ASSOCIATION OF SERUM MAGNESIUM WITH HAEMATOLOGICAL HEALTH INDICES IN DOGS

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ABSTRACT

Magnesium (Mg) is the second most abundant trace element in the body of both humans and animals and is an essential element for health. Hypomagnaesemia and hypermagnaesemia have been reported to be common occurrences in critically ill patients and were postulated to determine the prognosis of disease. This study was designed to ascertain the relationship between haematological health indices and serum Mg levels. A total of 103 dogs from the 'dog market 'of Jos, Plateau State were sampled. Blood was collected for complete blood count (CBC) using standard manual procedures: Serum protein analysis was by the biuret colorimetric method, while serum Mg levels were determined with an atomic absorption spectrophotometer. Result showed that anaemic dogs (PCV < 35 %) had significantly ($p<0.05$) lower serum Mg (1.9 ± 0.15 mg/dL) than non-anaemic (PCV ≥ 35 %) dogs (2.6 ± 0.12 mg/dL). Dogs with regenerative anaemia (corrected reticulocyte count ≥ 1%) had significantly ($p<0.05$) lower serum Mg (2.3 ± 0.19 mg/dL) than dogs with non-regenerative anaemia (2.8 ± 0.12 mg/dL). Dogs with thrombocytopenia had significantly ($p<0.05$) lower serum Mg (2.00 ± 0.16 mg/dL) than those with normal platelet counts (2.94 ± 0.12 mg/dL). Animals with marked leukopenia, mild neutropenia and marked lymphopenia had significantly ($p<0.05$) lower serum Mg levels. In conclusion, dogs with poor haematological health indices; low PCV, low platelet counts, low lymphocyte counts and low serum protein were associated with significantly lower serum Mg levels.

Keywords: Dogs, Haematological health indices, Serum magnesium, Blood disorders

INTRODUCTION

Magnesium (Mg) is important in many biological functions as a coenzyme in the body of both humans and animals (Martin *et al.,* 1994). Mg is involved in more than 300 enzymatic reactions and is essential for life. Due to the proliferative nature of blood cells, the microenvironment which regulates the latency of the haematopoietic stem cells are key to the survival of animals in health and disease (Borelli *et al*., 2009; Mendelson and Frenette, 2014; Nogueira-Pedro *et al*., 2016; Oliveira *et al*., 2018). These cells multiply and differentiate to form leucocytes, erythrocytes and platelets and therefore require nutrients for their sustenance (Oliveira *et al*., 2018). Mg deficiency has been reported in 20 – 65 % of patients in an intensive care unit (ICU) (Reinhart and Desbiens, 1985; Ryzen *et al*., 1985; Deheinzelin *et al.,* 2000).

Interestingly, human patients who develop Mg deficiency in the ICU had mortality rates 2 to 3 times higher and more prolonged hospitalization compared with those who were not Mg deficient (Fiaccadori *et al*., 1988). Hypomagnesaemia has been reported to be the most common altered element during long admissions in the ICUs (Deheinzelin *et al.,* 2000). Haematological health indices are essential in monitoring patients' disease status (Lobato *et al.,* 2005) and these too may be associated with disturbances in Mg levels. Hypomagnesaemia is more common than hypermagnesaemia; however, hypermagnesaemic human patients in the ICU were also more likely to die than those with normal serum Mg levels (Martin *et al.,* 1994; Humphrey *et al.,* 2015). Few reports are available in small animal practice of conditions related to Mg disturbances. These therefore require that predictors of Mg alterations in veterinary practice be established. Haematological parameters are good indicators of the physiological status of animals (Khan and Zafar, 2005). Some interventions given to manage certain disease conditions may be detrimental to the Mg status of the body (Quamme and de Rouffignac, 2000). Therefore, monitoring Mg levels in dogs may improve the outcome of clinical interventions. This work was therefore designed to investigate the relationship between serum Mg levels and some key haematological indicators of health in dogs.

MATERIALS AND METHODS

Animals: A total of 103 adult Nigerian indigenous breed of dogs in the Dog Market, Jos, Plateau State, Nigeria, were used in the study. They were sampled randomly.

Blood and Serum: Blood (2 ml) was collected by jugular venipuncture into sample bottles containing EDTA for haematology. 5 ml of blood was collected into plain sample bottles for separation of serum. Samples were transported to the laboratory in cold packs. The clotted blood was centrifuged at 1500 g for 10 minutes. The supernatant serum was collected into Eppendorf tubes and stored in the freezer (-20^{o}C) pending analysis.

Haematology and Serum Protein Analyses: Complete blood counts (CBC) were done using standard manual procedures: PCV by the microhaematocrit method, platelet and total WBC counts with the haematocytometer as described by Jain (1986). Differential WBC counts were by examination of Leishman - stained blood smears (100 cells). Serum protein analysis was carried out by the Biuret colorimetric method (Jain, 1986). Dogs with regenerative anaemia were determined when corrected reticulocyte count was ≥ 1 % and PCV <35 %, while non-regenerative anaemic dogs were those with PCV ≥35 % and corrected reticulocyte count of <1 % (Jain, 1993).

Serum Magnesium Determination: Each serum sample was diluted 50 fold with Milli Q deionized water. The diluted samples were then measured for Mg using the Atomic Absorption Spectrophotometer (PerkinElmer Analyst 200) fitted with Winlab 32 Software.

Statistical Analyses: Data were analyzed descriptively for their central tendencies with

Graph Pad Prism version 7 (Graph Pad Software, San Diego, CA) and results expressed as Mean ± SE. One-way ANOVA was used for comparison data, followed by the post hoc Newman-Keuls Multiple Comparison test. P<0.05 was considered statistically significant.

RESULTS

Relationship between Serum Magnesium and PCV and Platelets Counts: Anaemic dogs (PCV <35 %) had significantly lower (1.9 ± 0.15 mg/dL) serum Mg levels than non-anaemic (PCV ≥35 %) dogs (serum Mg 2.6 ± 0.12 mg/dL) (Table 1). Dogs with regenerative anaemia (corrected reticulocyte count ≥ 1 %) had statistically significant lower serum Mg levels (2.3 ± 0.19 mg/dL) than those with non-regenerative anaemia (corrected reticulocyte count <1 %) (serum Mg of 2.8 ± 0.12 mg/dL). Dogs that were thrombocytopenic had significantly (p<0.05) lower serum Mg (2.00 ± 0.16 mg/dL) than dogs with normal platelet counts (serum Mg levels 2.94 ± 0.12 mg/dL) (Table 1).

Relationship between the Leukogram and Serum Magnesium Levels in Dogs: Dogs with leukocyte counts below 6×10^3 /µL had significantly (p<0.05) higher serum Mg levels (2.73 ± 0.07 mg/dL) than those with leukocyte counts ≥ 6×10^3µL (serum Mg levels 2.29 ± 0.11 mg/dL). Dogs with segmented neutrophil counts ≥ 3×10^3/µL had significantly (p<0.05) lower serum Mg levels 2.29 ± 0.08 mg/dL than those with segmented neutrophil counts below 3×10^3 /µL with serum Mg levels of 2.62 ± 0.10 mg/dL. Dogs with band neutrophil counts less than 0.3×10^3 had significantly (p<0.05) lower serum Mg levels of 2.04 ± 0.08 mg/dL than those with neutrophilic left shifts. Dogs with lymphopenia had significantly (p<0.05) lower serum Mg levels (2.0 ± 0.01 mg/dL) when compared to those with normal lymphocyte counts (serum Mg 2.5 ± 0.20 mg/dL) (Table 2).

Serum Proteins and their Relationship with Serum Magnesium Levels in Dogs: Dogs with total serum protein less than 5.5 g/dL had significantly (p<0.05) lower serum Mg levels (1.9 ± 0.22 g/dL) compared to normoproteinaemic and hyperproteinaemic dogs. However, there was no statistical difference (p>0.05) between serum Mg levels of dogs with normoproteinaemia and hyperproteinaemia. Serum Mg levels were significantly (p<0.05) higher (2.7 ± 0.06 mg/dL) in dogs with normoalbuminaemia compared to dogs that were hypoalbuminaemic (serum magnesium levels 2.0 ± 0.02 mg/dL). Also, hyperglobulinaemic dogs had significantly (p<0.05) lower serum magnesium (2.3 ± 0.24 mg/dL) than normoglobulinaemic dogs (2.7 ± 0.08 mg/dL) (Table 3).

DISCUSSION

This study has shown that anaemic dogs were hypomagnesaemic, while non-anaemic dogs were normomagnesaemic. This implies that dogs with low serum Mg are likely to respond poorly to a disease condition. Elin *et al.* (1980) in a study observed that rats fed Mg-deficient diet became significantly anaemic. This was attributed to a decreased mean cell age of erythrocytes in the circulation due to hypomagnesaemia. A study found that Mg depletion could induce hemolytic anaemia and Mg-deficient red blood cells had shorter survival duration (Zhan *et al.*, 2014). Xu *et al.* (2017) in a study observed that serum Mg is inversely associated with anaemia, with higher serum Mg associated with a lower prevalence of anaemia. This implies that correcting Mg deficiency may also have a beneficial role in ameliorating anaemia. Mg is essential to protect against oxidative damage of the erythrocyte membrane and haemoglobin, thereby increasing the RBC lifespan. Cosens *et al.* (1977) stated that the role of Mg in protein synthesis is compatible with haemoglobin synthesis. Thus, deficiency in Mg will lead to reduction in haemoglobin causing Mg deficiency anaemia.

Carroll *et al.* (1990) in a study observed a progressive increase in serum Mg levels in dogs following severe haemorrhage. They postulated the possible use of serum Mg as an index of the severity of shock. The lower serum Mg levels in dogs with regenerative anaemia can be attributed to an increased concentration of Mg, normal pyrophosphatase and appropriate pyruvate kinase activities within young erythrocytes (Elin *et al.,* 1980).

Table 1: Association between serum magnesium levels, PCV and platelets of adult indigenous breed of dogs from Jos, Plateau State, Nigeria

Parameters	Levels	N	Serum (mg/dL)
PCV (%)	≥35	20	2.6 ± 0.12
	<35	20	1.9 ± 0.15*
Regenerative anaemia	CRC ≥1	8	2.3 ± 0.19*
Non-regenerative anaemia	CRC <1	16	2.8 ± 0.12
Platelets (×10^3/µL)	<200	20	2 ± 0.16
	≥200	20	2.94 ± 0.12*

**=p<0.05, CRC = Corrected Reticulocyte Count*

Table 2: Association between serum magnesium and white blood cells of adult indigenous breed of dogs from Jos, Plateau State, Nigeria

Parameters	Levels	N	Mean Serum (mg/dL)
TWBC (×10^{3}µL)	≥6.0	37	2.29 ± 0.11*
	<6.0	66	2.73 ± 0.07
Seg Neutrophils (×10^3/ µL)	≥3.0	60	2.29 ± 0.08
	<3.0	43	2.62 ± 0.10*
Band Neutrophil (×10^3 /µL)	<0.3	59	2.04 ± 0.08^{a}*
	0.3-0.85	29	2.60 ± 0.12^{b}
	>0.85	15	2.60 ± 0.19^{b}
Lymphocyte(×103/µL)	≥1.0-4.8	16	2.5 ± 0.20
	<1.0	87	2.0 ± 0.01*

**= p <0.05, N = number of serum samples, TWBC = Total white blood cells*

Table 3: Association between serum magnesium and protein levels of adult indigenous breed of dogs from Jos, Plateau State, Nigeria

Parameters	Levels	N	Mean Serum (mg/dL)
	5.5 - 7.5	75	2.5 ± 0.07b
	<5.5	11	1.9 ± 0.22a*
Albumin (g/dL)	2.6 - 4.0	86	2.7 ± 0.06*
	<2.6	17	2.0 ± 0.20*
Globulin(g/dL)	>4.0	13	2.3 ± 0.24b
	>3.7	47	2.3 ± 0.10b
	≤3.7	43	2.7 ± 0.08a*
A/G Ratio	≥0.8	38	2.4 ± 0.07
	<0.8	65	2.3 ± 0.13

**= p <0.05, N= number of samples*

The present study also observed significantly higher serum Mg levels in dogs with higher platelets counts. This suggests that Mg may play a vital role in homeostasis. Therefore, dogs with low Mg levels could be vulnerable to disease conditions affecting coagulation. Mg has a well-known antiaggregatory effect on platelets and it was employed for platelet enumeration in capillary blood before the use of EDTA as an anticoagulant (Choccalingam *et al.,* 2017). *In vivo*, Mg inhibits the action of thromboxane A_2, prostaglandin I_2, and 12-hydroxyeicosatetraenoic acid which are important platelet aggregatory agents (Hwang *et al.,* 1992). It also inhibits the normal clotting action of factors VIIa, IXa, and proteins C and S. Mg is a natural calcium antagonist. It competes with calcium for binding sites on prothrombin, hence inhibiting coagulation (James and Neil, 1995; Rukshin *et al.,* 2001; Rukshin *et al.,* 2002). Inhibition of fibrinogen binding to the platelet membrane glycoprotein IIb/IIIa by altering membrane fluidity of platelets and inhibition of intracellular calcium mobilization are the main mechanisms of the antiaggregatory action of Mg *in vitro* (Brecher and Cronkite, 1950; Gries *et al.,* 1999; Gulliksson *et al.,* 2003; Schuff-Werner *et al.,* 2013). Therefore, hypomagnesaemic animals may be prone to disseminated intravascular coagulopathy leading to thrombocytopenia. It was observed that Mg deficiency is associated with platelet hyperactivity, thereby increasing the risk of intravascular thrombosis (Shechter *et al.,* 2000; Baradaran and Nasri, 2005; Nasri *et al.,* 2006; Nasri and Baradaran 2006; Tauszig *et al.,* 2012).

This study also showed that lower total leukocyte and segmented neutrophil counts were associated with significantly higher serum Mg levels, while those with low serum Mg, and were associated with increased total white blood cells count. Therefore, response to infectious disease by dogs with low serum Mg levels might have worse consequences. Several lines of evidence support a role for inflammation as the major origin of oxidative stress. The inflammatory response has been proposed to be responsible for oxidative damage during Mg deficiency (Mazur *et al.,* 2007). Indeed, numerous studies reported indices of the oxidative stress in Mg-deficient animals: enhanced tissue erythrocyte and lipoprotein peroxidation, oxidative modification of proteins, reduced antioxidant status, and increased plasma nitric oxide (NO) (Freedman *et al.,* 1991; Bussiere *et al*., 2002; Petrault *et al.,* 2002). These observations indicated that Mg-deficient animals are more sensitive to oxidative stress. Van Orden *et al.* (2006) observed that dietary Mg influences both serum and plasma Mg levels. They noticed a two fold increase in circulating leukocytes in rats fed Mg deficient diet. Malpuech-Brugere *et al.* (2000) also confirmed the occurrence of an inflammatory response in Mg deficient rats. Blood leukocytes response and changes in leukocyte subpopulations are also consequences of Mg deficiency (Kashiwa and Hungerford, 1958; Kurantsin-Mills *et al.,* 1997). Another study with a spin-trapping technique also indicated that Mg deficiency results in an increase in production of free radicals (Rock *et al.,* 1995).

This study also revealed significantly lower serum Mg levels in dogs that were lymphopenic, while dogs with normal lymphocyte counts had higher serum Mg levels. The possibility that animals with compromised immune status will have hypomagnesaemia exists. Lower Mg levels are not accompanied by a rise in lymphocyte counts and the slight decrease in the proportion of CD5 and CD8 cells might reflect a decrease of humoral immunity concomitant with thymus alterations (Malpuech-Brugere *et al.,* 1999). One of the most remarkable results, regarding effects of Mg deficiency is the higher level of apoptosis shown in thymuses from Mg-deficient rats compared with controls (Malpuech-Brugere *et al*., 1999). Mg-dependent functions in the synthesis, release and activity of cells of immune system have been reported *in vivo* and *in vitro* studies. Mg deficiency in animals is associated with impairments of both humoral and cell mediated immunity. Production and activity including cell adhesion, of granulocyte and mononuclear phagocytes are affected by the availability of Mg (Kubenam, 1994). Levels of immunoglobulin G (IgG) may be reduced and IgE increased during Mg deficiency.

Hypoproteinaemic and hypoalbuminaemic dogs had low serum Mg while those with normal and higher protein and albumin levels had higher Mg levels. This shows that animals with low protein and albumin are prone to hypomagnesaemia and thus incompetent to successfully combat infections. Reduction in the serum albumin will also decrease the total serum Mg, since about 25 % of the Mg is bound to albumin (Elin, 1988). Kroll and Elin (1985) and Cheungpasitporn *et al.* (2015) noted that albumin and Mg are linearly related implying that Mg concentration is dependent on albumin concentration. They estimated that 25 % of Mg is bound by albumin, 8 % bound to globulin and 32 % to total protein. Thus, of the Mg bound to protein, about three-quarters is bound to albumin and the rest to globulin. Nielsen (1969) stated that Mg binds preferentially to albumin and alpha-2 globulin. Therefore, it appears that the lower the albumin level, the lower the level of serum Mg; which was observed in this study.

This work also showed that dogs with globulin levels within reference values are associated with significantly higher serum Mg than those with increased globulin levels. This may be because hyperglobulinaemic dogs may be undergoing an immunologic response, resulting in higher demands for Mg in the body.

Conclusion: This work has shown that local dogs with poor haematological health indices; low PCV, platelet counts, lymphocyte numbers and low serum total protein and albumin had significantly altered serum Mg levels.

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