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Research Article

EVALUATION OF SYMMETRIC DIMETHYLARGININE AS A BIOMARKER FOR EARLY DIAGNOSIS OF CHRONIC RENAL DISEASE IN DOGS

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ABSTRACT

The study was conducted with the objective to ascertain the early diagnosis of chronic renal disease with help of symmetric dimethylarginine (SDMA). Dogs aged above five years with history, clinical signs and laboratory findings suggestive of renal disease were taken up for the study. Based on the creatinine values, animals were selected and divided into 3 groups Group-I (At risk group), Group-II (Renal failure group) and Group-III (Control). SDMA was measured by IDEXX catalayst one machine by using canine SDMA kit. There was a significant difference (P d" 0.05) in mean values of SDMA between Group-II and Group-I and between Group-II and Group-III while there was non-significant difference (P > 0.05) in mean between Group-I and Group-III (control). However, out of the 10 animals of Group-I, nine animals showed elevated SDMA levels as compared to control and three animals had elevated levels of SDMA as compared to normal reference range of IRIS guidelines. Therefore, it was concluded that elevated serum SDMA levels in individual animals suspected for CKD in Group-I are indicative of early onset of renal disease.

Keywords: Dogs, Biomarker, Renal failure, SDMA

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Elevation of serum creatinine is still considered the gold standard for diagnosis of renal disease in dogs along with analysis of serum urea concentration, urine specific gravity and UPC. However, creatinine concentration becomes elevated only after over 75% of nephrons are damaged thus making it unreliable in early diagnosis of renal disease. Nonetheless, early diagnosis has atmost clinical significance as early intervention can delay disease progression, potentially improving quality of life and extending survival (Tenhündfeld *et al.*, 2009). Thus, the lack of an accurate, simple, and minimally invasive marker of GFR is a limiting factor in the diagnosis of renal disease and newer, more precise alternatives are constantly sought. SDMA is an amino acid that is released by cells during protein degradation and was first discovered in human urine in 1970 (Kakimoto and Akazawa 1970). SDMA clearance is primarily accomplished through glomerular filtration. Serum SDMA values are reported to be are elevated much before serum creatinine values and as early as 20-40% of nephrons being damaged (Hall *et al.*, 2016). Since the introduction of commercially available SDMA assays for use in dogs, serum SDMA has been increasingly used as an indirect marker of GFR. Hence keeping in view above facts, the present study was carried out with the objective to determine the usefulness of SDMA as a biomarker in the early diagnosis of renal disease.

MATERIALS AND METHODS

The study was conducted in the Department of Veterinary Medicine, Veterinary College Science Bangalore, KVAFSU, Bidar (Karnataka). Twenty-two dogs aged above five years with history, clinical signs and laboratory findings suggestive of renal disease were taken up for the study. Blood was collected from these animals and the serum creatinine values were determined. The samples were immediately processed for hematological and biochemical (BUN) the remaining serum samples were frozen in aliquots at -80 °C for analysis of SDMA which was carried out after a month of clinical study with good prognosis. Based on the creatinine values, animals were selected and divided into 3 groups.

International renal interest society (IRIS) had modified the guidelines for staging of CKD for dogs in 2019. In these discrepancies between creatinine and SDMA was taken into consideration. As per new guidelines if serum or plasma SDMA is persistently ≤ 18 µg/dl in a dog whose creatinine is <1.4 mg/dl (IRIS CKD stage 1 based on creatinine), this canine patient should be staged and treated as an IRIS CKD Stage 2 patient. If serum or plasma SDMA is persistently ≤ 35 µg/dl in a dog whose creatinine is between 1.4 and 2.8 mg/dl (IRIS CKD stage 2 based on creatinine), this canine patient should be staged and treated as an IRIS CKD Stage 3 patient. If serum or plasma SDMA is persistently 54 µg/dl in a dog whose creatinine is between 2.9 and 5.0 mg dl (IRIS CKD stage 3 based on creatinine), this canine patient should be staged and treated as an IRIS CKD Stage 4 patient while stage 4 SDMA values are ≥ 54 µg/dl.

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At risk group (Group-I)

Comprised of ten dogs with clinical signs suggestive of renal disease with normal serum creatinine values of ≤ 1.4 mg/dl were selected to determine the possibility of early renal disease being present in these animals.

Renal failure group (Group-II)

Comprised of six dogs with clinical signs of renal failure and serum creatinine values of >1.4 mg/dl for comparison of creatinine and SDMA values.

Healthy control group (Group-III)

Comprised of six apparently healthy dogs with serum creatinine values of ≤1.4 mg/dl were selected as control group.

Estimation of concentration of SDMA in serum samples

SDMA was estimated by using IDEXX Catalyst One Chemistry Analyzer. Procedure followed was as per manufacturer's instructions. Sample information was entered on the IDEXX Vet. Lab station and then the patient and sample type were selected on the Catalyst Dx chemistry analyzer. The sample was the loaded into the slot along with the chip, SDMA slide and reagent. The appropriate button was then tapped as per instruction and the result was displayed IDEXX Vet. Lab station which was recorded in µg/dl.

Statistical analysis

Statistical analysis (One way ANOVA) of collected data was performed with IBM SPSS software (New York, USA) and GraphPad Prism 8.4 software (GraphPad Software, CA, USA).

RESULTS AND DISCUSSION

In the present study predominant signs observed in Group-I and Group-II is depicted in Table 1. The clinical signs observed were anorexia, diarrhea, vomition and polyuria and polyphagia.

There was significant difference (P ≤ 0.05) in the mean values of TEC, hemoglobin, PCV, TLC, neutrophil, lymphocyte and platelet count in Group-II when compared to Group-I and Group-III (Table 2) except TLC. Devipriya *et al.* (2018) has also reported lower values for TEC, Hb and PCV in dogs with renal failure, suggesting that affected dogs were suffering from anemia possibly due to deficient production of erythropoietin which commonly reported in CKD (Silverberg *et al.*, 2002).

Sumit *et al.* (2018) has also reported that leukocytosis with neutrophilia and lymphopenia in animals with renal failure. Increased TLC count with neutrophilia and lymphopenia that was observed in the present study could be because of the presence of inflammation, which may either have an infectious or a non-infectious cause (Osborne and Finco 1995). Gafter *et al.* (1987) attributed thrombocytopenia in renal failure to insufficient thrombopoietic activity.

Table 1

Clinical signs exhibited by dogs of Group I and Group II

Clinical signs	Group I (n = 10)		Group II (n = 6)	
	No. of animals	(%)	No. of animals	(%)
Anorexia	8	80	6	100
Diarrhea	6	60	4	66.6
Vomiting	8	80	4	66.6
Polyuria and polydipsia	1	10	3	50
Oral ulceration	1	10	4	66.6
Pallor of mucous membrane	3	30	4	66.6
Melena	1	10	5	83.3
Lethargy	5	50	5	83.3
Recumbency	-	-	3	50

There was significant difference (P ≤ 0.05) in the mean values of serum creatinine and BUN in Group-II when compared to Group-I and Group-III. The normal levels of creatinine in blood are indicators of ability of kidney to eliminate nitrogenous waste products successfully. According to the classification of IRIS (2019) creatinine values of ≤1.4 mg/dl and SDMA values ≤ 18 µg/dl is considered as stage I renal failure. Therefore, some of animals in Group-I with clinical signs suggestive of renal failure and with normal creatinine levels (≤ 1.4 mg/dl) could be in stage I renal failure (Table 3). Creatinine inversely correlates with GFR and is a better indicator of renal function but creatinine is insensitive to detect early renal insufficiency because the values increase above the reference ranges only when the damage to the kidneys is more than 60-75 per cent (Lefebvre, 2011).

In the present study, SDMA was measured by IDEXX Catalyst one machine by using canine SDMA kit. There was a significant difference (P ≤ 0.05) in mean values between Group-II and Group-I and Group-II and Group-III (Table 3). This is in agreement with findings of Hall *et al.* (2016); Supreet (2017); Ernst *et al.* (2018); Sonu (2019) and Sueur *et al.* (2019) wherein all of these workers reported low levels of serum SDMA in healthy control group as compared to much higher values in animals with renal disease. IRIS guidelines 2019 of renal failure suggested a normal value of SDMA in stage is < 18 µg/dl. Further, the increased serum SDMA in all animals of

Table 2

Values of hematological parameters in dogs of Group I, II and III

Parameters	Group I	Group II	Group III
TLC ($\times 10^3/\mu L$)	23.96 ± 3.45^{ax}	25.18 ± 4.36^{ax}	11.86 ± 1.00^{aw}
Hb (g/dL)	14.18 ± 0.35^{aw}	9.43 ± 0.70^{ax}	14.20 ± 0.52^{aw}
PLT ($\times 10^3/\mu L$)	343 ± 45.5^{ay}	191.9 ± 32.2^{az}	364.8 ± 31.6^{ay}
TEC ($\times 10^6/\mu L$)	6.58 ± 0.23^{ay}	5.35 ± 0.17^{az}	6.91 ± 0.24^{ay}
PCV (%)	41.30 ± 1.59^{ay}	28.20 ± 1.90^{ax}	43.72 ± 1.44^{ay}
Neutrophil (%)	73.33 ± 2.73^{ay}	85.03 ± 0.94^{ax}	72.76 ± 2.36^{ay}
Lymphocyte (%)	19.96 ± 1.17^{ay}	13.98 ± 4.94^{ax}	24.7 ± 1.01^{ay}

Note: a, b, c, d Mean values within a row having dissimilar superscripts differ significantly ($P \leq 0.05$)
w, x, y, z Mean values within a column having dissimilar superscripts differ significantly ($P \leq 0.05$)

Group-II in the present study correlated well with increased creatinine values thus proving that serum SDMA is a good index to predict renal failure. The physiological and the biochemical role of SDMA in diagnosis of renal disorders has been described by Schwedhelm and Boger (2011), wherein these workers have stated that SDMA is eliminated from the body primarily by renal excretion with more than 90 per cent through glomerular filtration without tubular reabsorption or secretion and serum concentrations of SDMA are increased in patients with renal failure. Further it has been shown that serum SDMA concentrations are inversely correlated with glomerular filtration rate (GFR). Thus, the increased SDMA level in Group-II animals in the present study is a confirmation of renal disease, as was also evident by increased serum creatinine values.

There was no significant difference ($P > 0.05$) in mean values of SDMA between Group-I and Group-III (control) (Table 3). However, of the 10 animals of Group-I, 9 animals showed elevated SDMA levels as compared to control and 3 animals had elevated levels of SDMA as compared to normal reference range of IRIS (2019) (Table 4). According to Dahlem *et al.* (2017) SDMA represents the GFR and compared to creatinine, serum SDMA has higher sensitivity for detecting renal function and progression of renal disease in dogs, correlating strongly with GFR in dogs with progressive kidney disease. According to Hall *et al.* (2016) SDMA values increases with 25 to 40 per cent reduction in GFR, whereas increase in creatinine levels cannot be detected until GFR is reduced by 75 per cent. Therefore, it can be concluded that elevated serum SDMA levels in individual animals in Group-I are indicative of early onset of renal failure. This is in agreement with Nabity *et al.* (2015) wherein it was stated that use of cut off for serum creatinine is still an unreliable method for detecting early decrease in kidney function as it detected the reduced function much later as compared to SDMA. Authors further stated that SDMA detected decreasing GFR earlier than all other methods and hence was a more sensitive biomarker and could add value in early detection of renal disease particularly if evaluating the dog for the first time without the benefit of previous testing.

Table 3

Values of serum creatinine and SDMA in dogs of Group I, II and III

Parameters	Group I	Group II	Group III
Serum creatinine (mg/dl)	1.20 ± 0.06^{ax}	5.30 ± 0.87^{ax}	1.10 ± 0.06^{aw}
Serum SDMA (µg/dL)	$14.7\pm1.38aw^{*}$	41.5 ± 10.51^{ax}	9.5 ± 0.76^{aw}
Serum BUN (mg/dl)	22.4 ± 2.51^{az}	79.4 ± 8.93^{ay}	19.0 ± 2.64^{az}

Note: a, b, c, d Mean values within a row having dissimilar superscripts differ significantly ($P \leq 0.05$)
w, x, y, z Mean values within a column having dissimilar superscripts differ significantly ($P \leq 0.05$)
* According SDMA 2019 guidelines if serum or plasma SDMA is persistently ≤ 18 µg/dl in a dog whose creatinine is <1.4 mg/dl (IRIS CKD stage 1 based on creatinine)

CONCLUSION

It was thereby concluded that, SDMA was an effective marker for detecting and tracking reduced renal function in dogs. Hence, SDMA proved to be a promising endogenous GFR marker, which could be particularly useful to overcome the shortfalls associated with sCr assessment. Additional research is warranted in dogs with the application of ultrasonography, urine culture examination and characterization of renal diseases to ascertain the cases in which SDMA can be helpful as well as to determine how soon SDMA values can detect declining renal function in a clinical environment relative to sCr with a larger sample size.

REFERENCES

Dahlem, D.P., Neiger, R., Schweighauser, A., Francey, T., Yerramilli,

M., Obare, E. and Steinbach, S.M.L. (2017). Plasma SDMA concentration in dogs with acute kidney injury and chronic kidney disease. *J. Vet. Intern. Med.* **1**: 1-6.

Devipriya, K., Lavanya, C., Selvaraj, P. and Ezakialnapolean, R. (2018). Early diagnosis of renal insufficiency in dogs with haemato-biochemical findings. *J. Entomol. Zool.* **6(5)**: 703-705.

Ernst, R., Ogeer, J. and Mccrann, D. (2018). Comparative performance of IDEXX SDMA test and the DLD SDMA ELISA for the measurement of SDMA in canine and feline serum. *PLoS One*. **13(10)**: 0205030.

Gafter, U., Bessler, H. and Malachi, T. (1987). Platelet count and thrombopoietic activity in patients with chronic renal failure. *Nephron.* **45(3)**: 207-210.

Hall, J.A., Yerramilli, M., Obare, E. and Jewell, D.E. (2016). Serum concentrations of symmetric dimethylarginine and creatinine in dogs with naturally occurring chronic kidneydisease. *J. Vet. Intern. Med.* **30(3)**: 794–802.

International Renal Interest Society. (2019). IRIS Staging of CKD (modified 2019). http://www.iris-kidney.com.

Kakimoto. Y and Akazawa. S. (1970). Isolation and identification of N-G, N-G- and N-G, N'-G- dimethyl-arginine, N-epsilon-mono-, di-, and trimethyllysine, and glucosylgalactosyl- and galactosyl-delta-hydroxylysine from human urine. *J. Biol. Chem.* **245**: 5751-5758.

Lefebvre, H. 2011. Renal function testing. In: Nephrology and urology of small animals. (Edt.), Bartges, J. and Polzin, D. Wiley-Blackwell, USA. pp. 91-96.

Nabity, M.B., Lees, G.E., Boggess, M.M., Yerramilli, M., Obare, E., Yerramilli, M. Rakitin, A., Aguiar, J. and Relford. R. (2015). Symmetric Dimethylarginine Assay Validation, Stability, and Evaluation as a Marker for the Early Detection of Chronic Kidney Disease in Dogs. *J. Vet. Intern. Med.* **29**: 1036-1044.

Osborne, C.A. and Finco, D.R. (1995). Renal failure and uremia. Canine and feline nephrology and urology. Williams and Wilpins, Baltimore.

Schwedhelm, E. and Boger, R.H. (2011). The role of symmetric and asymmetric dimethylarginines in renal disease. *Nat. Rev. Nephrol.* **7**: 275-285.

Silverberg, D.S., Wexler, D. and Blum, M. (2002). The correction of anemia in severe resistant heart failure with erythropoietin and intravenous iron prevents the progression of both the heart and the renal failure and markedly reduces hospitalization. *Clin. Nephrol.* **58(1)**: 37-45.

Sonu (2019). Study on biomarkers in diagnosis of renal failure and its management in dogs. M.V.Sc. thesis. Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India.

Sueur, A., Geraldes, S.S., Melchert, A., Takahira, R.K., Coyne, M., Murphy, R., Szlosek, D. and Okamoto, C.G. (2019). Symmetric dimethylarginine concentrations in dogs with International Renal Interest Society stage 4 chronic kidney disease undergoing intermittent hemodialysis. *J. Vet. Intern. Med.* **1**: 1-9.

Sumit, Parveen G., Praveen, K., Dinesh, G., Ricky, J., Neelesh, S. and Chaudhary, R.N. (2018). Haematobiochemical and serum electrolytes alteration in dogs with chronic kidney disease. *J. Pharm. Innov.* **7(11)**: 302-306.

Supreet, S. (2017). Studies on the early diagnostic and prognostic markers of chronic kidney disease in dogs. M.V.Sc. thesis. GADVASU, Ludhiana, Punjab, India.

Tenhündfeld, J., Wefstaedt, P. and Nolte, I.J. (2009). A randomized controlled clinical trial of the use of benazepril and heparin for the treatment of chronic kidney disease in dogs. *J. Am. Vet. Med. Assoc.* **234**: 1031-1037.

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