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Case report

SYMMETRIC DIMETHYLARGININE IS A PROMISING BIOMARKER FOR THE EARLY DETECTION OF AGE-RELATED KIDNEY DYSFUNCTION IN ZOO FELIDS

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Chronic renal failure is one of the leading causes of death in African lions, cheetahs, and tigers. Conventional methods to measure renal dysfunction include measuring serum creatinine and blood urea nitrogen (BUN). Symmetric dimethylarginine (SDMA) measurement is a reliable predictor of renal dysfunction in the domestic cat because SDMA serum levels increase early when the kidneys are damaged. Serum SDMA levels were assessed and correlated with creatinine as well as BUN from healthy captive Bengal tigers and lions at the Everland Zoo in Korea. Serum SDMA concentrations were increasingly associated with increased age in lions. However, SDMA concentrations were higher in some young Bengal tigers than in older ones, which may allow for earlier renal dysfunction detection in these young cats than would be BUN and creatinine alone. In Bengal tigers, the correlation between the SDMA and BUN was slightly higher than that between SDMA and creatinine. In lions, SDMA correlated better with creatinine than with BUN concentration. These results show that serum SDMA concentration can be used as a biomarker for age-associated renal dysfunction. SDMA measurement may be an essential preventive management method in zoos.

Key words: African lion, Bengal tiger, Symmetric dimethylarginine (SDMA)

INTRODUCTION

The population of wild Felidae is reducing worldwide, and the International Union for Conservation of Nature (IUCN) lists lions as Vulnerable species (VU) and Bengal tigers as an Endangered species (EN) [1-3]. There are several causes of wild Felidae's population decline. Particularly, chronic kidney disease (CKD) is a common cause of mortality in domestic, nondomestic, and wild felines [4]. Post-mortem CKD evidence

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was detected in 12% of domestic cats [5]. Renal diseases have also been reported in cheetahs, leopards, lions, and tigers [6-9]. In many zoos, intra-tubular concrements, necrosis, tubular degeneration and neoplasia were the most reported renal pathology [10,11]. Noninfectious and traumatic factors, respiratory and digestive disorders were the leading causes of mortality in captive Felidae in Korea in the past (1976–2001) [12]. Captive animals have a longer life expectancy than wild animals [13,14], so age-related diseases are essentially emerging issues. The CKD incidence increases with age in domestic cats [15,16]. Older captive nondomestic felids are most affected compared with younger animals globally. CKD often occurs with age-related comorbidities, including degenerative diseases [17,18].

Many methods are used to diagnose CKD, including symptoms, blood and urine tests, and diagnostic imaging. Creatinine (Cre), urea, Symmetric dimethylarginine (SDMA) are the most used serum biomarkers to predict kidney disease in carnivores and other species [19]. Many biomarkers are increased in the serum as a response to reduced glomerular filtration rate (GFR), such as SDMA, which is highly sensitive; SDMA concentrations increase even before Cre concentration [20,21]. SDMA concentrations increase when 25%–40% of kidney function is impaired, whereas Cre concentration does not increase until ~70% of kidney function is lost [20,21]. Cre is also affected by muscle mass, protein intake, age, and sex [22,23]. Thus, SDMA is a promising biomarker for the early diagnosis of kidney disease in nondomestic felids [24-26].

SDMA is a critical early serum predictor of kidney disease in humans and small animals. SDMA was first used to diagnose non azotemic CKD. Recent studies showed that SDMA is also suitable for diagnosing acute and CKD in dogs. However, SDMA cannot discriminate between acute and chronic renal changes [20,21]. This study was conducted to monitor serum concentrations of SDMA in captive nondomestic felids as a predictor of renal dysfunction at the Everland Zoo. If kidney dysfunction can be detected early, it may be possible to reduce the progression through proper nutritional and medical interventions.

MATERIALS AND METHODS

Study area and animals

This study was conducted at the Everland Animal Zoo in the Republic of Korea in 2018 and 2019. Eleven lions (seven males and four females) with ages ranging from 2–16 years and seven Bengal tigers (two males and five females) aged 7–13 years were included in this study. The collection of blood from all animals was conducted during regular annual health evaluations. Radiological examination was conducted to examine dental disease, joint and spine abnormalities in either under three years old or older than ten years of age. To remove internal and external parasites, Felidae were treated with anthelmintic drugs (ivermectin, albendazole, and flubendazole) every 2–3 months.

Animals were vaccinated against feline calicivirus, feline rhinotracheitis virus, and feline panleukopenia (Nobivac Tricat Trio, Intervet international B.V, 5831 AN Boxmeer, Netherland) once a year. The daily diet included 3–4-kg whole chicken per day and 500-g ground beef along with nutritional supplements. A multivitamin is given once a day for nutrition, and vitamin E as well as calcium are given once a week.

Anesthesia and animal restraint

Physical examination and blood collection were conducted while the cats were under general anesthesia. For each cat, anesthesia was performed 12–16 h after fasting. All animals were anesthetized after weighing. Anesthetics were injected intramuscularly using a blowgun or pipe with 3-mg/kg ketamine (Yuhan Corporation, 74, Noryangjin-Ro, Daebang-Dong, Dongjak-Gu, Seoul, Korea) and 30 μg/kg of medetomidine hydrochloride (Domitor, Orion Corporation Orion Pharma, Orionitie 1, Espoo, Finland, 02200) for lions and tigers. Blood was collected about 5–10 min after injection and after confirming a stable respiratory rate of 20–30 cycles/min. After examination, atipamezole hydrochloride (Antisedan, Orion Corporation Espoo, Finland, 250-ug/kg) was given intravenously to reverse the effects of medetomidine.

Hematology and serum biochemistry

Blood samples were obtained from the medial saphenous vein or femoral vein, placed into EDTA, plain and heparin-coated vacutainer tubes, and immediately transported to the zoo animal hospital laboratory. Blood samples in a plain tube for the SDMA test were allowed to clot for 20–30 min and centrifuged for five minutes at 1008 x g. Blood samples for measuring SDMA were mailed to the IDEXX laboratory (174-10 jagongno, Gangnam-gu, Seoul, Republic of Korea) in the Republic of Korea. Blood in EDTA and heparin-coated vacutainer tubes were centrifuged at 1008 x g for five minutes to separate the Plasma. A complete Plasma hemogram was conducted on 18 samples (Table 1) using the Everland hospital blood analyzer (VETSCAN HM5, Abaxis Inc., USA). Chemistry analysis was conducted using an automated chemistry analyzer (FUJI DRI-CHEM, 3500i, Fujifilm, Japan).

Statistical analysis

For all graphs, Microsoft Excel (2017) program was used. Graphpad Prism 8 for Windows 64-bit (San Diego, CA 92108, USA) was used to make xy graph and an R-square analysis Microsoft Excel (2017) program was used. R-square analysis was conducted on the correlation between SDMA, BUN, and Cre of Bengal tiger and lion.

Table 1. Hematology and Biochemistry analytes in captive tiger and lion.

Complete Blood Cell Count	Blood Chemistry
White Blood Cell (WBC)	Sodium (Na), Potassium (K), Chloride (Cl)
Lymphocyte (LYM)	Lactate Dehydrogenase (LDH)
Monocyte (MON)	Blood Urea Nitrogen (BUN)
Neutrophil (NEU)	Creatinine (CRE)
Eosinophil (EOS)	Creatine Phosphokinase (CPK)
Basophil (BAS)	Magnesium (Mg)
Red Blood Cell (RBC)	Albumin (ALB)
Hemoglobin (HGB)	Alkaline Phosphatase (ALP)
Hematocrit (HCT)	Lipase (vLip),
Mean Corpuscular Volume (MCV)	Amylase (AMYL)
Mean Corpuscular Hemoglobin (MCH)	Total Protein (TP)
Platelet (PLT)	Gamma Glutamyltransferase (GGT)
Mean Plasma Volume (MPV)	Total Cholesterol (TCHO)
Mean Corpuscular Hemoglobin Concentration (MCHC)	Triglyceride (TG), Total Bilirubin (TBIL)
	Alanine aminotransferase (ALT)
	Aspartate aminotransferase (AST)
	Phosphorus (IP), Calcium (Ca)
	Glucose (GLU)

RESULTS

Cre, BUN, and SDMA correlations in the lion and Bengal tiger

Cre, BUN, and SDMA serum concentrations correlated better in lions than in Bengal tigers (Figure 1). Moreover, the correlation between SDMA and BUN was slightly higher than the correlation between SDMA and Cre in Bengal tigers (Figure 1. B&C). However, SDMA concentration in lions correlated better with Cre than with BUN (Figure 1 E & F). In the case of SDMA and BUN levels in the Bengal tiger, SDMA concentrations in different ranges can be seen even at high BUN levels. Alternatively, in the case of the lion, the SDMA value is high on average, and the BUN range seems to vary.

Cre, BUN, and SDMA associations with age

Cre, BUN, and SDMA concentrations increased with age in tigers and lions (Figure 2). In lions, SDMA concentration sharply increased at the age of two, gradually increased until the age of six, and remained at steady levels from 6–14 years, before increasing again at the age of 15 years (Figure 2 B). Increased SDMA concentration was strongly

associated with increased age in lions (Figure 2 B). However, in tigers, some old animals indicated lower SDMA concentrations than concentrations in younger animals (Figure 2 A).

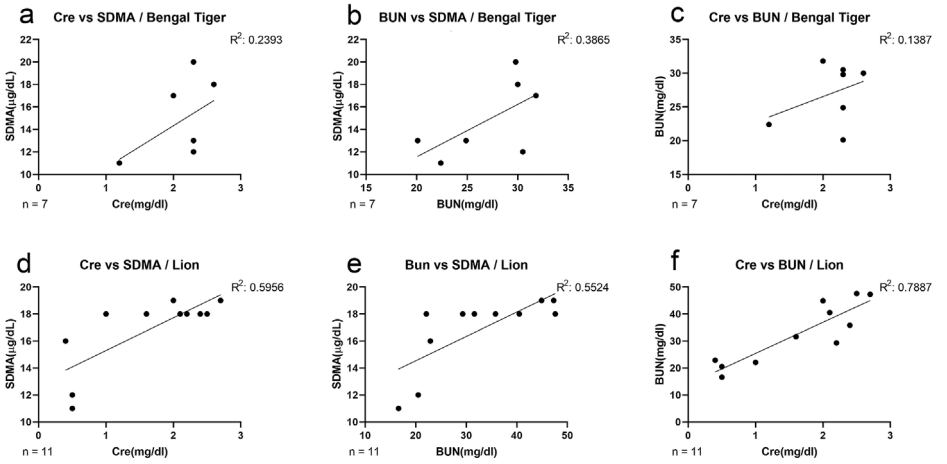

Figure 1. Correlation between serum BUN, creatinine, and SDMA concentrations in Bengal tigers and lions at Everland Zoo in the Republic of Korea.

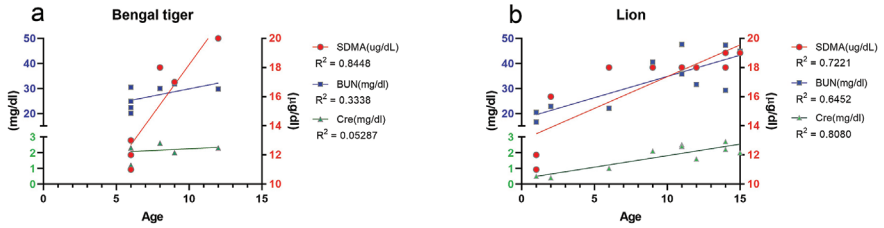

Figure 2. Serum BUN, creatinine, and SDMA concentrations and their correlation with increased age in Bengal tigers and lions at Everland Zoo in the Republic of Korea.

DISCUSSION

This study examined the use of SDMA in evaluating renal health in tigers and lions and investigated the possibility of its role as an indicator for proper health management with increasing age. Domestic and nondomestic cats both exhibit high frequency of renal disease, especially with age [9,11,17,27]. As the animal husbandry techniques of nondomestic Felidae improve, the number of old animals gradually increases. Old large cats will have different degenerative diseases, including CKD [13]. Normally, degenerative diseases, paralysis or lethargy and reduced activity, develop in big cats around 10–19 years [27]. When a degenerative disease in the captive Felidae is already in progress, slowing the progression can be challenging [8,27].

This study confirmed that SDMA concentrations of zoo Felidae were already elevated compared to BUN and Cre before the age of ten. However, since the data of SDMA is insufficient, more research is needed to determine whether the SDMA concentration is high or whether it rises faster than BUN and Cre. In addition, there were no significant results in blood tests other than BUN, Cre, and SDMA.

This is also because the SDMA concentration is hardly affected by age compared to the BUN [28]. SDMA increased earlier than the initial reduction in GFR even in domestic cats and was widely used as an early detection biomarker of renal function [21,29,30]. Therefore, the recommended age for more frequent health screening for captive cats in zoos is about 6–7 years. Early detection of renal disease permits dietary and husbandry changes that will improve the quality of life for large cats and enhance longevity.

Creatinine and SDMA have been used to diagnose chronic renal failure. Creatinine is a product of muscle metabolism, usually associated with animal muscle mass. It is mainly excreted in the kidneys and may increase as the GFR reduces. However, in animals with low muscle mass, levels may appear normal even though GFR is reduced due to the low metabolic rate of creatinine. The reduction in GFR and rise in Cre levels are indirectly proportional, but rather a rapid increase in Cre values as the GFR reduces. It suggests that even if the Cre value increases slightly from the initial kidney problem, the actual kidney function may be significantly worse [5].

Evaluation of SDMA concentrations from nondomestic Felidae like African lions has been previously rare. Thus, this study was conducted to evaluate the relationship among three biomarkers to predict kidney injury, Cre, BUN, and SDMA, in captive Felidae (Bengal tigers and lions) at the Everland Zoo in the Republic of Korea. Additionally, this study was conducted because comparative analysis will be possible when looking at the histopathological results of animals who were subjected to blood tests in the future. All three biomarkers were elevated in association with increased age in lions. But one study found that age did not affect SDMA in tigers [28]. Relationships between SDMA and BUN and between SDMA and Cre were more remarkable in lions than in Bengal tigers. In a study on SDMA measurement of cheetahs, the correlation between SDMA and Cre was higher than others [9].

The SDMA measurement of domestic cats is well established. Non azotemic cats with SDMA concentrations of >14-μg/dL ultimately progressed to azotemic CKD [29]. Another study indicated that serum SDMA concentration was positively correlated with renal dysfunction; thus, serum SDMA concentration can be used to diagnose CKD in domestic cats [24]. Likewise, a recent study indicated that SDMA is a sensitive indicator of kidney disease in older cats and is strongly associated with GFR [11]. All animals in our study were healthy without obvious renal symptoms and were not diagnosed with renal disease. SDMA is known to be associated with GFR in dogs and cats but is not correlated with GFR, BUN, or creatinine in cheetahs [5,26]. Therefore, a comparative analysis will be required to determine if there are any differences to

correlate SDMA with biopsy and/or GFR values in tigers and lions. As serum SDMA increases before Cre in domestic cats, dogs, and cheetahs with kidney disease [9,[21,29,32], Cre levels in lions and tigers should be measured and monitored regularly in the zoo.

One of the limitations of this study is that blood was obtained during anesthesia and that GFR was not measured. If blood is obtained through medical training like Positive Reinforcement training without anesthesia at regular check-ups, it seems that changes in blood composition or risks induced by anesthesia can be minimized. As the cheetah study evaluated GFR, it seems useful to use the creatinine clearance technique for GFR in the future in captive Felidae [28,33]. Additionally, histopathological kidney diagnosis has not been made because no animals have died yet, which is for future study.

In the results of this study, a difference in creatinine concentration between lions and tigers can also be seen. In the case of tigers, there were also differences in creatinine concentration in subspecies due to intestinal absorption differences or GFR [28]. In a similar age group, the Cre concentration of the tiger is slightly higher than that of the lion, and Bengal tigers and Amur tigers seem to have a little similar Cre value. It looks like other domestic cat breeds [34]. There is a difference in the increase of lions by age; so more research is required to determine whether there is a difference from actual clinical symptoms. In addition to blood Cre and SDMA concentration, many efforts have been made to predict CKD in felines, such as homocysteine [35].

In this study, the serum SDMA concentration in these animals was elevated in correlation with the age progression, showing that SDMA can be used to indicate age-related renal function loss. Note that some young tigers exhibited higher serum SDMA concentrations than the older tigers. This may show that these animals possibly had a congenital renal dysfunction. Considering all the data and previous studies, SDMA is a promising biomarker that can predict renal dysfunction associated with increased age. Further research is needed to clarify these findings. Recently, research on SDMA concentration has been actively conducted in dogs and cats and in nondomestic felids, such as tigers and cheetahs and other animals like draft horse [9,28,36]. In some case studies, biological changes on SDMA and Cre concentration in domestic cats and dogs were considered [32,37]. But, in this study, urinalysis for measuring the specific gravity of urine or blood pressure, ultrasound examination was not performed. There will be an attempt to establish a reference range for blood SDMA concentration in tigers and lions and predict CKD through more continuous research activities. In the future, we hope that SDMA research on different animals will positively affect the diagnosis of kidney disease and based on animal welfare.

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Authors' contributions

SHY have made experiment and analyzed data, wrote drafts. AKE, MJC, JKS analyzed data. EJ, KYS, HJS, JYY, KSH, and DHC monitored, handled animal, performed experiments and analyzed data. JHY, JYS, EJL, and IHH revised manuscripts and interpretation of figures. SHY and JKS discussed projects, conceived the study, designed the study and made finalyzed drafts. All authors of Zoo members and KNU members approved the final manuscript.

Declaration of conflicting interests

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

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SIMETRIČNI DIMETILARGININ PREDSTAVLJA POTENCIJALNI BIOMARKER ZA RANU DETEKCIJU DISFUNKCIJE BUBREGA KOD STARIH FELIDA U ZOO VRTOVIMA

Soong-Hee YOUN, Ahmed K. ELFADL, Myung-Jin CHUNG, Eun JUNG, Ki-Yong SHIN, Hyeon-Joo SHIN, Joon-Young YANG, Kwang-Seok HEO, Dong-Hee CHUNG, Jae-Hyuk YIM, Ji-Yoon SON, Eun-Joo LEE, Il-Hwa HONG, Kyu-Shik JEONG

Hronično otkazivanje bubrega predstalja jedan od vodećih uzroka uginjavanja Afričkih lavova, geparda i tigrova. Konvencionalne metode za merenje disfunkcije bubrega obuhvataju merenje kreatinina u serumu kao i azota iz ureje u krvi (BUN). Merenje simetričnog dimetilarginina (SDMA) je pouzdani biomarker koji može da predvidi disfunkciju bubrega kod domaćih mačaka zato što se nivoi SDMA u serumu povećavaju u ranoj fazi oštećenja bubrega. U studiji je obavljena procena nivoa SDMA u serumu kao i korelacija ovih vrednosti sa kreatininom i BUN-om kod zdravih Bengalskih ti-

grova i lavova u Everland zoo vrtu u Koreji. Povećane koncentracije SDMA u serumu su bile asocirane sa starošću lavova. Međutim, kod nekih mladih Bengalskih tigrova, koncentracije SDMA su bile veće u poređenju sa starijim životinjama. Ovaj podatak omogućava da se postavi rana detekcija disfunkcije bubrega kod ovih mladih mačaka u poređenju samo sa podacima o BUN-u i kreatininu. Kod Bengaslih tigrova, korelacija između SDMA i BUN, bila je neznatno veća nego što je to bio slučaj korelacija između SDMA i kreatinina. Kod lavova, korelacija SDMA bila je jača sa kreatininom nego sa koncentracijama BUN-a. Dobijeni rezultati ukazuju da se koncentracija SDMA u serumu može koristiti kao biomarker u proceni disfunkcije bubrega koja je povezana sa starenjem. Merenje SDMA moglo bi da bude korisna preventivna metoda u zoo vrtovima.