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Plasma and Erythrocyte Glutathione Peroxidase Activity, Serum Selenium Concentration, and Plasma Total Antioxidant Capacity in Cats with IRIS Stages I–IV Chronic Kidney Disease

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Background: Serum selenium concentrations and the activity of plasma glutathione peroxidase (GPx) decrease with the progression of chronic kidney disease (CKD) in human patients. Selenium is considered a limiting factor for plasma GPx synthesis. Plasma total antioxidant capacity (TAC) is decreased in CKD cats in comparison to healthy cats.

Hypothesis: Serum selenium concentrations and plasma and erythrocyte GPx activity in cats with CKD are lower than in healthy cats. Serum selenium concentrations, the activity of enzymes, and plasma TAC progressively decrease with the progression of kidney disease according to IRIS (International Renal Interest Society) classification.

Animals: Twenty-six client-owned cats in IRIS stages I–IV of CKD were compared with 19 client-owned healthy cats.

Methods: A CBC, serum biochemical profile, urinalysis, plasma and erythrocyte GPx activity, serum selenium concentration, and plasma TAC were measured in each cat.

Results: Cats in IRIS stage IV CKD had a significantly higher ($P = .025$) activity of plasma GPx (23.44 ± 6.28 U/mL) than cats in the control group (17.51 ± 3.75 U/mL). There were no significant differences in erythrocyte GPx, serum selenium concentration, and plasma TAC, either among IRIS stages I–IV CKD cats or between CKD cats and healthy cats.

Conclusions and Clinical Importance: Erythrocyte GPx activity, serum selenium concentration, and plasma TAC do not change in CKD cats compared with healthy cats. Selenium is not a limiting factor in feline CKD. Increased plasma GPx activity in cats with stage IV CKD suggests induction of antioxidant defense mechanisms. Antioxidant defense systems might not be exhausted in CKD in cats.

Key words: Antioxidants; Cats; Oxidative stress; Renal failure.

Chronic kidney disease (CKD) is a common condition in geriatric cats.[1] It is characterized by progressive loss of kidney function, which results in renal fibrosis, uremia, and death.[2] In human uremic patients, a state of imbalance between pro-oxidative and antioxidative processes has been established, and defined as oxidative stress with increased amounts of reactive oxygen species (ROS) present.[3–5] ROS are involved in cell signaling and cause activation of proinflammatory and mitogenic cellular pathways, which enhance the progression of renal fibrosis and cause a progressive decline in renal function.[5–7] Glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and nitric oxide synthase are the most important antioxidant enzymes that detoxify ROS molecules in the kidneys.[4]

Two forms of GPx have been identified in human blood: plasma and erythrocyte GPx. The major synthetic site of plasma GPx is the proximal tubule in the kidneys.[8] Plasma GPx also may function as an antioxidant in the renal tubules and extracellular space.[9] The activity of GPx is decreased in human CKD patients. Plasma GPx activity therefore is an important diagnostic and prognostic factor of CKD in humans.[3,10,11]

Abbreviations:

CAT	catalase
CKD	chronic kidney disease
ETAAS	electrothermal atomic absorption spectrometry
FeLV	feline leukemia virus
FIV	feline immunodeficiency virus
GPx	glutathione peroxidase
HSD	honestly significant difference
IRIS	International Renal Interest Society
ROS	reactive oxygen species
SOD	superoxide dismutase
TAC	total antioxidant capacity

Erythrocyte GPx is predominantly present in erythrocytes, kidney, and liver.[9,12] Some studies report that erythrocyte GPx activity is significantly increased early in the development of CKD, but remains stable until progression to end-stage kidney disease.[3] Other studies showed no significant differences in erythrocyte GPx activities between CKD patients and healthy controls.[10,13] In contrast, other studies reported a significant decrease in erythrocyte GPx activity in CKD patients in comparison to a control group.[13–15]

Selenium is an integral part of several selenoproteins, including glutathione peroxidases, and is present in food rich in protein.[16] Cats have higher blood selenium concentrations than other species.[17] They maintain selenium homeostasis by modulating its urinary excretion.[18,19] In human uremic patients, whole blood and serum concentrations of selenium are decreased because of either lower dietary intake or increased

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excretion. Furthermore, plasma selenium and plasma GPx concentrations have been found to be significantly lower in humans with end-stage kidney disease.[13,20]

To date, 2 studies evaluating oxidative stress in cats with CKD have been published.[21,22] Yu and Paetau-Robinson[21] investigated oxidative stress and the effects of dietary antioxidant supplementation on the oxidative status of cats with CKD. Their study showed that antioxidant supplements may be beneficial in cats with CKD.[21] Keegan and colleagues studied oxidative stress parameters and neutrophil function in cats with CKD.[22] Their results indicated that antioxidant defense mechanisms were activated in diseased cats.[22]

The purpose of the present study was to investigate plasma and erythrocyte GPx activity, serum selenium concentration, and plasma total antioxidant capacity (TAC) in cats with IRIS[23] stages I–IV CKD. The objective of the present study was to test the hypotheses that serum selenium concentrations, plasma and erythrocyte GPx activity, and plasma TAC in cats with CKD are lower than in healthy cats. These parameters decrease with the progression of CKD according to the IRIS classification.

Materials and Methods

Selection of Cats

The study was performed at the Clinic for Surgery and Small Animal Medicine at the Veterinary Faculty in Ljubljana from September 2009 to March 2011. A detailed history was taken, and physical examination, CBC, serum biochemistry, and urinalysis were performed. Based on the examination and laboratory findings, the cats were divided into 2 groups: a control group and a group of cats with CKD.

Inclusion Criteria

Cats of all ages, breeds, and both sexes were considered for enrollment. Using available history, physical examination, hematology, biochemistry, urinalysis, and urine protein/creatinine ratio (UPC) data, cats were divided into 2 groups: a control group and cats with CKD. To be diagnosed with CKD, cats had to have serum creatinine concentration above the reference range (140 μmol/L) on at least 2 occasions (except IRIS stage I cats) and show clinical signs such as polyuria and polydipsia, and palpably small kidneys. In cats with IRIS stage 1 CKD, serum creatinine concentration was within the reference range. Cats with CKD were classified into 4 stages following IRIS guidelines: stage I (serum creatinine concentration < 140 μmol/L), stage II (serum creatinine concentration: 140–249 μmol/L), stage III (serum creatinine concentration 250–439 μmol/L), and stage IV (serum creatinine concentration >440 μmol/L).[23]

Cats in the control group were determined to be healthy based on history, physical examination, and the results of hematology, biochemistry, and urinalysis. Urine specific gravity in healthy cats had to be >1.035.

All owners signed an informed consent form before enrolling their cats in the study. All procedures compiled with the relevant Slovenian governmental regulations (Animal Protection Act, Official Gazette of the Republic of Slovenia, No. 43/2007).

Exclusion Criteria

Cats with acute kidney injury, prerenal or postrenal azotemia, nephropathy of toxic or infectious origin within the previous 28 days, urinary tract obstruction, acute systemic inflammation, liver disease, chronic heart failure, cancer, or those that tested positive for feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), or both were excluded from the study.

Blood and Urine Sampling and Processing

Blood samples were collected from the jugular vein and transferred to serum separator tubes[a] for the determination of serum biochemical profiles and for serologic detection of FeLV and FIV. The tubes were stored for 30 minutes at room temperature to clot, and then centrifuged at 1,300 × *g* for 10 minutes at room temperature to separate the serum. Serum samples were assayed on the day of collection. Biochemical profiles were determined with an automated biochemistry analyzer (RX Daytona[b]). An enzyme-linked immunosorbent assay (ELISA) for the detection of antigen to FeLV and specific antibody to FIV were performed according to the manufacturer's instructions[c] on the day of collection.

Blood samples for hematologic analysis were collected into ethylenediaminetetraacetic acid (EDTA)–containing tubes.[d] EDTA blood samples were stored at room temperature and analyzed from 1 to 3 hours after sampling. Hematologic analyses were performed with an automated laser hematology analyzer ADVIA 120[e] using species-specific software.

Blood samples for determining plasma GPx activity and plasma TAC were collected into 2 mL tubes containing lithium heparin[a] and immediately centrifuged at 1,500 × *g* for 15 minutes at 4°C. Plasma was separated and immediately frozen at −80°C until analysis. Activity of plasma GPx and plasma TAC in all samples collected was determined at the end of the study. The activity of human plasma GPx was shown to be stable for at least 4 years in samples stored at −76°C.[24] Human serum or plasma TAC results were shown to be stable for 12 months when samples were stored at −70/80°C (or liquid nitrogen).[25] Our personal data (unpublished results), based on several studies, showed that plasma TAC, determined in canine and equine plasma samples, is stable for at least 2 years.

Blood samples for determining GPx activity in erythrocytes were collected into 2-mL tubes containing lithium heparin.[a] Erythrocyte lysates were prepared immediately after blood collection, according to the manufacturer's (Ransod kit[b]) instructions, and stored at −80°C until analysis. Hemoglobin concentration in erythrocyte lysate was determined spectrophotometrically by the cyano-methemoglobin method, using an automated biochemistry analyzer (RX Daytona).[b] Activity of erythrocyte GPx in all samples collected was determined at the end of the study. The activity of human erythrocyte GPx was shown to be stable for 21 months when samples were stored at −80°C.[26]

Blood samples for serum selenium concentration determination were collected into 1.5-ml Eppendorf tubes.[f] They were stored for 30 minutes at 4°C to allow the blood to clot, and the tubes then were centrifuged at 1,300 × *g* for 10 minutes. Serum was frozen at −80°C until analysis. Serum selenium concentration in all samples collected was determined at the end of the study. Freezing at −20°C (or lower) for long-term storage of human whole blood, plasma, and serum samples for trace element analysis (including selenium) for up to 2 years is recommended.[27]

Urine samples were collected by cystocentesis or catheterization. After collection, urinalysis was performed within 1–2 hours of sample collection. Analysis included measurement of specific

gravity with a refractometer, use of a standard multitest urine dipstick, and microscopic examination of urine sediment. Urine samples were centrifuged at 800 × *g* for 10 minutes at room temperature. Urine supernatants were used for determination of protein and creatinine concentrations to calculate UPC. Protein and creatinine concentrations were measured with an automated biochemistry analyzer (RX Daytona)[b] using colorimetric pyrogallol red[b] and colorimetric picric acid[b] methods, respectively. Protein concentrations were not determined in urine samples of cats when the urine sample was grossly contaminated with blood or contained spermatozoa.

Determination of GPx Activity

Plasma and erythrocyte GPx activity was measured spectrophotometrically with an automated biochemistry analyzer (RX Daytona)[b] using a commercial Ransel kit,[b] which is based on the method of Paglia and Valentine.[28] The activity of plasma GPx was expressed as units per mL (U/mL) and the activity of erythrocyte GPx as units per grams of hemoglobin (U/gHgb).

Determination of Serum Selenium Concentrations

Serum selenium concentrations were measured by electrothermal atomic absorption spectrometry (ETAAS) using a Varian Spektr AA-800 electrothermal atomic absorption spectrometer.[g]

Determination of Plasma TAC

Plasma samples were assayed for TAC with an automated biochemistry analyzer (RX Daytona)[b] with a commercial total antioxidant status (TAS) kit,[b] following the instructions of the kit. The assay[29] is based on the reduction of free radicals (ABTS*$^+$ −2,2'–azinobis-(3-ehylbenzothiazoline-6-sulfonate)) measured as a decrease of absorbance at 600 nm at 3 minutes by antioxidants. The results are expressed as mmol/L of Trolox equivalents.

Statistical Analysis

Data were analyzed using the SPSS computer program.[h] Descriptive statistics were used to describe the baseline data of the 2 groups of cats. The Shapiro–Wilk test was performed to test whether the data were normally distributed or not. Results are expressed as means ± standard deviation, unless stated otherwise. Values of selected blood parameters (serum selenium concentration, plasma and erythrocyte GPx, plasma TAC) were compared among groups of cats (4 groups of cats with different stages of feline CKD and healthy cats) using one-way ANOVA and Tukey's HSD posthoc test. An unpaired *t*-test was used to compare ages between the controls and clinical groups of cats (healthy versus IRIS stages I–IV). Posthoc power analysis using the general linear model/univariate approach also was performed. A value of $P < .05$ was considered significant.

Results

The characteristics of cats with CKD and healthy controls, as well as the details of the laboratory findings and signalment for each group of cats, are summarized in Tables 1 and 2, respectively. In the present study, 58 cats were evaluated; however, 2 of the cats with CKD were excluded from the study because they were positive for FeLV/FIV. In addition, 11 healthy cats were excluded because of inability to collect urine samples. Overall, 45 client-owned cats were included in the study. Nineteen healthy cats were included in the control group. The group of cats with CKD consisted of 26 cats. Five cats were classified as stage I, 9 as stage II, 7 as stage III, and 5 as stage IV. The mean age of the healthy cats differed significantly (Table 1) in comparison with the mean age of stages I–IV CKD cats.

Laboratory findings (Table 1) indicated that mean serum creatinine concentrations in individual groups of

Table 1. Summary of general characteristics for control cats and cats in IRIS stages I–IV of CKD.

	Control (n = 19)	IRIS Stage I (n = 5)	IRIS Stage II (n = 9)	IRIS Stage III (n = 7)	IRIS Stage IV (n = 5)
Age					
Mean ± SD (y)	5.1 ± 5.1[a]	11.0 ± 5.4[b]	12.6 ± 6.6[b]	13.4 ± 3.6[b]	11.6 ± 3.6[b]
Range	6264 m–19 y	4–19 y	3–21 y	10–20 y	7–17 y
P value		.027	.002	.000	.011
Body weight (kg)	4.2 ± 1.5	4.1 ± 0.7	4.5 ± 1.2	3.1 ± 0.6	3.0 ± 0.7
Mean ± SD					
Sex (F/M)	10/9	2/3	4/5	4/3	5/0
Female intact	4	0	0	0	0
Female spayed	6	2	4	4	5
Male intact	4	0	1	0	0
Male neutered	5	3	4	3	0
Clinical signs					
Decreased appetite	0/19	1/5	0/9	4/7	5/5
Vomiting	0/19	1/5	2/9	4/7	3/5
Polyuria/polydipsia	0/19	1/5	2/9	3/7	2/5

The *P* value indicates the significance of differences ($P < .05$; an unpaired *t*-test) in age among clinical groups of cats (healthy versus. IRIS stages I–IV).

IRIS, International Renal Interest Society; CKD chronic kidney disease; n, number of cats; m, months; y, years; F/M, number of female/male cats.

Table 2. Summary of laboratory findings for control cats and cats in IRIS stages I–IV of CKD.

	Control, Mean ± SD	IRIS Stage I, Mean ± SD	IRIS Stage II, Mean ± SD	IRIS Stage III, Mean ± SD	IRIS Stage IV, Mean ± SD
Biochemical profile	(n = 19)	(n = 5)	(n = 9)	(n = 7)	(n = 5)
Urea (mmol/L)	10.6 ± 3.5	12.8 ± 9.6	12.1 ± 3.3	27.9 ± 10.4	76.8 ± 37.5
Creatinine (μmol/L)	118.7 ± 23.0	122.2 ± 20.3	179.6 ± 31.8	296.9 ± 65.0	1081.3 ± 823.7
AP (U/L)	47.6 ± 43.9	25.2 ± 12.9	27.7 ± 7.3	28.0 ± 14.7	19.3 ± 5.0
ALT (U/L)	64.5 ± 41.1	68.2 ± 52.2	73.5 ± 31.7	120.8 ± 75.3	72.0 ± 40.3
CBC	(n = 19)	(n = 5)	(n = 9)	(n = 7)	(n = 5)
WBC ($\times 10^9$/L)	9.1 ± 3.6	8.0 ± 1.6	8.3 ± 3.9	15.1 ± 7.8	13.0 ± 10.0
RBC ($\times 10^{12}$/L)	9.0 ± 1.3	7.8 ± 1.4	8.2 ± 1.3	7.2 ± 2.1	5.3 ± 1.4
HCT (L/L)	0.40 ± 0.06	0.36 ± 0.05	0.37 ± 0.06	33.1 ± 10.9	0.25 ± 0.06
HGB (g/L)	140.5 ± 66.1	112.0 ± 14.9	112.6 ± 18.5	103.9 ± 33.4	95.4 ± 25.7
PLT ($\times 10^9$/L)	255.6 ± 69.5	280.8 ± 98.5	318.7 ± 103.8	383.9 ± 196.7	336.4 ± 212.2
Urinalysis	(n = 19)	(n = 4)	(n = 6)	(n = 7)	(n = 5)
Specific gravity	1.062 ± 0.012 (n = 11)	1.051 ± 0.020 (n = 3)	1.043 ± 0.016 (n = 5)	1.023 ± 0.006 (n = 5)	1.019 ± 0.005 (n = 4)
UPC	0.170 ± 0.104	6.341 ± 9.271	0.182 ± 0.129	0.747 ± 0.431	1.236 ± 0.939

IRIS, International Renal Interest Society; CKD, chronic kidney disease; n, number of cats; AP, alkaline phosphatase; ALT, alanine aminotransferase; CBC, complete blood count; WBC, white blood cells; RBC, red blood cells; HCT, hematocrit; HGB, hemoglobin; PLT, platelets; UPC, urine protein-to-creatinine ratio.

Table 3. Serum selenium concentration, plasma and erythrocyte GPx activity, and plasma TAC level in cats in IRIS stages I–IV of CKD.

	Serum Selenium (mg/L)	Plasma GPx (U/mL)	Erythrocyte GPx (U/gHgb)	Plasma TAC mmol/L
IRIS Stage I (n = 5)	0.465 ± 0.053	21.86 ± 2.04	591.18 ± 56.74	1.47 ± 0.18
P value (versus control)	.807	.534	1.000	.990
P value (versus IRIS stage II)	.934	.913	.998	1.000
P value (versus IRIS stage III)	.904	1.000	1.000	.581
P value (versus IRIS stage IV)	.833	.719	.960	.954
IRIS Stage II (n = 9)	0.426 ± 0.042	18.67 ± 1.66	582.51 ± 80.79	1.46 ± 0.16
P value (versus control)	.999	.940	1.000	.964
P value (versus IRIS stage I)	.934	.912	.998	1.000
P value (versus IRIS stage III)	1.000	.912	.992	.482
P value (versus IRIS stage IV)	.994	.175	.818	.916
IRIS Stage III (n = 7)	0.419 ± 0.173	20.87 ± 2.56	603.92 ± 64.42	1.30 ± 0.13
P value (versus control)	1.000	.464	.998	.134
P value (versus IRIS stage I)	.904	1.000	1.000	.581
P value (versus IRIS stage II)	1.000	.912	.992	.482
P value (versus IRIS stage IV)	.991	.612	.967	.232
IRIS Stage IV (n = 5)	0.404 ± 0.077	23.44 ± 6.28	639.81 ± 27.78	1.68 ± 0.40
P value (versus control)	.999	.025	.840	.992
P value (versus IRIS stage I)	.833	.719	.960	.954
P value (versus IRIS stage II)	.994	.175	.818	.916
P value (versus IRIS stage III)	.999	.612	.967	.232
Control group (n = 19)	0.415 ± 0.091	17.51 ± 3.75	591.0 ± 111.21	1.51 ± 0.17

The *P* value indicates the significance of differences ($P < .05$; one-way ANOVA) in measured parameters among clinical groups of cats (healthy versus IRIS stages I–IV and between IRIS stages I–IV).

CKD, chronic kidney disease; GPx, glutathione peroxidase; TAC, total antioxidant capacity; n, number of cats.

cats varied according to IRIS guidelines for feline CKD stages I–IV.[23] In healthy cats, the results of all laboratory parameters, with the exception of platelets, were within normal reference ranges.

Results for serum selenium concentration, activity of plasma and erythrocyte GPx, and plasma TAC are summarized in Table 3. There was no significant difference in serum selenium concentrations in CKD patients in comparison to the control group or among individual groups of CKD cats.

Significantly ($P = .025$) higher activity of plasma GPx was observed in stage IV CKD cats in comparison to the control group. However, there was no significant difference in plasma GPx activity between stages I–III CKD patients and the control group and among the 4 groups of CKD cats. Moreover, there was no

significant difference in erythrocyte GPx activity and plasma TAC among any of the groups of cats.

Discussion

In the present study, a significantly higher activity of plasma GPx in stage IV CKD cats than in the control group was found. However, there were no significant differences in serum selenium concentration, plasma TAC, and erythrocyte GPx, either in cats at any stage of CKD in comparison to the control group or among individual groups of CKD cats.

The selenium concentrations in our study corresponded to those found in the healthy control group of cats in a study that compared selenium status and GPx activity in healthy and hyperthyroid cats.[17] Furthermore, the study cited found no correlation between plasma GPx activity and selenium concentrations in the healthy or hyperthyroid cats. A correlation between selenium concentration and GPx activity was found only if the selenium intake was below the recommended amount. As soon as selenium concentrations reached recommended values, GPx activity reached its plateau value and did not increase further.[17] In another study, the results showed no increase in erythrocyte and plasma GPx activities with increasing selenium intake at the amounts used in that study.[18] It also was found that the selenium concentration in cat food was well above minimum recommended values. There seems to be enough selenium in the diet to fulfill the needs of diseased cats, and this trace element is not a limiting factor in feline CKD or in the synthesis of plasma GPx.[17]

In contrast, there is significant plasma and whole blood selenium deficiency in human end-stage kidney disease patients.[10,20] In human patients with kidney disease, plasma selenium concentrations decrease as the disease progresses.[3,10,20] The decrease in selenium concentrations is ascribed to low protein intake and increased protein loss attributable to proteinuria, which develops over the course of the disease.[30] Lower selenium concentrations therefore could explain lower plasma GPx activity in human CKD patients. As carnivores, cats consume a diet that has a high concentration of proteins and therefore selenium is ingested in relatively high amounts. Cats therefore can tolerate greater selenium concentrations than other species.[19] This may be the reason why cats with CKD do not have low serum selenium concentrations as human CKD patients do.

Several studies have reported decreased activity of plasma GPx in human chronic kidney disease patients, regardless of the stage of the disease.[3,11,13,14] Decreased GPx activity may be because of an increased rate of lipid peroxidation, a decrease in functional renal mass,[3,14,20] the interference of uremic toxins,[14,20] plasma GPx consumption and inactivation by biochemical modification, or an abnormality in the hexose monophosphate pathway.[20] Furthermore, the decrease in GPx activity also has been ascribed to selenium deficiency, because this microelement is a limiting factor for the synthesis of the enzyme.[20] Other studies, however, have not suggested decreased GPx activity in association with low selenium intake.[3,13] The results of the present study showed no significant difference in the activity of plasma GPx in cats with stages I–III CKD, but significantly higher activity in cats with stage IV CKD when compared with a healthy cat population. The latter might reflect the presence of an adaptation mechanism by which the antioxidant system of these cats is enhanced and adapted to conditions of increased oxidative stress. The results indicate that antioxidant defense systems are not exhausted in stage IV feline CKD. A similar conclusion was reached by Keegan and Webb[22] in a study performed on 20 cats with CKD. On the other hand, our results contrast with previous reports on plasma GPx activity in human CKD patients.[3,11,13,14] This might be because of differences in selenium metabolism between cats and humans. Selenium deficiency occurs in human CKD patients.[10,20] However, there seems to be no deficiency in cats with CKD, and the synthesis of plasma GPx is not impaired.

Total antioxidant capacity is a biochemical parameter suitable for evaluating the overall antioxidant status of plasma and body fluids resulting from antioxidant intake or production, and their consumption by normal or increased amounts of ROS production. The capacity of known and unknown antioxidants and their synergistic interaction therefore are assessed, thus giving insight into the delicate balance between oxidants and antioxidants in vivo.[31,32] The results of the present study demonstrated no significant difference in plasma TAC in cats with CKD in comparison to controls or among individual groups of CKD cats, thus indicating that systemic antioxidant defense systems might not be exhausted in CKD cats. Our result is in contrast with the study of Keegan and Webb[22] who reported a significant decrease in antioxidant capacity in CKD cats compared with the healthy group of cats.[22] The difference might be attributable to differences in the study design, because cats with CKD were not further classified in IRIS stages I–IV of CKD in the previous report.[22] In part, the difference might be because of variations among the cats studied and because of differences in the spectrophotometric kits used for plasma TAC determination.

Erythrocyte GPx is considered the principal antioxidant enzyme for the detoxification of hydrogen peroxide in human erythrocytes.[33] In human renal patients, its activity has been found to be increased,[3] decreased,[13,14] or not significantly different from healthy individuals.[10,34,35] In the present study, the values of erythrocyte GPx activity in the control group and stages I–IV CKD patients were in agreement with published results in cats.[36] No significant difference in its activity was found, either between the control group and the stages I-IV CKD group or among individual groups of cats with CKD. This suggests that erythrocyte GPx activity does not depend on the pathophysiologic course of feline CKD.

Although the study reported here indicated that antioxidant defense systems might not be exhausted in feline CKD, this study had some limitations. These include low number of cats included in groups of IRIS

stages I–IV of CKD, no age-matching between control group and 4 groups of cats with CKD, and the use of only 1 method for plasma TAC determinations. Because low numbers of cats were included in groups of IRIS stages I–IV of CKD, posthoc power analyses were conducted. Statistically nonsignificant results may be the result of insufficient statistical power.[37,38] Posthoc power analyses indicated low power coefficients (less than the recommended 0.80)[37] for statistically nonsignificant results, that is for serum selenium (0.118), erythrocyte GPx (0.123), and plasma TAC (0.468), which suggests ambiguity. On the other hand, much greater power was determined for plasma GPx (0.722), the parameter that was significantly higher in stage IV CKD patients in comparison to the control group. It would have been ideal to have had larger numbers of cats across the IRIS stages of CKD such that the difference in measured parameters would be greater and overlaps would not occur in the values measured.

To conclude, antioxidant defense systems might not be exhausted in feline CKD. Moreover, significantly higher plasma GPx activity in cats with stage IV of CKD suggests induction of the antioxidant enzyme defense system. Furthermore, there was no significant difference in serum selenium concentration, plasma TAC, or erythrocyte GPx activity, either when comparing the control group with CKD patients or among different stages of CKD patients. Selenium is therefore not a limiting factor in feline CKD, and erythrocyte GPx may not have a role in the course of this disease.

Footnotes

[a] Vacuette; Greiner Bio-One, Kremsmunster, Austria
[b] Randox Laboratories, Crumlin, UK
[c] SNAP feline triple, IDEXX Laboratories Inc, Westbrook, ME
[d] BD Microtainer; Becton, Dickinson and Company, Franklin Lakes, NJ
[e] SIEMENS, Munich, Germany
[f] Eppendorf tubes, Brand, Wertheim, Germany
[g] Varian Australia Pty Ltd, Mulgrave, Victoria, Australia
[h] SPSS 17.0 for Windows, Chicago, IL

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