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EVALUATION OF SERUM SYMMETRIC DIMETHYLARGININE (SDMA) IN CLINICALLY HEALTHY GERIATRIC HORSES

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ABSTRACT

In recent years, there is a necessity for horses to be evaluated for kidney function as equine kidney disease is becoming an increasing problem. As we know, horses are often treated on everyday basis with drugs that can be nephrotoxic in some instances. This is why practitioners need to be familiar with different kidney biomarkers and their normal values in all horse age groups, as different age groups might have slight or significant differences in normal values. In this article we evaluate equine serum SDMA, which is becoming widely used and accepted in clinical practice as a biomarker for early detection of kidney injury. We evaluate its serum concentration in the geriatric group of horses in order to bring more insight into its use in this age group.

Key words: horses, geriatric, SDMA, creatinine, urea.

Introduction

In recent years, according to Makris et Spanou (2016) the term Acute Kidney Injury (AKI) has widely replaced the term Acute Renal Failure (ARF). AKI is a clinical syndrome that presents with a rapid decrease in the glomerular filtration rate (GFR) and can be a result from either structural or functional damage to the kidneys (Makris et Spanou 2016; Levey et James 2017; Siwinska *et al.* 2021). According to Savage *et al.* (2019) the occurrence of AKI in a number of hospitalized horses admitted to Rossdales Equine Hospital between August 2015 and January 2019 was 14,8%. However, these numbers can vary based on the selected horses for evaluation and selected criteria for diagnostics.

Everyday use of potentially nephrotoxic substances (such as phenylbutazone, flunixin meglumine, gentamycin, and oxytetracycline) and the numerous risk factors (dehydration, diarrhea, colic, and endotoxemia) for the development of AKI in horses require development of better diagnostic means for kidney disease in this species. Recently, researchers focused their attention in using symmetric dimethylarginine (SDMA) in order to detect both AKI and chronic kidney disease (CKD) at their early stages. So far there is no data report on SDMA levels in confirmed CKD cases in horses (Galen *et al*. 2022).

In horses the diagnosis of kidney disease is based on finding elevated serum creatinine (sCr) levels but this is not considered efficient for early detection of impaired kidney function as sCr concentration does not increase until there is a 75% reduction in glomerular filtration rate (GFR) (Braun *et al.* 2003; Hokamp et Nabity 2016; Scott *et al.* 2021). This is why more research was conducted to provide a more reliable blood parameter. That is believed to be SDMA. A study in

cats showed elevated SDMA levels in this species when there was 40% reduction in the GFR and in some instances the reduction was only 25% (Hall *et al.* 2014).

SDMA is listed as a uremic toxin (Vanholder *et al.* 2003; Tain and Hsu 2017). According to research (Raijmakers *et al.* 2007) it is a structural isomer of asymmetric dimethylarginine (ADMA). Unlike ADMA, SDMA is excreted almost entirely via the kidneys (Kakimoto et Akazawa 1970; Olivia-Damaso 2019). This makes it a suitable renal biomarker for clinical practice. However, it should be noted that according to research the liver is also capable of eliminating SDMA (Nijveldt 2003; Siroen 2005; Schepers *et al.* 2014; Olivia-Damaso *et al.* 2019).

Although SDMA is now widely used in equine clinical practice, there are not many scientific papers available on SDMA. According to Galen *et al.* (2022) there are nine studies in total on SDMA in horses. These studies involve determination of diagnostic relevance of SDMA in horses (Lo, 2022) and determination of SDMA levels in healthy horses, horses with AKI (Siwinska *et al.* 2020; Siwinska *et al.* 2021), as well as horses at risk of developing AKI (Savage *et al.* 2019). There is data on SDMA levels in horses with dehydration (Lo *et al.* 2022), in Endurance horses (Ertelt *et al.* 2021), in healthy neonatal Thoroughbreds (Gough et McGovern, 2021), in healthy neonatal foals and mares (Bozorgmanesh *et al.* 2021), and in healthy draft horses (Schott II *et al.* 2021). There is another research regarding SDMA in horses treated with non-nitrogenous biphosphonates (Edwards et Magdesian 2023). According to Galen *et al.* (2022), application of new diagnostic biomarkers in veterinary medicine needs full validation as some extrarenal factors such as breed, age, sex, and body weight can influence the reference interval of certain parameters. To our knowledge, there is no study available that evaluates serum SDMA levels in older horses. In this report we evaluate serum SDMA concentrations in 25, randomly chosen, clinically healthy geriatric horses. The objective of this study was to assess serum SDMA levels in healthy geriatric horses. The aim was to check if old age influences serum SDMA levels.

Materials and methods

Animals

For the purpose of the study we collected blood samples from clinically healthy geriatric horses. All of the horses were clinically examined before sample collection. There was a total of 25 horses of autochthonous, sport and draft horse breeds and one riding pony included in this study. There were 5 horses between the age of 17 and 19 years old, 18 horses between the age of 20 and 30 years old, and 2 horses above 30 years of age. In this group there was 1 stallion, 15 geldings and 9 mares. One of the mares was 10 months pregnant.

Horses' health was determined via basic clinical examination that included measurement of heart and respiratory rate, internal body temperature, mucous membrane, capillary refill time determination, mandibular lymph node palpation and peristalsis.

Sample collection protocol and evaluation

Blood samples were collected from the jugular vein of all of the horses. We used 20G $1^1/_2$" needles (Vacutest® KIMA S.R.L., Italy) and blood was collected in 6 ml vacutainer tubes (Vacutest® KIMA S.R.L., Italy) containing clot activator. Within 6 hours of obtaining the samples, the serum (1 mL) was separated in Eppendorf tubes and frozen at -20°C. All of the samples were evaluated within 14 days after collection. Sample collection was performed from 28th of March to 10th

of April 2024). Samples were evaluated in an external veterinary laboratory (Laboklin GmbH & Co. KG, Germany) via photometric studies.

Statistical Analysis

Data were statistically analyzed using one-way ANOVA, and the level of statistical significance calculated according to the Tukey-Kramar test ($P<0.05$). The results are presented as mean ± standard error of the mean (SEM).

Results

Clinical examination of the tested horses did not show any signs of systemic disease. All horses had their heart rate, respiratory rate and internal body temperature measured. They were all examined for changes in the color of the mucous membranes, capillary refill time, size of the lymph nodes and changes in peristalsis. The results are shown in Table 1. Based on the obtained results the horses were considered suitable for the purpose of this article. None of the evaluated horses had any of the evaluated parameters below or above the reference range interval for this species. Given the aforementioned, the selected horses for the article are considered clinically healthy.

Table 1: Age and Clinical Examination of the selected horses

Age (years old), median (range), (n)	Heart rate (bpm), median (range)	Respiratory rate (bpm), median (range)	Internal body temperature (°C), median (range)	Mucous membranes (color),	Capillary refill time (s)	Mandibular lymph node (reactivity)	Peristalsis (normal or abnormal)
25,24±0,91 (17-34), n=25	33,6±0,48 (30-40)	10,2±0,35 (8-14)	37,3 (37,0-37,7°C)	Pale pink	<2 s	Not reactive	Normal, present in all quadrants

The study included a total of 25 horses, with median age 25,24±0,91, and range 17–34 years old. The mean value of serum SDMA in the evaluated horses was 0,47±0,03μmol/L, and range 0,13–0,72μmol/L. This suggests that the serum SDMA values in geriatric horses do not differ from the values that are obtained in grown healthy horses.

Discussion

Diagnosis of kidney injury in horses is mostly relied on finding elevated serum Creatinine (sCr) levels (Siwinska *et al.* 2021). However, Delanaye *et al.* (2017) mention in their work that sCr levels in people are influenced by extrarenal factors such as muscle mass (Heymsfield 1983; Spender 1986; Perrone *et al.* 1992), daily protein intake (Crim *et al.* 1975; Heymsfield 1983; Mayersohn *et al.* 1983; Perrone *et al.* 1992; Preiss *et al.* 2007), and possibly by extrarenal clearance via intestinal bacteria (Mitch et Walser 1978). In horses it is reported that sCr concentration is increased in some normal Quarter Horses that have more muscle mass and is lower in cachectic horses that lack reasonable amount of muscle mass (Smith *et al.* 2001; Galen *et al.* 2022). This makes sCr hard to interpret in some instances. Moreover, this requires that selected biomarkers for clinical use should be well investigated.

The expected results were that old age will not affect SDMA concentration in horses. According to available literature (Galen *et al.* 2022) SDMA is not influenced by extrarenal factors such as sex (Siwinska *et al.* 2020; Gough et McGovern 2021; Fraczkowska *et al.* 2021; Schott *et al.* 2021), age in fully grown horses (Siwinska *et al.* 2020; Schott *et al.* 2021; Fraczkowska *et al.* 2021), weight (Siwinska *et al.* 2020; Schott *et al.* 2021), and body condition score (Schott *et al.* 2021). The reference range intervals that have been determined so far showed that foals have significantly higher normal values for SDMA than adult horses (Galen *et al.* 2022). Gough et McGovern (2021) reported that SDMA concentration in equine neonates is higher than that reported in adults. Another study in neonate foals and their dams also showed that SDMA levels are higher in neonates than the upper reference range of adult horses (Bozorgmanesh *et al.* 2021).

In adults, the reference range intervals is reported to be 0,3-0,8 μmol/L (Siwinska *et al.* 2020; Ertelt *et al.* 2021; Ertelt *et al.* 2021; Galen *et al.* 2022). The mean serum SDMA concentration of the horses in our study is 0,47±0,03 μmol/L, range (0,13-0,72 μmol/L) which is within the reference adult range interval in these species. Moreover, according to the upper reference range given by the LABOKLIN laboratory (<0,75 μmol/L) all of the examined horses had SDMA values within the accepted range for adult horses. This suggests that increasing age does not influence the SDMA levels in healthy geriatric horses.

From our point of view there are some limitations of the study. Probably the number of evaluated horses is not enough and might not be considered representative. However, no single horse had SDMA concentrations above the upper reference limit and thus the study is considered to be of reasonable value.

Conclusion

Given the aforementioned results, we concluded that the likelihood of older horses having differences in the reference range interval in regards of SDMA concentration is low. Our findings show that the SDMA levels in horses of old age are not higher that the upper reference limit established for this species. It will be beneficial if future studies are performed on a greater number of horses as this will support or will reject our finding and statement.

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References

1. Bozorgmanesh R., Thornton J., Snyder J., Fletcher C., Mack R., Coyne M., Murphy R., Hegary E., Slovis N. (2021). *Symmetric dimethylarginine concentrations in healthy neonatal foals and mares.* Journal of Veterinary Internal Medicine, 35(6):2896.
2. Braun J. P., Lefebvre H. P., Watson A. D. J. (2003). *Creatinine in the dog: a review.* Veterinary Clinical Pathology, 32:162–179.
3. Cri M.C., Calloway D.H. Margen S. (1975). Creatinine metabolism in men: urinary creatinine and creatinine excretions with creatine feeding. Journal of Nutrition, 105:428–438.
4. Delanaye P., Cavalier E., Pottel H. (2017). *Serum Creatinine: Not So Simple!* Nephron, 136:302–308.

5. Edwards L., Magdesian K.G. (2023). Retrospective evaluation of acute kidney injury in horses treated with non nitrogenous bisphosphonates (2013–2020): 8 cases. Journal of Veterinary Emergency and Critical Care (San Antonio), 33(6):685–695.
6. Ertelt A., Merle R., Stumpff F., Bollonger L., Liertz S., Weber C., Gehlen H. (2021). *Evaluation of Different Blood Parameters From Endurance Horses Competing at 160 km.* Journal of Equine Veterinary Sciences 104103687.
7. Ertelt A., Stumpff F., Merle R., Kuban S., Bollinger L., Liertz S., Gahlen H. (2021). *Asymmetric dimethylarginine – a potential cardiac biomarker in horses.* Journal of Veterinary Cardiology, 33:43–51.
8. Fraczkowska K., Trzebuniak Z., Zak A., Siwinska N. (2021). Measurement of Selected Renal Biochemical Paramethers in Healthy Adult Donkeys Considering the Influence of Gender, Age and Blood Freezing. Animals, 11(6), 1748.
9. Galen G., Olsen E., Siwinska N. (2022). Biomarkers of Kidney Disease in Horses: A Review of the Current Literature. Animals, 12, 2678.
10. Gough R., McGovern K. (2021). Chronic kidney disease in adult horses: causes, diagnosis and management. UK–Vet–Equine, 5(5):195.
11. Hall J.A., Yerramilli M., Obare E., Yerramilli M., Yu S., Jewell D.E. (2014). Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in healthy geriatric cats fed reduced protein foods enriched with fish oil, L–carnitine, and medium–chain triglycerides. Veterinary Journal, 202(3):588–596.
12. Heymsfield S.B., Arteaga C., McManus C., Smith J., Moffitt S. (1983). *Measurement of muscle mass in humans: validity of the 24–hour urinary creatinine method.* American Journal of Clinical Nutrition, 37:478–494.
13. Hokamp J.A., Nabity M.B. (2016). *Renal biomarkers in domestic species.* Veterinary Clinical Pathology, 45:28–56.
14. Kakimoto, Y. 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 glucosyl galactosyl– and galactosyl–delta–hydroxylysine from human urine. Journal of Boilogy and Chemistry, 245, 5751–5758.
15. Levey A.S., James M.T. (2017). *Acute kidney injury.* Annals of Internal Medicine, 167:66–80
16. Lo H.C. (2022). Diagnostic relevance of symmetric dimethylarginine and renal function analysis in horses. Dissertation.
17. Lo H.C., Winter J.C., Merle R., Gahlen H. (2022). *Symmetric dimethylarginine and renal function analysis in horses with dehydration.* Equine Veterinary Journal, 54:670–678.
18. Makris K., Spanou L. (2016). Acute kidney injury: definition, pathophysiology and clinical phenotypes. Clinical Biochemistry Rev, 37:85–98,
19. Mayersohn M., Conrad K.A., Achari R. (1983). *The influence of a cooked meat meal on creatinine plasma concentration and creatinine clearance.* British Journal of Clinical Pharmacology, 15:277–230.
20. Mitch W.E., Walser M. (1978). A proposed mechanism for reduced creatinine excretion in severe chronic renal failure. Nephron, 21:248–254
21. Nijveldt, R. (2003). The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA). Clinical Nutrition, 22, 17–22.

22. Olivia–Damaso E, Olivia–Damaso N, Rodriguez–Esparragon F, Payan J, Baamonde–Laborda E, Gonzalez–Cabrera F, Santana–Estupinan, Rodriguez–Perez C. (2019). *Asymmetric (ADMA) and Symmetric (SDMA) Dimethylarginines in Chronic Kidney Disease: A Clinical Approach.* International Journal of Molecular Sciences, 20, 3668.
23. Perrone R.D., Madias N.N., Levey A.S. (1992). *Serum creatinine as an index of renal function: new insights into old concepts.* Clinical Chemistry, 38:1933–1953.
24. Preiss D.J., Godber I.M., Lamb E.J., Dalton R.N., Gunn I.R. (2007). *The influence of a cooked–meat meal on estimated glomerular filtration rate.* Annal of Clinical Biochemistry, 44(pt 1):35–42
25. Raijmakers R., Zendman A.J., Egberts W.V., Vossenaar E.R., Raats J., Soede–Huijbregts C., Rutjes F.P., van Veelen P.A., Drijfhout J.W., Pruijn G.J. (2007). Methylation of arginine residues interferes with citrullination by peptidylarginine deiminases in vitro. Journal of Molecular Biology, 367, 1118–1129.
26. Savage V.L., Marr C.M., Bailey M., Smith S. (2019). *Prevalence of acute kidney injury in a population of hospitalized horses.* Journal of Veterinary Internal Medicine, 33:2294–2301.
27. Schepers E., Speer T., Bode–Boger S.M., Flisher D., Kielstein J.T. (2014). *Dimethylarginines ADMA and SDMA: The Real Water–Soluble Small Toxins?* Seminars in Nephrology, 34, 97–105
28. Schott H.C. II, Gallant L.R., Coyne M., Murphy R., Cross J., Strong–Townsend M., Szlosek D., Yerramilli M., Li J. (2021). *Symmetric dimethylarginine and creatinine concentrations in healthy draft horses.* Journal of Veterinary Internal Medicine, 35:1147–1154.
29. Siroen M.P., Van Der Sijp J.R.M., Teerlink T., Van Schaik C., Nijveldt R., Van Leeuwen P.A.M. (2005). *The human liver clears both asymmetric and symmetric dimethylarginine.* Hepatology, 41, 559–565.
30. Siwinska, N., Zak, A. & Paslawska, U. (2021). Detecting acute kidney injury in horses by measuring the concentration of symmetric dimethylarginine in serum. Acta Veterinaria Scandinavia, 63, 3.
31. Siwinska N, Zak A, Slowikowska M, wt al. (2020). Serum symmetric dimethylarginine concentration in healthy horses and horses with acute kidney injury. BMC Veterinary Research, 16:396.
32. Smith B.P., van Metre D.C., Pusterla N. (2019). *Large Animal Internal Medicine,* Elsevier Health Sciences: St. Louis, MO, USA.
33. Spender K. (1986). Analytical reviews in clinical biochemistry: the estimation of creatinine. Annal of Clinical Biochemistry, 23(pt 1):1–25.
34. Tain Y.L., Hsu C.N. (2017). Toxic Dimethylarginines: asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). Toxins, 9(3), 92.
35. Vanholder, R; De Smet, R; Glorieux, G; Argiles, A; Bauermeister, U; Brunet, P; Clark, W; Cohen, G; De Deyn, P.P; Deppisch, R; *et al.* (2003). *Review on uremic toxins: Classification, concentration, and interindividual variability.* Kidney International, 63, 1934–1943.