A THESIS FOR THE DEGREE OF MASTER

Effect of oligo-fucoidan, fucoxanthin, and L-carnitine on chronic kidney diseases in dogs: a retrospective study

개의 만성 신부전에서 올리고-푸코이단, 푸코잔틴, L-카르니틴의 효과: 후향적 연구

2023년 8월

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수의학과 임상수의학(수의내과학) 전공
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이 논문을 수의학 석사 학위논문으로 제출함

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홍나은의 석사 학위논문을 인준함

2023년 7월

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Abstract

Effect of oligo-fucoidan, fucoxanthin, and L-carnitine on chronic kidney diseases in dogs: a retrospective study

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Chronic kidney disease (CKD) commonly occurs in old dogs and cats. Oligo-fucoidan, fucoxanthin, and L-carnitine (OFL) compounds have a variety of reno-protective properties, including anti-inflammatory, anti-oxidative, and anti-fibrotic effects. Because their effects have not been investigated in naturally occurring canine CKD, their reno-protective activities in dog patients with CKD were examined.

A total of 57 patients (OFL, n=32; control, n=25) were included in the analysis. A significant difference was identified in serum blood urea nitrogen (BUN) and creatinine (CREA) concentrations between the control and OFL groups at 6 months (BUN, $p=0.0058$; CREA, $p<0.0001$). No significant difference in electrolytes was found between the groups. A significant difference was identified in serum CREA concentration between the control and OFL groups in azotemic (CKD

IRIS stage 2–4) at 6 months ($p<0.0001$).

The OFL compounds showed a reno-protective effect, consistent with previous animal studies. The OFL combination can potentially delay the progression of canine CKD and be used as an adjuvant therapy.

Keywords: Dog, Chronic kidney disease, Reno-protective, Oligo-fucoidan, Fucoxanthin, L-carnitine

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1. Introduction

Chronic kidney disease (CKD) is common in old dogs and cats. Patients with CKD have impaired renal structures and decreased renal function. Because renal degeneration is generally irreversible, treatment of CKD is focused mainly on conserving the remaining renal function [1]. The median survival age of dogs with CKD requires further research, but previous studies have suggested 174–336 days [2-4].

Fucoidan is a sulfated polysaccharide extracted from echinoderms or marine plants such as brown algae [5]. Fucoidan has a variety of effects, including antioxidant, anti-coagulant, immunomodulatory, anti-inflammatory, and anti-tumor properties [6]. It has also demonstrated reno-protective anti-inflammatory, anti-oxidative, and anti-fibrotic effects in previous studies [5]. For example, fucoidan alleviates renal-associated blood levels in CKD animal models by reducing renal fibrosis [7]. Fucoxanthin is a carotenoid abundant in brown algae and has many pharmacological activities, including anti-inflammatory, anti-tumor, and antioxidant properties, through which it exerts renal protective effects [8, 9]. L-carnitine is a quaternary amino acid involved in many metabolic pathways in the body [10]. It is obtained from dietary sources or synthesized from lysine and methionine in the liver and kidney [11]. L-carnitine alone does not reduce renal fibrosis, but L-carnitine provides synergistic effects on renal function when combined with fucoidan and fucoxanthin [12].

Although fucoidan, fucoxanthin, and L-carnitine (OFL) compounds have

potential kidney benefits, their combined effect on naturally occurring CKD in dogs has not been investigated. To explore these effects, the renal protective activities of these OFL compounds in dog patients with CKD was investigated.

2. Materials and Methods

2.1. Case selection

This retrospective multi-center study reviewed the medical data of canine patients with CKD. The Veterinary Medical Teaching Hospital of Seoul National University and the VIP Animal Medical Center in the Republic of Korea participated in the study. The data between January 1, 2020, and October 31, 2022, were reviewed through an electronic charting program (E-friends: Pet Network Veterinarian, Seoul, Korea). All patients' owners signed informed consent forms at the first visit.

The study enrolled canine CKD patients who received the OFL-based supplement (Fuco K, Hi-Q Marine Biotech International Ltd., Taipei, Taiwan) and regularly visited for check-ups on health status and kidney-related blood analysis for more than 6 months. Forty-three patients were selected as the test group (Fuco K–receiving group), but eleven were excluded for not meeting the study criteria (lost to follow-up or discontinuance of supplements). Twenty-five patients who did not receive the supplement were selected as a control group. In total, fifty-seven patients were included in the analysis.

Through e-charts, patients' information (breed, sex, and age), medical records, and concurrent disease data were collected. Each patient's vital signs (rectal temperature, heart rate, respiratory rate, and blood pressure), body weight, blood analysis (including serum chemistry and electrolytes), urinalysis, radiographs, and abdominal ultrasound results were reviewed. The kidney value such as blood urine

nitrogen (BUN), creatinine (CREA), calcium (CA) and inorganic phosphate (IP) were collected monthly for 6 months. The diagnoses, stages, and substages of CKD were determined using the International Renal Interest Society (IRIS) criteria.

Oligo-fucoidan, fucoxanthin, and L-carnitine were administrated using an OFL-based supplement (Fuco K). The dose of Fuco K was administered following the manufacturer's recommendations: 1 capsule for dogs of 1–5 kg or 2 capsules for dogs of 6–10 kg, once a day, with and without food. Each capsule of Fuco K contained 125 mg of oligo-fucoidan, 125 mg of high-soluble fucoxanthin, and 50 mg of L-carnitine.

2.2. Statistical analysis

GraphPad Prism software version 6.01 (GraphPad, Inc., La Jolla, CA, USA) was used for statistical analysis. Normality was evaluated using the Shapiro–Wilk test. Results of data is presented as the mean ± standard deviation. Differences among groups were evaluated using the Mann–Whitney test or the Kruskal–Wallis test. In all comparisons, a probability value of $p < 0.05$ was considered statistically significant unless otherwise stated.

3. Results

3.1. Study population

The OFL group comprised 43 dog patients (compound-receiving group). Of the 43 dogs, 4 discontinued compound usage, and 7 died before 6 months (3 died from worsening CKD and 4 from other causes); these 11 dogs were excluded from the study. As a control group, 25 CKD patients who did not receive the OFL compounds were selected. A total of 57 patients were included in the analysis.

The characteristics of the patients are summarized in Table 1. Maltese, Pomeranian, Poodles, and Shih-tzu were the most common breeds in both the OFL and control groups. In the OFL group, 14 dogs were male (12 castrated, 2 intact) and 18 were female (15 spayed, 3 intact). In the control group, 15 dogs were male (13 castrated, 2 intact) and 10 were female (all spayed). The median age was 13 years (range: 4–17 years) in the OFL group and 14 years (range: 8–18 years) in the control group. The median body weight was 3.3 ± 9 kg in the OFL group and 4.2 ± 3 kg in the control group.

The median International Renal Interest Society (IRIS) CKD stage was 2 for both groups. In the OFL group, 7, 22, 2, and 1 were IRIS stages 1, 2, 3, and 4, respectively. In the control group, 5, 19, 1, and 0 were IRIS stages 1, 2, 3, and 4, respectively. The median systolic blood pressure was 150 ± 24.2 mmHg in the OFL group and 150 ± 28.2 mmHg in the control group. In the OFL group, 8 dogs were normotensive, 7 pre-hypertensive, 9 hypertensive, 3 severely hypertensive, and 5

were unknown due to lack of blood pressure records. In the control group, 7 dogs were normotensive, 10 pre-hypertensive, 5 hypertensive, and 3 were severely hypertensive. For assessing proteinuria, urinalysis was reviewed. In the OFL group, 5 dogs were proteinuric, 2 borderline proteinuric, 10 non-proteinuric, and 15 were unknown due to lack of a urine protein creatinine ratio (UPC) value. In the control group, 8 dogs were proteinuric, 1 borderline proteinuric, 3 non-proteinuric, and 13 were unknown.

The most common concurrent diseases were canine valvular heart disease (CVHD) (n=32), tracheal collapse (n=25), and chronic pancreatitis (n=9). Of the dogs with CVHD, some received concurrent medications, including pimobendane (n=22), loop-diuretics (n=18), spironolactone (n=12), and Angiotensin-converting enzyme inhibitor (ACEi) (n=23). Of the dogs with tracheal collapse, 17 were receiving theophylline. For the management of CKD, subcutaneous fluid was the most common treatment (n=22). Polyunsaturated fatty acids supplement (n=20), renal supportive supplement (Renal Advanced, Candioli Pharma; Beinasco, Italy) (n=20), and probiotics (Azodyl, Vetoquinol USA; Texas, USA) (n=14) were the next most common.

3.2. Effect of oligo-fucoidan, fucoxanthin, and L-carnitine on renal function in CKD dogs

All of the 57 dogs in this study were evaluated for pretreatment serum BUN and CREA. At 6 months, 30 dogs in the OFL group (93%) and 25 dogs in the control group (100%) were evaluated for serum BUN and CREA levels. In the OFL group,

20 (62%) dogs visited the hospital every month, 2 (6.25%) dogs missed 1 visit, 4 (12.5%) dogs missed 2 visits, 3 (9.38%) dogs missed 3 visits, and 3 (9.38%) dogs missed more than 4 visits. In the control group, 12 (48%) dogs visited the hospital every month; 6 (24%) dogs missed 1 visit; 4 (16%) dogs missed 2 visits; 2 (8%) dogs missed 3 visits; and 1 (4%) dog missed 4 visits.

The mean pretreatment serum BUN levels were 32.27 ± 14 mg/dL in the OFL group and 41.6 ± 16 mg/dL in the control group (reference range: 9.6–31.4). Until 5 months, no significant difference was found in serum BUN between the OFL and control groups (Figure 1). However, at 6 months, the mean serum BUN levels in the OFL group (41.13 ± 36 mg/dL) and control group (60.2 ± 36 mg/dL) were significantly different ($p=0.0058$). The mean pretreatment serum CREA levels were 1.66 ± 0.63 mg/dL in the OFL group and 1.61 ± 0.54 mg/dL in the control group (reference range: 0.4–1.3). Until 5 months, no significant difference in serum CREA level was found between the OFL and control groups (Figure 1). However, at 6 months, the mean serum CREA levels in the OFL group (1.75 ± 0.88) and the control group (2.46 ± 1.28) were significantly different ($p<0.0001$).

For most of the 57 dogs in this study, serum CA (n=50) and IP (n=52) were evaluated at the pretreatment point. The mean pretreatment serum CA levels were 11.24 ± 5.24 mg/dL in the OFL group and 10.16 ± 1.29 mg/dL in the control group (reference range: 9.0–11.9). The mean pretreatment serum IP levels were 4.32 ± 1.8 mg/dL in the OFL group and 4.15 ± 1.15 mg/dL in the control group (reference range: 2.3–6.3).

3.3. Assessment of electrolytes after treatment with oligo-fucoidan,

fucoxanthin, and L-carnitine

For most of the 57 dogs in this study, the serum electrolytes sodium ion (Na^+) (n=49), potassium ion (K^+) (n=49), and chloride (Cl^-) (n=48) were evaluated at the pretreatment point. The mean pretreatment serum Na^+ levels were 147.2 ± 4.19 mmol/L in the OFL group and 145.5 ± 3.34 mmol/L in the control group (reference range: 145.1–152.6). The mean pretreatment serum K^+ levels were 4.67 ± 0.61 mmol/L in the OFL group and 4.87 ± 0.67 mmol/L in the control group (reference range: 3.6–5.5). The mean pretreatment serum Cl^- levels were 115.0 ± 4.97 mmol/L in the OFL group and 113.73 ± 4.08 mmol/L in the control group (reference range: 113.2–122.9). No significant difference in electrolytes was found between the two groups during the study period (Figure 2).

3.4. Effect of oligo-fucoidan, fucoxanthin, and L-carnitine on renal function in CKD dogs according to the non-azotemic and azotemic groups

Of the 35 dogs in the OFL group, 7 were non-azotemic (CKD IRIS stage 1) and 25 were azotemic (CKD IRIS Stage 2–4). Of the 25 dogs in the control group, 5 were non-azotemic (CKD IRIS stage 1) and 20 were azotemic (CKD IRIS Stage 2–4). Among the non-azotemic dogs, the differences in BUN and CREA between the OFL and control groups were not statistically significant (Figure 3). Among the azotemic dogs, the difference in BUN between the OFL and control groups was not statistically significant; however, the difference in serum CREA between the two groups at 6 months was statistically significant (1.87 ± 0.89 in the OFL group and

2.77 ± 1.22 in the control group) ($p<0.0001$).

3.5. Adverse reactions

To assess adverse reactions to the OFL compounds, all dogs in the OFL group were reviewed for history, clinical signs, and vital signs. None of the dogs was reported to have adverse effects after administration of the OFL compounds.

4. Discussion

This study aimed to determine the changes in kidney-related blood factors when OFL compounds are administered in canine CKD patients and to investigate the possibility of using these compounds as an adjuvant therapy for patients with naturally occurring CKD.

Comparing the changes in serum BUN and CREA for 6 months, the control group showed a greater increase in serum BUN and CREA than the OFL group (Figure 1). The difference between the two groups was not significant until 5 months, but statistical significance was identified at 6 months. As CKD progressed, serum BUN and CREA levels increased in the control group. These results suggest that an OFL-based supplement could delay increases in serum BUN and CREA levels; this effect appears after at least 6 months of administration.

After applying the OFL compound to dogs with naturally occurring CKD, the treatment mechanism has not been studied. Therefore, further research is needed to elucidate the mechanism of prolonging the renal disease delay caused by the OFL compound. However, studies have been conducted in mouse models to clarify this mechanism. Previous studies showed that fucoidan and fucoxanthin have protective effects on the kidney. Fucoidan significantly decreased the levels of serum CERA and BUN in a rat model of chronic renal failure [13, 14]. Although more research is needed, the anti-inflammatory effect of fucoidan was exerted in vitro through decreased cytokine levels (TNF-α, IL-1β, and IL-6) and suppression of signal pathways (MAPK, NF-κB) in vivo [15]. Fucoxanthin reduces apoptosis of renal

tubular cells in a CKD mouse model via increasing expression of the Na^+/H^+ exchanger isoform 1 [8]. Furthermore, using fucoidan and fucoxanthin together has synergistic effects, including reducing serum CREA in CKD mice, inhibiting renal fibrosis, and reducing reactive oxygen species generation and apoptosis [9, 12].

L-carnitine plays an essential role in the utilization of fatty acids in the mitochondria [16]. The kidney synthesizes and metabolizes L-carnitine in animals, and a beneficial effect of L-carnitine in the kidneys has been suggested. L-carnitine inhibits gentamicin-induced apoptosis of renal tubular cells in a rat cell line via PGI2-mediated PPARα activation [17]. In CKD mice, L-carnitine treatment reduces serum CREA levels at doses of 50 or 100 mg/kg/day [12]. In addition, the OFL combination shows greater anti-fibrosis effects on renal function in mouse CKD models [12].

When the patients were divided into azotemic (CKD IRIS stage 1) and non-azotemic (CKD IRIS stage 2–4) dogs, no significant differences in serum BUN and CREA levels were found between the OFL and control groups in non-azotemic dogs (Figure 3). In azotemic dogs, the serum BUN of the control group was greater than that of the control group for 6 months but not statistically significant. In contrast, the change in serum CREA levels among azotemic dogs was greater in the control group than in the OFL group, and the difference was significant at 6 months. This result suggests that the efficacy of OFL-based supplements could be confirmed more clearly in the azotemic stage of canine CKD.

This study has some limitations. The study population was relatively small, and thus a larger sample size is required to confirm the findings. In addition, this study measured the effect of the OFL compounds by dividing the patients into two

large groups: non-azotemic (CKD IRIS stage 1) and azotemic (CKD IRIS stage 2–4). Further research is needed to evaluate the effects of the OFL compounds according to more-subdivided CKD IRIS stages. Because the average age of the patients participating in the study was > 10 years old, various senile diseases were common; thus, dogs taking various medications participated in the study, and the effects of these drugs on CKD may have biased the results. Lastly, monitoring was performed for 6 months after the dogs were diagnosed with chronic renal failure, but further monitoring requires a longer period. Despite these limitations, it was found that the administration of the OFL compounds significantly decreased the BUN and CREA levels compared to the study's control group.

5. Conclusion

In this study, the effects of oligo-fucoidan, fucoxanthin, and L-carnitine in canine CKD patients was evaluated retrospectively. The supplements were supplied for 6 months and showed a reno-protective effect, consistent with previous animal model studies. Based on our results, the combination of oligo-fucoidan, fucoxanthin, and L-carnitine has the potential to delay the progression of canine CKD and be used as an adjuvant therapy.

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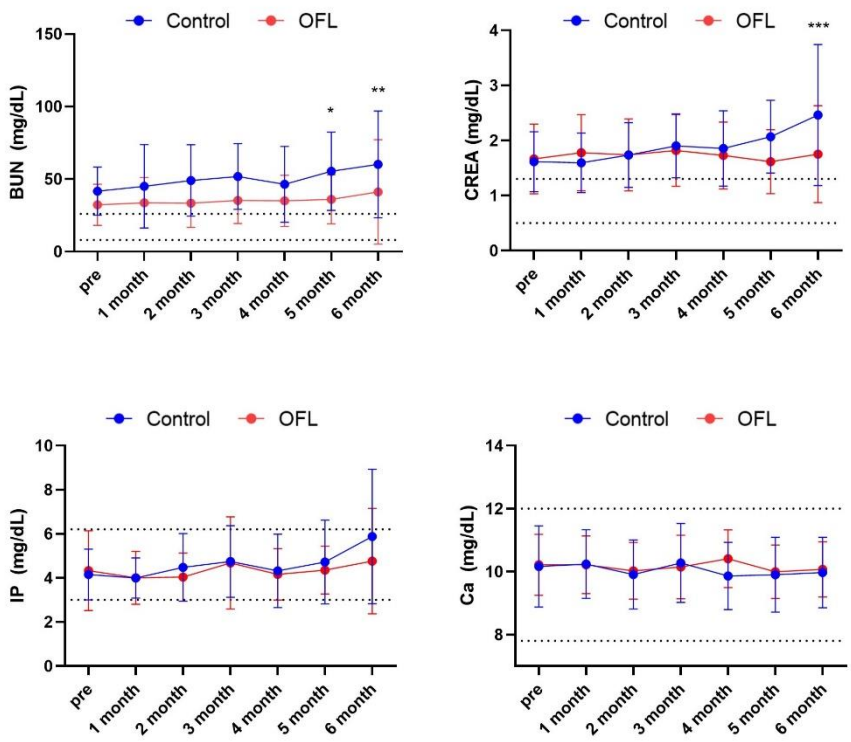

Figure 1. Comparison of serum BUN, CREA, IP and Ca concentration in control and OFL group. A significant difference was identified in serum BUN concentration between the control group and OFL group at 5 and 6 month. In addition, A significant difference was identified in serum CREA concentration between each group at 6 month. Abbreviations: BUN, blood urea nitrogen; Ca, Calcium; CREA, creatinine; IP, inorganic phosphate

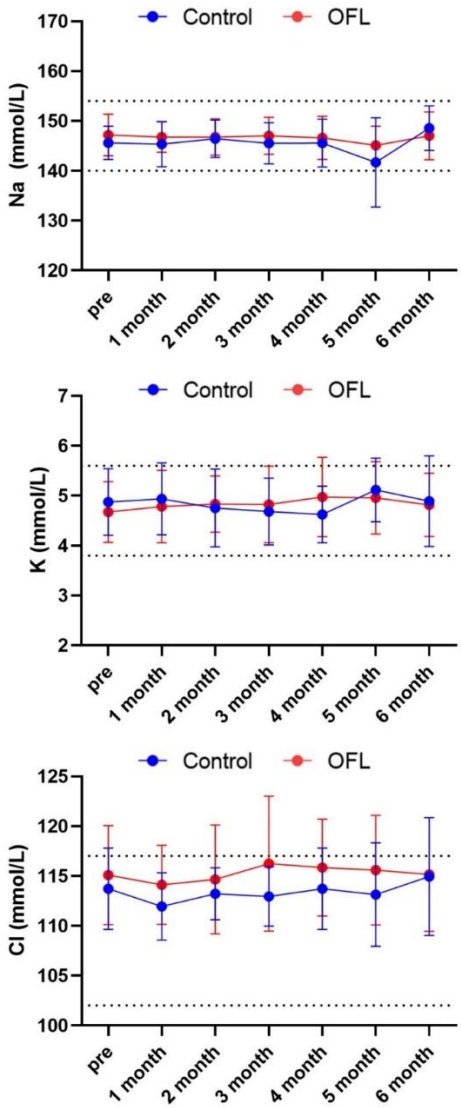

Figure 2. Comparison of electrolyte concentration in control and OFL group.

There are no significant differences between groups. Abbreviation: Na, Sodium; K, Potassium; Cl, Chloride

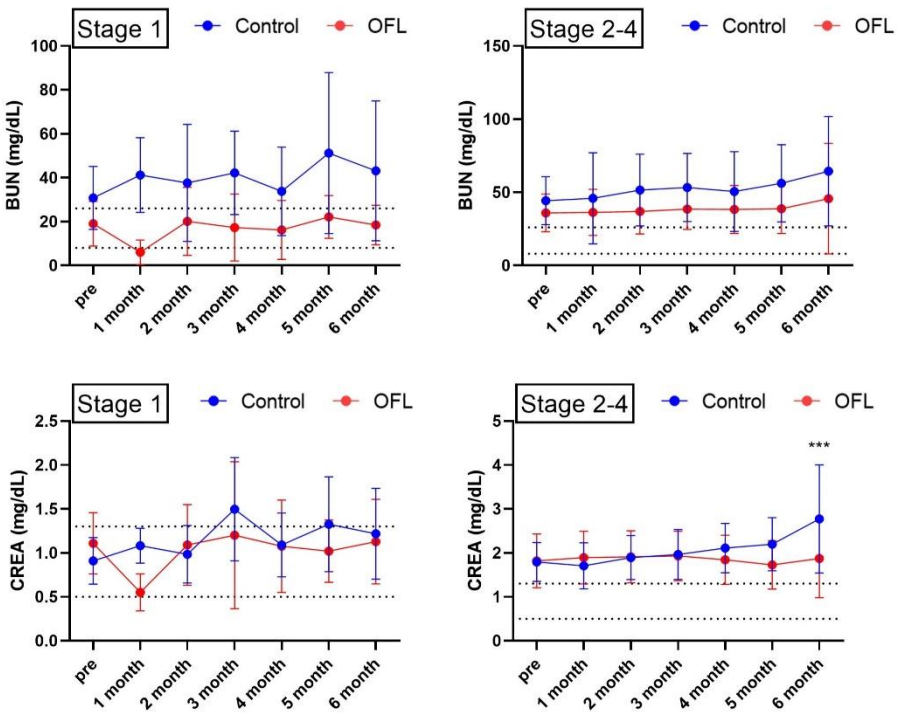

Figure 3. Comparison of serum BUN and CREA concentration in control and OFL group according to non-azotemic (CKD IRIS Stage 1) and azotemic (CKD IRIS stage 2-4) group. A significant difference was identified in serum CREA concentration between the control group and OFL group in azotemic (CKD IRIS stage 2-4) group at 6 month. Abbreviations: BUN, blood urea nitrogen; CREA, creatinine

Table 1. Baseline participant characteristics.

Variables	Reference ranges	Group	
		Control (n =25)	OFL (n=32)
Breed	**NA**	Maltese (3), Pomeranian (4), Poodle (2), Shih-tzu (4), Yorkshire terrier (2), Chihuahua (1), Spitz (1), Cocker spaniel (1), Coton de tulear (1), Dachshund (1), Miniature pinchers (1), White terrier (1), Mongrel (3)	Maltese (9), Pomeranian (5), Poodle (5), Shih-tzu (4), Yorkshire terrier (2), Welsh corgis (1), Doberman pinscher (1), Chihuahua (1), Spitz (1), Mongrel (3)
Sex	**NA**	CM (13), M (2), SF (10), F (0)	CM (12), M (2), SF (15), F (3)
Weight (kg)		4.2 ± 3	3.3 ± 9
Age, years	**NA**	14 (8-18)	13 (4-17)
Systolic blood pressure	**NA**	150 ± 28.2	150 ± 24.2
Proteinuria	**NA**	Proteinuric (8), borderline (1), Non-proteinuric (3), Unknown (13)	Proteinuric (5), borderline (2), Non-proteinuric (10), Unknown (15)
Systemic hypertension	**NA**	Normotensive (7), Pre-hypertensive (10), Hypertensive (5), Severely hypertensive (3)	Normotensive (8), Pre-hypertensive (7), Hypertensive (9), Severely hypertensive (3), Unknown (5)
CKD IRIS stage	**NA**	Stage 1 (5), Stage 2 (19), Stage 3 (1), Stage 4 (0)	Stage 1 (7), Stage 2 (22), Stage 3 (2), Stage 4 (1)

Value of ages is presented as median with range. Values of weight, systolic blood pressure are presented as means ± S.D. CM, Castrated male; F, Female; M, Male; SF, Spayed female;

7. 국문초록

개의 만성 신부전에서 올리고-푸코이단, 푸코잔틴, L-카르니틴의 효과: 후향적 연구

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만성 신부전증(CKD)은 노령의 개와 고양이에서 흔히 발생하는 질환이다. 올리고 푸코이단, 푸코잔틴, L-카르니틴(OFL) 화합물은 항염증, 항산화 및 항섬유화 작용을 포함한 다양한 신장 보호 성질을 가지고 있다. 그러나 OFL 화합물의 효과는 자연발생한 개의 만성 신부전증에서 조사되지 않았으며, 따라서 본 연구에서 신장 보호 효과를 평가하였다.

총 57마리의 환자(OFL, n=32; 대조군, n=25)가 본 연구에 참여하였다. 6개월 후, 대조군과 OFL 군 간 혈청 blood urea nitrogen (BUN) 및 크레아티닌 (CREA) 농도에서 유의한 차이가 확인되었다 (BUN, p=0.0058; CREA, p<0.0001). 두 그룹 간 전해질의 유의한 차이는 확인되지 않았다. 또한 질소혈증이 존재하는 경우(CKD IRIS stage 2-4), 6개월차에 대조군과 OFL 군 간 혈청 CREA 농도에서 유의한 차이가 확인되었다 (p<0.0001).

이전 동물 연구에서 확인된 바와 같이, OFL 화합물은 신장 보호 효과를 나타냈다. 본 연구를 통해 OFL 화합물은 개 CKD의 진행을 지연시킬 수 있으며, 보조요법으로 사용될 수 있을 것으로 예상된다.

주요어: 개, 만성 신부전, 신장 보호, 올리고-푸코이단, 푸코잔틴, L-카르니틴

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