



Serum and erythrocyte oxidative stress in dogs with acanthocytosis. Estresse oxidativo sérico e eritrocitário em cães com acantocitose.

[Laura Arduino Vasconcelos](#)¹, [Maria Fernanda Fink de Almeida](#)², [Paula Lima de Oliveira](#)^{2,3}, [Vanessa Vieira de Freitas](#)², [Mariana Orlandini Mendonça](#)¹, [Tainara de Oliveira Martins](#)¹, [Beatriz Perez Floriano](#)⁴, [Breno Fernando Martins de Almeida](#)^{1,3*}

¹ Department of Veterinary Medicine, Roque Quagliato Veterinary Hospital, University Center of the Integrated Faculties of Ourinhos (Unifio), Ourinhos, São Paulo State, Brazil. *Corresponding author: Dr. Breno Fernando M. Almeida, BR 153 road, km 338, Água do Cateto, 19909-100 – Ourinhos, São Paulo State, Brazil. Phone No.: +55 14 33026400. E-mail address: bfmalmeida@yahoo.com.br; breno.fernando@unifio.edu.br

² Hospital Veterinário Ourinhos (HVO), Ourinhos, São Paulo State, Brazil.

³ Faculty of Veterinary Medicine of Araçatuba, São Paulo State University, (FMVA-UNESP), Araçatuba, São Paulo State, Brazil.

⁴ Department of Small Animal Clinic, Centre of Rural Sciences, Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul State, Brazil.

Abstract

Oxidative stress occurs as a result of an increase in oxidants and/or a reduction of antioxidants in the body, causing oxidation of cellular constituents and cellular dysfunction, which can lead to premature cell death. This study aimed to evaluate serum and red blood cell (RBC) oxidative stress in dogs with acanthocytosis. Sixteen dogs with acanthocytosis confirmed on blood smear, regardless of the associated pathological condition, and 17 healthy dogs without clinical and laboratorial alterations were selected. Complete blood counts (CBC) were performed by an automated cell counter, biochemical and oxidative stress analyzes were performed by an automated photocolormeter. Dogs with acanthocytosis showed lower RBC, hemoglobin, hematocrit and mean corpuscular hemoglobin concentration (MCHC), and higher red cell distribution width (RDW) compared with healthy dogs. On the leukogram, higher concentrations of band neutrophils and lower numbers of lymphocytes were observed in dogs with acanthocytosis. On serum biochemical parameters, a significant increase in ALP activity, cholesterol and globulin levels, and decreased albumin concentration were observed in dogs with acanthocytosis. Regarding oxidative stress parameters, dogs with acanthocytosis showed lower serum total antioxidant capacity (TAC) by cupric reduction (TAC-CUPRAC) and higher TAC by ferric reduction (TAC-FRAP), total oxidant capacity (TOC) and lipid peroxidation, while RBC showed higher TAC by ABTS cation reduction alone (TAC-ABTS) and associated with peroxidase (TAC-ABTS+HRP), TAC-CUPRAC, TOC and lipid peroxidation compared with those of control dogs. Dogs with acanthocytosis demonstrate serum and erythrocyte oxidative stress, which may be one of the factors involved in erythrocyte damage.

Keywords: Acanthocytes. Anemia. Antioxidants. Oxidants. Canine.

Resumo

O estresse oxidativo ocorre como resultado do aumento de oxidantes e/ou redução de antioxidantes no organismo, causando oxidação de constituintes celulares e disfunção celular, o que pode levar à morte celular prematura. O presente estudo teve como objetivo avaliar o estresse oxidativo sérico e eritrocitário em cães com acantocitose. Foram selecionados 16 cães com acantocitose detectada em esfregaço sanguíneo, independente da condição patológica associada, e 17 cães saudáveis sem alterações clínicas e laboratoriais. O hemograma foi realizado em contador de células automatizado, as análises bioquímicas e de estresse oxidativo foram realizadas em fotocolormetro automatizado. Cães com acantocitose apresentaram menores valores de eritrócitos, hemoglobina, hematócrito e concentração hemoglobínica corpuscular média (CHCM) e maior amplitude de distribuição eritrocitária (RDW) que cães saudáveis. No leucograma, maiores concentrações de neutrófilos bastonetes e menor número de linfócitos foram observados em cães com acantocitose. Nos parâmetros bioquímicos, foi observado aumento significativo da atividade FA, teor de colesterol e de globulina, além de diminuição da concentração de albumina nos cães com acantocitose. Em relação aos parâmetros de estresse oxidativo, cães com acantocitose apresentaram menor capacidade antioxidante total (CAT) sérica por redução cúprica (CAT-CUPRAC) e maior CAT por redução férrica (CAT-FRAP), capacidade oxidante total (COT) e peroxidação lipídica, enquanto nos eritrócitos foi detectada maior CAT pela redução do cátion ABTS sozinho (CAT-ABTS) e associado à peroxidase (CAT-ABTS+HRP), CAT-CUPRAC, COT e peroxidação lipídica em comparação com os cães controle. Cães com acantocitose apresentam estresse oxidativo sérico e eritrocitário, que pode ser um dos fatores envolvidos no dano eritrocitário.

Palavras-chave: Acanócitos. Anemia. Antioxidantes. Oxidantes. Canino.



Introduction

Oxidative stress is defined as a condition in which there is a significant reduction in antioxidant agents compared with oxidizing agents, thus resulting in an imbalance between the consumption and production of oxidants and antioxidants (FERREIRA; MATSUBARA, 1997). Oxidants are produced during normal cell aging and can also be associated with pathological conditions, causing changes on the membranes of erythrocytes, responsible for protecting against free radicals, which therefore results in disorders on microcirculation. However, cell death can occur when antioxidant systems are ineffective or deficient as a result of oxidative damage, such as during lipid peroxidation that causes hemolysis (YANG et al., 2006).

Erythrocytes are extremely susceptible to oxidative damage, as their cell membranes have a high content of polyunsaturated fatty acids (PUFA), in addition to high cellular concentrations of oxygen and hemoglobin (CLEMENS et al., 1987; SCOTT et al., 1993). In dogs, conditions related to erythrocyte oxidative damage include, for example, ingestion of garlic and onion, which makes erythrocytes susceptible to the formation of Heinz bodies and eccentrocytes, thereby leading to hemolysis of the affected cells (DESNOYERS, 2010; HARVEY, 2008; LEE et al., 2000). Regarding preventing oxidative stress in the body, erythrocytes contribute by controlling the elimination of free radicals through maintenance of an antioxidant system such as the glutathione system, methemoglobin reductase and catalase enzymes. However, when erythrocytes establish this function, they become susceptible to oxidative injuries (RICHARDS et al., 1998), which can cause anemia. In chronic metabolic diseases, in which there is oxidant-antioxidant imbalance, erythrocytes are more susceptible to morphological changes due to presence of oxidative stress in most cases (BAYNES, 1991). In humans, erythrocyte morphological changes have already been associated with oxidative stress in dialyzed nephropathic patients (MCGRATH et al., 1995) and patients with metabolic syndrome (GYAWALI et al., 2015), thalassemia (CHAICHOMPOO et al., 2019), chronic fatigue syndrome (RICHARDS; WANG; JELINEK, 2007), and even autism (CICCOLI et al., 2013). In dogs, to our knowledge, there are no studies that have determined the role of oxidative stress on canine erythrocyte morphological changes, only the relationship between oxidative stress and lower erythrocyte survival in dogs with babesiosis (OTSUKA et al., 2002).

Although the pathogenesis of some oxidative lesions of erythrocytes during the development of anemia is well established, few studies have determined oxidative stress in poikilocytosis, especially in dogs. Therefore, the purpose of the present study was to verify whether dogs presenting pathologies associated with acanthocytosis show altered serum and erythrocyte oxidative stress parameters.

Material and Methods

Approval by the Ethics Committee

The research project was approved by the Ethics Committee on Animal Usage of the University Center of the Integrated Faculties of Ourinhos (Unifio) under Protocol No. 023/2020, thereby taking place in accordance with ethical principles. Control dogs used in the experiment were included upon authorization by their tutor, through signing an informed consent form, while samples

from dogs with acanthocytosis came from the routine practice of the Veterinary Clinical Pathology Laboratory of the Veterinary Hospital at the same institution.

Animal selection

Thirty-three dogs were selected and divided into two experimental groups:

Control group: 17 adult dogs (2-13 years old) of both sexes (11 females and 6 males) considered healthy based on clinical and laboratory evaluation, which comprised complete blood count (CBC) and serum determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), albumin, globulin, total protein, total cholesterol, triglycerides, creatinine and urea.

Acanthocytosis group: 16 dogs aged 1-13 years of both sexes (10 females and 6 males) that showed acanthocytosis on CBC, performed during routine practice at the Veterinary Hospital. The number of acanthocytes was estimated on a stained blood smear using light microscopy, where at least 500 red blood cells (RBC) were counted on the monolayer, followed by determination of the percentage of RBC with acanthocytosis. Subsequently, the concentration of blood acanthocytes was obtained from the concentration of the animal's RBC. All selected dogs had only acanthocytosis as a morphological alteration in the erythrogram cytology evaluation. The records of these animals were analyzed to establish the main diagnosis.

Animals treated with any type of medication, particularly drugs that have antioxidant and/or anti-inflammatory action, were not included in this study.

Collection of samples and laboratory analysis

Blood samples were obtained by jugular puncture into tubes with K₂EDTA (BD Vacutainer[®], Becton-Dickson, New Jersey, USA) to perform CBC and obtain RBC, and one tube with a clot activator (BD Vacutainer[®], Becton-Dickson, New Jersey, USA) to perform biochemical and oxidative stress analysis using serum, which was stored at -20°C protected from light until determinations were performed, for a maximum period of 15 days.

CBC was performed using an automated veterinary cell counter (ABX Micros ESV 60, Paris, France), morphological analyzes and differential leukocyte counts were performed on blood smears under optical microscopy following previously methodology (SOUZA et al., 2022).

After performing CBC, blood samples with K₂EDTA were centrifuged at 3,000 rpm for 5 minutes, the plasma was discarded, and the tube was filled with saline solution (0.9% sodium chloride solution) followed by homogenization. The sample was centrifuged again and the supernatant was discarded again, to achieve three erythrocyte washes. Thereafter, in order to obtain a final RBC lysate for oxidative stress analysis, 200 µL of RBC concentrate were added to 400 µL of distilled water, followed by homogenization. The lysate was stored at -20°C under protection from light until determinations were performed for a maximum period of 15 days. All determinations of RBC lysate were normalized by the sample hemoglobin content determined by a photocolormeter (BIO 2000, BioPlus, São Paulo, Brazil) using the hemoglobin cyanide biochemical method with commercial reagents (Labtest Diagnóstica SA, Minas Gerais, Brazil) according to the manufacturer's instructions.

Serum biochemical analyzes were performed using an automated photocolormeter (AS-160, Bioelab, China) and commercial reagents (Labtest Diagnóstica SA, Minas Gerais, Brazil) according to the manufacturer's recommendations as previous reports (BONATTO et al., 2021; OLIVEIRA et

al., 2020; SILVA et al., 2019). Total cholesterol and triglyceride levels were determined by the enzymatic Trinder method, and ALT and AST by the ultraviolet (UV) kinetic method. Albumin was analyzed by the colorimetric method using bromocresol green, creatinine by the alkaline picrate colorimetric method, and ALP by Bowers and McComb's modified kinetic procedure, GGT by the Szasz modified method, amylase by the substrate 2-chloro-p-nitrophenyl-alpha-D-maltotrioxide, total protein by biuret colorimetric method, urea by UV enzymatic method and the globulin content was quantified by subtracting the albumin from total proteins.

Serum or RBC lysate oxidative stress parameters were also determined by an automated photocolormeter (AS-160, Bioelab, China) as previously described (ALMEIDA et al., 2021; BONATTO et al., 2021; SOUZA et al., 2022). Total antioxidant capacity (TAC) was determined using four different methods: by inhibiting the reduction of the ABTS cation alone (TAC-ABTS) (EREL, 2004) or in association with peroxidase (TAC-ABTS+HRP) (RUBIO et al., 2016a), using the cupric ion reducing antioxidant capacity assay (TAC-CUPRAC) (RUBIO et al., 2016b) and the ferric reducing antioxidant power assay (TAC-FRAP) (BENZIE; STRAIN, 1996). TOC was determined by the colorimetric method of xylenol orange (EREL, 2005), while lipid peroxidation was determined using thiobarbituric acid reactive substances (TBARS) (HUNTER; NLEMADIM; DAVIDSON, 1985). All the reagents were from Sigma-Aldrich Chemical Co.

Statistical analysis

Data were first evaluated for normality using the Shapiro-Wilk test and mean comparisons were performed using unpaired t test or Mann-Whitney test. Analyses were performed using a computer software (GraphPad Prism, v.6.00 for Windows, GraphPad Software, La Jolla, CA, USA, <https://www.graphpad.com>) and significance was considered when $P < 0.05$.

Results

Dogs with acanthocytosis were diagnosed with infectious diseases (31.25%) such as monocytic ehrlichiosis, parvovirus or canine distemper virus infection; and neoplasms (18.75%) including mast cell tumor and lymphoma alone or in association with other conditions. Most dogs (50%) showed multiple causes and one dog (6.25%) did not have a specific diagnosis established. These dogs showed mean count of $1.95 \pm 1.15 \times 10^{12}/L$ of acanthocytosis (Table 1). None of the dogs selected for the control group had acanthocytes.

On erythrogram variables, dogs with acanthocytosis showed lower RBC, hemoglobin, HCT and MCHC and higher RDW than control dogs. The leukogram of these dogs showed only an increase in band neutrophils and a decreased lymphocyte concentration. None of the other CBC parameters showed significant difference (Figure 1).

On clinical biochemistry, dogs with acanthocytosis showed higher ALP activity, globulin and total cholesterol and lower albumin compared with healthy dogs, with no significant differences in the other parameters evaluated (Figure 2).

Regarding the serum oxidative stress parameters, dogs with acanthocytosis showed reduced TAC-CUPRAC and increased TAC-FRAP, TOC and lipid peroxidation when compared with control dogs. On RBC lysate, dogs with acanthocytosis showed higher TAC-ABTS, TAC-ABTS+HRP, TAC-CUPRAC, TAC-FRAP, TOC and lipid peroxidation than control dogs (Figure 3).

Table 1 – Acanthocyte count and main diagnoses of dogs with acanthocytosis (n=16).

Dog	Gender	Age (years)	Racial pattern	Acanthocytes (%)	Acanthocytes ($10^{12}/L$)	Diagnosis
1	F	13	German Shepherd	37.6	1.97	Phlegmon
2	M	9	Maltese	46	2.44	Hyperadrenocorticism and CKD
3	F	1	Mongrel	57.6	3.02	Parvovirus and monocytic ehrlichiosis
4	F	1	Pitbull	29	0.90	Foreign body ingestion
5	M	10	Mongrel	47.4	3.01	Lymphoma and monocytic ehrlichiosis
6	M	8	Rottweiler	11.2	0.39	Mast cell tumor
7	F	8	Beagle	15	0.77	Pyometra
8	F	2	Pug	25	1.30	Pregnancy and monocytic ehrlichiosis
9	F	7	Mongrel	72.8	2.23	Monocytic ehrlichiosis
10	F	13	Mongrel	66.4	4.25	CKD
11	M	2	Mongrel	24.6	1.71	Gastroenteritis of undetermined cause
12	F	9	Maltese	51.2	3.13	Distemper
13	M	4	Maltese	47.4	3.22	Pulmonary fibrosis
14	F	12	Mongrel	13.6	0.55	Femur fracture
15	M	6	English Bulldog	25.8	1.39	Mast cell tumor
16	F	7	Mongrel	25	0.89	Undetermined

CKD: chronic kidney disease; F: female; M: male.

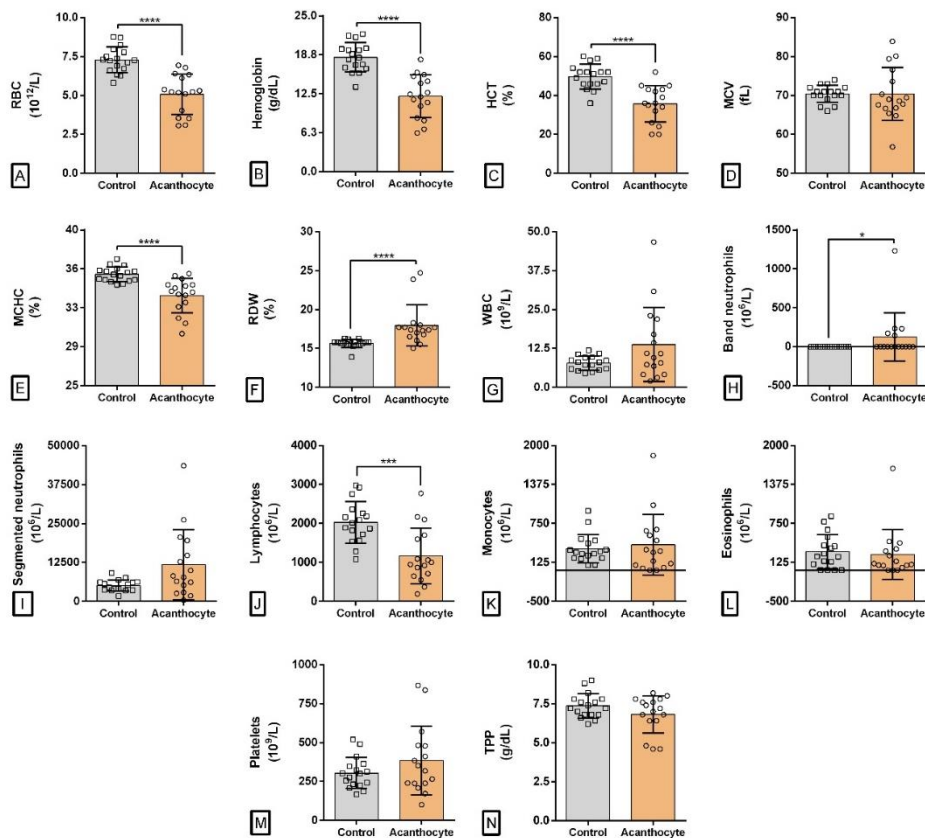


Fig. 1 - Red blood cells (RBC, **A**), hemoglobin (**B**), hematocrit (HCT, **C**), mean corpuscular volume (MCV, **D**), mean corpuscular hemoglobin concentration (MCHC, **E**), red blood cell distribution width (RDW, **F**), white blood cells (WBC, **G**), band neutrophils (**H**), segmented neutrophils (**I**), lymphocytes (**J**), monocytes (**K**), eosinophils (**L**), platelets (**M**) and total plasma protein (TPP, **N**) in healthy control dogs (n=17) and dogs with acanthocytosis (n=16). Graphs are represented by mean and standard deviation with individual values. The statistically significant difference is indicated by * (p<0.05), ** (p<0.01), *** (p<0.001) or **** (p<0.0001).

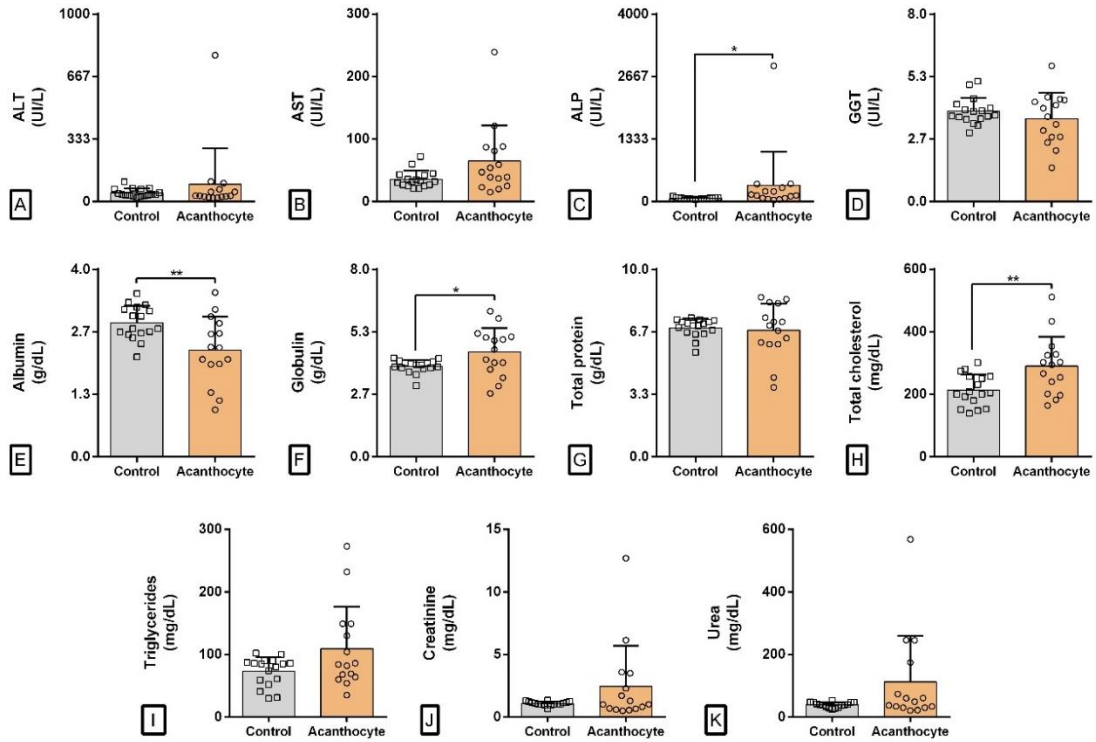


Fig. 2 - Alanine aminotransferase (ALT, **A**), aspartate aminotransferase (AST, **B**), alkaline phosphatase (ALP, **C**), gamma glutamyl transferase (GGT, **D**), albumin (**E**), globulin (**F**), total protein (**G**), total cholesterol (**H**), triglycerides (**I**), creatinine (**J**) and urea (**K**) in healthy control dogs (n=17) and dogs with acanthocytosis (n=16). Graphs are represented by mean and standard deviation with individual values. The statistically significant difference is indicated by * (p<0.05), ** (p<0.01), *** (p<0.001) or **** (p<0.0001).

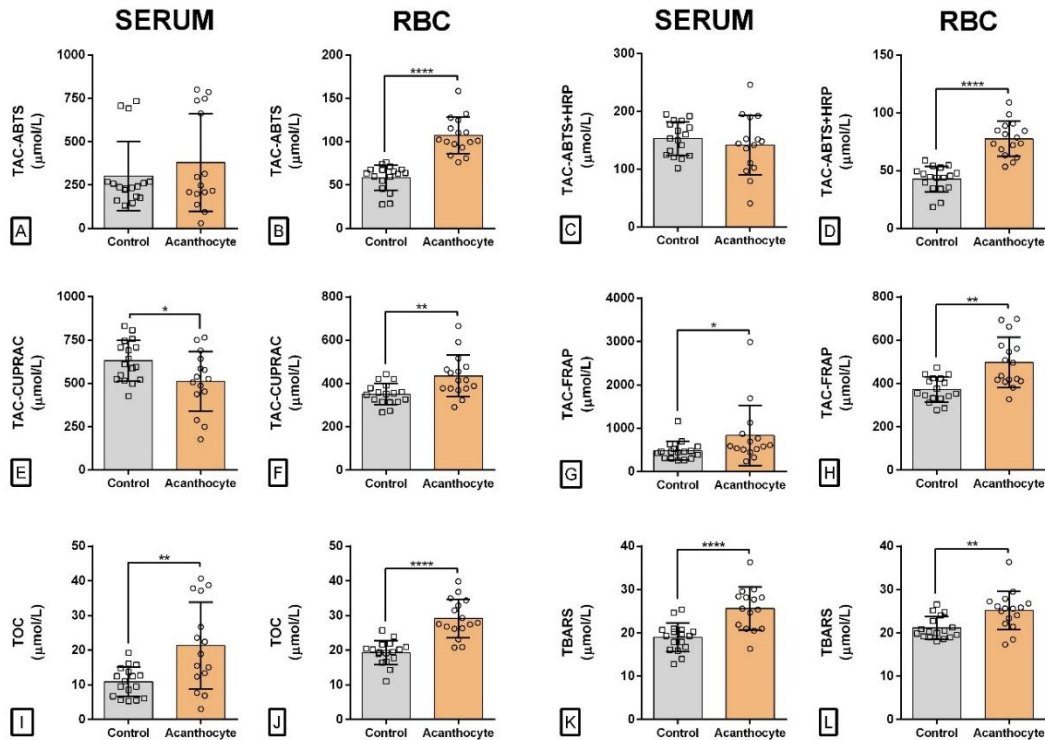


Fig. 3 - Serum and red blood cell (RBC) oxidative stress markers determined by TAC-ABTS (**A** and **B**), TAC-ABTS+HRP (**C** and **D**), TAC-CUPRAC (**E** and **F**), TAC-FRAP (**G** and **H**) TOC (**I** and **J**) and lipid peroxidation by thiobarbituric acid reactive substances (TBARS, **K** and **L**) in healthy control dogs (n=17) and dogs with acanthocytosis (n=16). Graphs are represented by mean and standard deviation with individual values. The statistically significant difference is indicated by * (p<0.05), ** (p<0.01), *** (p<0.001) or **** (p<0.0001).

Discussion

To date, there are few studies that have evaluated the involvement of oxidative stress with erythrocyte defects in dogs and there are no studies that have determined serum and erythrocyte oxidative stress in dogs with conditions associated with acanthocytosis. In this aspect, we observed that dogs with acanthocytosis showed oxidative stress with altered serum and erythrocyte markers, evidencing an increase in oxidants together with an increase in lipid peroxidation and a decrease or increase in serum TAC, depending on the method of analysis, in addition to an increase in RBC TAC in all evaluated methodologies.

As a result of their lower flexibility and fluidity, acanthocytes are more frequently destroyed and removed from the circulation when passing through capillary beds in the spleen, leading to extravascular hemolysis when these cells circulate in large numbers (WARRY et al., 2013). This could at least partially explain why dogs with acanthocytosis in this study showed lower values of RBC, hemoglobin and HCT. Winterbourn (1990) reported that the oxidation of hemoglobin in erythrocytes releases free radicals that induce oxidative damage on other cellular constituents, also justifying the anemia that is commonly seen in dogs with acanthocytosis.

Dogs with acanthocytosis showed neutrophil left shift with higher blood concentrations of band neutrophils compared with healthy dogs. Band neutrophils are young cells recruited during inflammatory response, an alteration compatible with the diagnoses of the dogs from the present study, which also showed hyperglobulinemia and hypoalbuminemia. In addition, a decrease in the concentration of circulating lymphocytes was also observed in dogs with acanthocytosis, which occurs in stress conditions with chronic release of corticosteroids such as systemic diseases, metabolic disorders or in response to pain, commonly associated with inflammatory diseases (THRALL et al., 2012), as observed in acanthocytosis group.

Regarding the changes on clinical biochemistry in dogs with acanthocytosis, we observed an increase in serum ALP activity and total cholesterol levels, with no changes on hepatocellular injury enzymes ALT and AST. These changes likely occurred as a result of cholestasis, as evidenced by higher serum ALP activity and lower hepatobiliary cholesterol excretion, without significant hepatocellular damage. Cholesterol is an important component of the cell membrane and changes in its metabolism, as well as changes in lipids and membrane proteins induced by the higher lipid peroxidation, could favor the occurrence of changes in RBC morphology (THRALL et al., 2012; WARRY et al., 2013), such as the acanthocytosis observed in the present study. Corroborating this finding, Gyawali et al. (2012) reported that humans with hypercholesterolemia also demonstrated membrane modifications such as acanthocytosis.

In humans, production of acanthocytes is commonly associated with congenital lipid disorders, liver cirrhosis or neoplasms (KELLER; MAJERUS; FINKE, 1971) and, as observed in the present study, many of the dogs with acanthocytosis had some type of neoplasm, as well as inflammatory processes caused by infectious agents. Harvey (2012) highlighted that the presence of acanthocytes may also be associated with chronic kidney disease and glomerulonephritis, disorders that reflect severe erythrocyte fragmentation. However, in the present study, biochemical markers of renal function such as urea and creatinine were not significantly different compared with the control group, although some dogs with acanthocytosis were diagnosed with renal disease and one showed severe azotemia.

Oxidative stress was another finding in dogs with acanthocytosis, demonstrated by increased oxidants and increased lipid peroxidation in both serum and RBC. Interestingly, TAC alterations varied according to the method of analysis and type of sample, being it serum or RBC. Erythrocyte oxidative stress has also been demonstrated in patients with renal conditions undergoing peritoneal dialysis or hemodialysis, evidenced by an increase in free radicals and decreased antioxidant enzymes glutathione reductase and glutathione peroxidase. The authors also observed an increase in erythrocyte membrane fluidity and suggested that such changes could contribute to the anemia seen in these patients, given that erythrocyte survival is impaired by oxidative lesions (MCGRATH et al., 1995). A relationship between morphological changes on erythrocytes and oxidative stress has also been proposed in human patients with chronic fatigue syndrome (RICHARDS; WANG; JELINEK, 2007). Also, in that same study, the authors found that erythrocytes from patients with chronic fatigue syndrome had higher levels of methemoglobin, an oxidized form of hemoglobin, as well as higher lipid peroxidation, which occurred concomitantly with increased stomatocyte formation.

The erythrocytes of dogs with acanthocytosis showed increased antioxidants levels, as evidenced by the higher TAC in all analyzed methodologies. Erythrocytes are rich in antioxidants, given the constant unavoidable injuries they suffer from reactive oxygen species generated during metabolic processes (FUJII et al., 2021). Therefore, one can assume that the higher erythrocyte TAC observed in dogs with acanthocytosis is due to the greater erythrocyte adaptability induced by increased oxidants, as evidenced by the higher TOC in dogs with acanthocytosis. Even so, this adaptation was insufficient to avoid oxidative damage, since serum and erythrocyte lipid peroxidation were higher in these dogs and could contribute to lower erythrocyte survival. This hypothesis is also supported by malondialdehyde, a product of lipid peroxidation, which induces its recognition by macrophages and predispose these cells to phagocytosis when present on the surface of RBC (HEBBEL; MILLER, 1984). Dogs infected with *Babesia gibsoni* showed oxidative damage in RBC, which was likely the cause exacerbating anemia in dogs with low parasitemia (OTSUKA et al., 2002), given that these changes on the RBC lipid bilayer induced by oxidative lesions would cause changes on the shape and rigidity of these cells (RICHARDS; WANG; JELINEK, 2007).

In sickle cells, a decrease was observed in glutathione peroxidase and catalase, together with increased levels of the enzyme superoxide dismutase and greater lipid peroxidation, thus suggesting involvement of oxidative stress in the occurrence of sickle cell morphology (DAS; NAIR, 1980). Therefore, the different antioxidants evidently do not behave in the same way under oxidative stress. In addition, chronic oxidative damage, as observed in most dogs of the present study, could induce a greater antioxidant response of certain erythrocyte antioxidants, which would be responsible for the higher RBC TAC observed in dogs with acanthocytosis. Using different methodologies for assessing TAC remains important because each method investigates different antioxidants, so different mechanisms of oxidative stress and different species could produce different results (ALMEIDA et al., 2021).

Although we investigated the potential involvement of oxidative stress in the development of acanthocytosis in dogs, it was not possible to determine whether oxidative stress was in fact responsible for such changes, since dogs with acanthocytosis selected in the present study presented different pathological conditions as primary diagnoses. Therefore, it is essential to carry out further studies to determine whether oxidative stress would be the cause or consequence of acanthocytosis or these pathological processes in dogs, considering the particularities of each disease process that may be involved.

Conclusions

Dogs with disease processes that progress with acanthocytosis presented changes in oxidative stress parameters, in both serum and RBC, suggesting that oxidative stress may be related to the development of acanthocytosis and anemia in these dogs.

Conflict of interest statement

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of this paper.

Ethics approval

The experiment was conducted according to the ethical principles of the Animal Research Ethics Committee of the University Center of the Integrated Faculties of Ourinhos (Protocol No. 023/2020).

Author Contributions

The study conception and design were performed by Laura Arduino Vasconcelos, Beatriz Perez Floriano and Breno Fernando Martins de Almeida. Animal selection, material preparation and sample collection were performed by Laura Arduino Vasconcelos, Maria Fernanda Fink de Almeida, Paula Lima de Oliveira, Vanessa Vieira de Freitas, Mariana Orlandini Mendonça, Tainara de Oliveira Martins and Breno Fernando Martins de Almeida. Laboratory analysis were performed by Laura Arduino Vasconcelos, Maria Fernanda Fink de Almeida, Paula Lima de Oliveira, Mariana Orlandini Mendonça, Tainara de Oliveira Martins and Breno Fernando Martins de Almeida. The first draft of the manuscript was written by Laura Arduino Vasconcelos and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by the São Paulo Research Foundation (FAPESP No. 2020/04749-0).

References

- ALMEIDA, B. F. M.; AMATTI, L. Z.; SOUZA, G. G.; GARCIA, L. V.; MONTECHIESI, D. F.; IGNÁCIO, F. S.; OLIVEIRA, P. L.; COSTA, L. R.; FLORIANO, B. P.; BOSCULO, M. R. M.; JOAQUIM, J. G. F.; RUBIO, C. P. Effect of uterine ozone therapy and anticoagulant sampling on oxidative stress parameters in mares. **Research in Veterinary Science**, v. 136, p. 503-511, 2021. <https://doi.org/10.1016/j.rvsc.2021.04.002>
- BAYNES, J. W. Role of oxidative stress in development of complications in diabetes. **Diabetes**, v. 40, n. 4, p. 405-412, 1991. <https://doi.org/10.2337/diab.40.4.405>
- BENZIE, I. F. F.; STRAIN, J. J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. **Analytical Chemistry**, v. 239, n. 1, p. 70-76, 1996. <https://doi.org/10.1006/abio.1996.0292>

- BONATTO, N. C. M.; OLIVEIRA, P. L.; MANCEBO, A. M.; COSTA, L. R.; BOSCULO, M. R. M.; BOSCO, A. M.; CIARLINI, P. C.; FLORIANO, B. P.; BARROS, L. D.; ALMEIDA, B. F. M. Postprandial lipemia causes oxidative stress in dogs. **Research in Veterinary Science**, v. 136, p. 277-286, 2021. <https://doi.org/10.1016/j.rvsc.2021.03.008>
- CHAICHOMPOO, P.; QILLAH, A.; SIRANKAPRACHA, P.; KAEWCHUCHUEN, J.; RIMTHONG, P.; PAIBOONSUKWONG, K.; FUCHAROEN, S.; SVASTI, S.; WORAWICHAWONG, S. Abnormal red blood cell morphological changes in thalassaemia associated with iron overload and oxidative stress. **Journal of Clinical Pathology**, v. 72, n. 8, p. 520-524, 2019. <http://dx.doi.org/10.1136/jclinpath-2019-205775>
- CICCOLI, L.; FELICE, C.; PACCAGNINI, E.; LEONCINI, S.; PECORELLI, A.; SIGNORINI, C.; BELMONTE, G.; GUERRANTI, R.; CORTELAZZO, A.; GENTILE, M.; ZOLLO, G.; DURAND, T.; VALACCHI, G.; ROSSI, M.; HAYEK, J. Erythrocyte shape abnormalities, membrane oxidative damage, and β -actin alterations: an unrecognized triad in classical autism. **Mediators of Inflammation**, v. 2013, p. 1-11, 2013. <https://doi.org/10.1155/2013/432616>
- CLEMENS, M. R.; RUESS, M.; BURSA, Z.; WALLER, H. D. The relationship between lipid composition of red blood cells and their susceptibility to lipid peroxidation. **Free Radical Research Communications**, v. 3, n. 1/5, p. 265-271, 1987. <https://doi.org/10.3109/10715768709069792>
- DAS, S. K.; NAIR, R. C. Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. **British Journal of Haematology**, v. 44, n. 1, p. 87-92, 1980. <https://doi.org/10.1111/j.1365-2141.1980.tb01186.x>
- DESNOYERS, M. Anemias Associated with Oxidative Injury. *In*: WEISS, D. J.; WARDROP, K. J. (Orgs.). **Schalm's Veterinary Hematology**. 6th ed. Ames: Blackwell Publishing Ltd, p. 239-245, 2010.
- EREL, O. A new automated colorimetric method for measuring total oxidant status. **Clinical Biochemistry**, v. 38, n. 12, p. 1103-1111, 2005. <https://doi.org/10.1016/j.clinbiochem.2005.08.008>
- EREL, O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. **Clinical Biochemistry**, v. 37, n. 4, p. 277-285, 2004. <https://doi.org/10.1016/j.clinbiochem.2003.11.015>
- FERREIRA, A. L. A.; MATSUBARA, L. S. Radicais livres: conceitos, doenças relacionadas, sistema de defesa e estresse oxidativo. **Revista da Associação Médica Brasileira**, v. 43, n. 1, p. 61-68, 1997. <https://doi.org/10.1590/S0104-42301997000100014>
- FUJII, J.; HOMMA, T.; KOBAYASHI, S.; WARANG, P.; MADKAIKAR, M.; MUKHERJEE, M. B. Erythrocytes as a preferential target of oxidative stress in blood. **Free Radical Research**, v. 55, n. 8, p. 781-799, 2021. <https://doi.org/10.1080/10715762.2021.1873318>
- GYAWALI, P.; RICHARDS, R. S.; BWITITI, P. T.; NWOSE, E. U. Association of abnormal erythrocyte morphology with oxidative stress and inflammation in metabolic syndrome. **Blood Cells, Molecules & Diseases**, v. 54, n. 4, p. 360-363, 2015. <https://doi.org/10.1016/j.bcmd.2015.01.005>
- GYAWALI, P.; RICHARDS, R. S.; UBA NWOSE, E. Erythrocyte morphology in metabolic syndrome. **Expert Review of Hematology**, v. 5, n. 5, p. 523-531, 2012. <https://doi.org/10.1586/ehm.12.47>
- HARVEY, J. W. The Erythrocyte: Physiology, Metabolism, and Biochemical Disorders. *In*: KANEKO, J.; HARVEY, J.; BRUSS, M. (Orgs.). **Clinical Biochemistry of Domestic Animals**. 6th ed. San Diego: Elsevier Inc., p. 173-240, 2008.
- HARVEY, J. W. **Veterinary Hematology: A Diagnostic Guide and Color Atlas**. 1st ed. Saint Louis: Saunders/Elsevier, 2012, 368p.

- HEBBEL, R. P.; MILLER, W. J. Phagocytosis of sickle erythrocytes: immunologic and oxidative determinants of hemolytic anemia. **Blood**, v. 64, n. 3, p. 733-741, 1984. <https://doi.org/10.1182/blood.V64.3.733.733>
- HUNTER, M. I. S.; NLEMADIM, B. C.; DAVIDSON, D. L. W. Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluid from multiple sclerosis patients. **Neurochemical Research**, v. 10, n. 12, p. 1645-1652, 1985. <https://doi.org/10.1007/BF00988606>
- KELLER, J. W.; MAJERUS, P. W.; FINKE, E. H. An unusual type of spiculated erythrocyte in metastatic liver disease and hemolytic anemia. **Annals of Internal Medicine**, v. 74, n. 5, p. 732-737, 1971. <https://doi.org/10.7326/0003-4819-74-5-732>
- LEE, K. W.; YAMATO, O.; TAJIMA, M.; KURAOKA, M.; OMAE, S.; MAEDE, Y. Hematologic changes associated with the appearance of eccentrocytes after intragastric administration of garlic extract to dogs. **American Journal of Veterinary Research**, v. 61, n. 11, p. 1446-1450, 2000. <https://doi.org/10.2460/ajvr.2000.61.1446>
- MCGRATH, L. T.; DOUGLAS, A. F.; MCCLEAN, E.; BROWN, J. H.; DOHERTY, C. C.; JOHNSTON, G. D.; ARCHBOLD, G. P. R. Oxidative stress and erythrocyte membrane fluidity in patients undergoing regular dialysis. **Clinica Chimica Acta**, v. 235, n. 2, p. 179-188, 1995. [https://doi.org/10.1016/0009-8981\(95\)06027-X](https://doi.org/10.1016/0009-8981(95)06027-X)
- OLIVEIRA, P. L.; BONATTO, N. C. M.; BOSCULO, M. R. M.; FLORIANO, B. P.; DE BARROS, L. D.; ALMEIDA, B. F. M. Effect of post-prandial lipemia on canine biochemical parameters. **Comparative Clinical Pathology**, v. 29, p. 763-775, 2020. <https://doi.org/10.1007/s00580-020-03130-y>
- OTSUKA, Y.; YAMASAKI, M.; YAMATO, O.; MAEDE, Y. The effect of macrophages on the erythrocyte oxidative damage and the pathogenesis of anemia in *Babesia gibsoni*-infected dogs with low parasitemia. **The Journal of Veterinary Medical Science**, v. 64, n. 3, p. 221-226, 2002. <https://doi.org/10.1292/jvms.64.221>
- RICHARDS, R. S.; ROBERTS, T. K.; DUNSTAN, R. H.; MCGREGOR, N. R.; BUTT, H. L. Erythrocyte antioxidant systems protect cultured endothelial cells against oxidant damage. **Biochemistry and Molecular Biology International**, v. 46, n. 5, p. 857-865, 1998. <https://doi.org/10.1080/15216549800204402>
- RICHARDS, R. S.; WANG, L.; JELINEK, H. Erythrocyte oxidative damage in chronic fatigue syndrome. **Archives of Medical Research**, v. 38, n. 1, p. 94-98, 2007. <https://doi.org/10.1016/j.arcmed.2006.06.008>
- RUBIO, C. P.; HERNÁNDEZ-RUIZ, J.; MARTINEZ-SUBIELA, S.; TVARIJONAVICIUTE, A.; ARNAO, M. B.; CERON, J. J. Validation of three automated assays for total antioxidant capacity determination in canine serum samples. **Journal of Veterinary Diagnostic Investigation**, v. 28, n. 6, p. 693-698, 2016a. <https://doi.org/10.1177/1040638716664939>
- RUBIO, C. P.; TVARIJONAVICIUTE, A.; MARTINEZ-SUBIELA, S.; HERNÁNDEZ-RUIZ, J.; CERÓN, J. J. Validation of an automated assay for the measurement of cupric reducing antioxidant capacity in serum of dogs. **BMC Veterinary Research**, v. 12, n. 137, p. 1-8, 2016b. <https://doi.org/10.1186/s12917-016-0760-2>
- SCOTT, M. D.; VAN DEN BERG, J. J. M.; REPKA, T.; ROUYER-FESSARD, P.; HEBBEL, R. P.; BEUZARD, Y.; LUBIN, B. H. Effect of excess alpha-hemoglobin chains on cellular and membrane oxidation in model beta-thalassemic erythrocytes. **The Journal of Clinical Investigation**, v. 91, n. 4, p. 1706-1712, 1993. <https://doi.org/10.1172/JCI116380>
- SILVA, N. L. T.; BONATTO, N. C. M.; OLIVEIRA, P. L.; VIEIRA, G. C.; FLORIANO, B. P.; BARROS, L. D.; BOSCULO, M. R. M.; ALMEIDA, B. F. M. Post-prandial lipemia and glycemia in dogs fed with industrialized pet food. **Comparative Clinical Pathology**, v. 28, n. 1, p. 253-258, 2019. <https://doi.org/10.1007/s00580-018-2824-0>

SOUZA, W. K. C.; MENDONÇA, M. O.; OLIVEIRA, T. M.; GONÇALVES, G. S.; VIEIRA, S. E.; MONZANI, G. S.; OLIVEIRA, P. L.; BOSCULO, M. R. M.; FLORIANO, B. P.; ALMEIDA, B. F. M. Effects of over-day and overnight fasting on the hematological, biochemical and oxidative profile of healthy dogs. **Archives of Veterinary Science**, v. 27, n. 1, p. 134-148, 2022. <http://doi.org/10.5380/avs.v1i1.80725>

THRALL, M. A.; WEISER, G.; ALLISON, R. W.; CAMPBELL, T. W. **Veterinary Hematology and Clinical Chemistry**. 2nd ed. Wiley-Blackwell: John Wiley & Sons, 2012, 762p.

WARRY, E.; BOHN, A.; EMANUELLI, M.; THAMM, D.; LANA, S. Disease distribution in canine patients with acanthocytosis: 123 cases. **Veterinary Clinical Pathology**, v. 42, n. 4, p. 465-470, 2013. <https://doi.org/10.1111/vcp.12097>

WINTERBOURN, C. C. Oxidative reactions of hemoglobin. **Methods in Enzymology**, v. 186, p. 265-272, 1990. [https://doi.org/10.1016/0076-6879\(90\)86118-F](https://doi.org/10.1016/0076-6879(90)86118-F)

YANG, H. L.; CHEN, S. C.; CHANG, N. W.; CHANG, J. M.; LEE, M. L.; TSAI, P. C.; FU, H. H.; KAO, W. W.; CHIANG, H. C.; WANG, H. H.; HSEU, Y. C. Protection from oxidative damage using *Bidens pilosa* extracts in normal human erythrocytes. **Food and Chemical Toxicology**, v. 44, n. 9, p. 1513-1521, 2006. <https://doi.org/10.1016/j.fct.2006.04.006>

Received on April 7, 2023

Returned for adjustments on July 26, 2023

Received with adjustments on July 29, 2023

Accepted on August 1, 2023