute FIC and found that serum concentrations of the pro-inflammatory cytokines and chemokines CXCL12, IL-12, IL-18, and Flt3L were increased in FIC-affected cats. Moreover, Brooks et al.[33] showed that Interferon gamma also binds to DS. IL-8, MIP-1α, and β (macrophage inflammatory peptides), and monocyte chemoattractant protein-1 (MCP-1) are the proteins that are able to bind to GAGs [23].

Therefore, pro-inflammatory processes in cats with non-obstructed LUTD may also increase some cytokines and chemokines, which later may have led to an increase in circulating DS in our non-obstructed LUTD cats.

The mean S-GAG levels in the male cats with non-obstructed LUTD were not statistically different from the healthy male cats' levels (3.69±0.26 ng/mL and 3.98±0.45 ng/mL, respectively). In the same vein, mean DS levels in the male cats with non-obstructed LUTD (28.67±7.79 ng/mL) were also not statistically differentiated (P>0.05), when compared to healthy male cats' levels (23.14±9.07 ng/mL). Also, no difference was found when comparing mean S-GAGs and DS levels between the neutered cats in both groups. However, the trend of increasing mean DS levels remained in the neutered cats with non-obstructed LUTD.

The studies investigating the relationship between GAGs in FIC and LUTD in cats mainly reported decreased urine or plasma GAGs. Therefore, one could expect a decreased concentration of circulating GAGs in this study's cats. In one study that evaluates the changes in total serum GAG levels in patients undergoing renal transplantation showed that measuring total serum GAG levels is more credible than measuring urinary GAG levels, since urine output may be compromised and does not accurately reflect the concentration of circulating GAGs, especially in patients with graft rejection [22]. This scenario may be valid for cats with FIC and LUTD.

However, our results showed that there were no differences of serum GAG levels between cats with non-obstructed LUTD and healthy cats. The main reason for this could be the duration of the disease. The cats in this study had their first LUTD diagnosis during their visits to the Veterinary Teaching Hospital. Contrary FIC the span of the inflammation could be the most important cause that may affect the circulating GAGs levels in non-obstructed LUTD in cats. Hence, a main limitation of the present study is that it was missing FIC patients. Further studies to confirm the suspected role of GAGs and their individual circulating levels in FIC and FLUTD are needed.

In conclusion, this study reports serum S-GAGs and DS levels in cats with non-obstructed LUTD against healthy cats. We believe that our findings may help develop a better understanding of the pathophysiology of FLUTD and FIC and the development of new treatment strategies.

Acknowledgements

The authors are especially thankful to Prof. Dr. Filiz Akdag for performing the statistical analyses of this study. We are appreciated to our clients for their special effort and thank to all of them for allowing their lovely cats to the present study.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

DP coordinated the study and drafted the manuscript. DP also participated in the collection and preparation of samples and participated in the design of the study. GC

carried out the out the immunoassays and participated in the design of the study. UO, YM and DD participated in the collection and preparation samples and all participated in the design of the study. All authors also approved the final version of the article.

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