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<Original Article>

Cardiocirculatory, biochemical and hemostatic evaluation of dogs with hyperadrenocorticism at diagnosis and after treatment

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Abstract: Hyperadrenocorticism (HAC) is a common endocrinopathy among dogs that causes multisystemic signs. This study was conducted to evaluate cardiocirculatory, biochemical, and hemostatic parameters in dogs with HAC at diagnosis, in addition to verifying whether abnormal parameters could be controlled by initial treatment with trilostane. Fifteen dogs with HAC were assessed by systolic blood pressure measurement, electrocardiography, Doppler echocardiography, serum concentration of troponin I, and biochemical and hemostatic profile at diagnosis and after trilostane therapy. Unlike biochemical parameters, hemostatic and cardiocirculatory parameters were not significantly influenced by the onset of treatment. The authors believe that clinical treatment with trilostane for 3 to 4 months might not be sufficient for the stabilization of cardiocirculatory abnormalities such as hypertension. Therefore, dogs with HAC must receive cardiocirculatory monitoring at diagnosis and during drug treatment.

Keywords: canine, cardiac troponin I, cardiology, endocrinology, trilostane

Introduction

Hyperadrenocorticism (HAC) is a common endocrinopathy among dogs, characterized by a constellation of clinical signs [3, 24]. Cardiovascular complications of HAC are the main cause of morbidity and mortality among humans with this condition, involving risk factors such as hypertension, dyslipidemia, and hypercoagulable states [6, 7]. Considering that hypercortisolism as a result of the disease causes multisystemic abnormalities, it is prudent to treat every patient with a confirmed diagnosis [27]. However, few studies have reported the influence of systemic parameters on the prognosis and survival of dogs with HAC [32].

The pathophysiology of cardiovascular complications of canine HAC and their influence upon the structure of the heart are still unclear. In recent years, many studies have assessed cardiocirculatory parameters in dogs with HAC, including echocardiography [5], arterial blood pressure [11, 21], and hemostasis [23, 28]. Nevertheless, there are no studies that evaluate all these parameters simultaneously, aside from electrocardiography (ECG), biochemical analysis, and measurement of cardiac troponin I (cTnI) levels.

The aim of this study was to evaluate cardiocirculatory, biochemical, and hemostatic parameters in dogs with HAC at diagnosis, in addition to verifying whether abnormal parameters could be controlled by initial clinical treatment with trilostane.

Materials and Methods

This was a prospective observational study of dogs diagnosed with naturally occurring HAC. The diagnosis of HAC was based on medical history, clinical signs, physical examination, clinical pathology, abdominal ultrasound examination, and HAC-compatible endocrine tests (low-dose dexamethasone suppression and/or adrenocorticotropic hormone stimulation test). The study protocol was approved by the Animal Research Ethics Committee of the Federal University of Rio Grande do Sul (protocol No. 25424) and the participation of the dogs in the study was authorized by their owners. The patients were assessed by systolic blood pressure (SBP) measurement, ECG, Doppler echocardiography, and blood tests. Two assessments were performed: the first one at diagnosis

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and the second one within 3 to 4 months after the onset of trilostane therapy at a dose recommended by the veterinary endocrinologist based on the patient's clinical picture (0.6 to 1.2 mg/kg twice a day).

Exclusion criteria were presence of systemic infections, endocrine diseases (*e.g.*, diabetes mellitus), myxomatous mitral valve disease with left atrial (LA) enlargement, cardiomyopathies, congenital heart diseases, renal failure, and treatment with drugs that could interfere with the assessed parameters (*e.g.*, vasodilators). A total of 32 dogs were evaluated in the study. Six dogs were excluded in the first assessment and 11 were excluded between the two assessments (five died). One dog had high serum urea in the first biochemical evaluation. However, this patient had no creatinine elevation or renal ultrasonographic changes and the urea concentration was within normal limits in the second evaluation, so the dog was not dropped from the study.

A Doppler ultrasound (DV610; MEDMEGA, Brazil) was used for SBP evaluation, following the standardization rules previously agreed upon by the American College of Veterinary Internal Medicine [4]. The mean value of five consistent measurements of SBP was considered for the statistical analysis.

In the Doppler echocardiographic examination using an ultrasound machine with a 2–5 MHz or 6–8 MHz phased array transducer (Mylab 40 Vet; Esaote, Italy), one-dimensional, two-dimensional, and Doppler wave modes were used. Dogs were unsedated and gently restrained in left and right lateral recumbency to obtain images of the right parasternal, left caudal (apical) parasternal and left cranial parasternal regions. All measurements were made by the same experienced cardiologist. The following parameters were evaluated: right atrial dimension; LA dimension; aortic root (Ao) diameter; LA/Ao ratio; interventricular septum thickness in diastole; left ventricular free wall thickness in diastole (LVWd); left ventricular internal diameter in systole (LVIDs) and diastole (LVIDd), and right ventricular internal diameter in diastole (RVIDd); fractional shortening; ejection fraction; and blood flows through mitral, tricuspid, aortic, and pulmonary valves. Diastolic function was assessed by the left ventricular filling pattern by measuring transmitral E/A wave velocity.

For ECG evaluation, the patients were positioned according to the standard protocol [34]. The ECG tracing was obtained from leads I, II, III, aVR, aVL, and aVF, and from precordial leads, using a computerized conventional electro-

Table 1. Cardiocirculatory parameters [mean ± SD, or median (minimum maximum)] in 15 dogs with hyperadrenocorticism (HAC) before and after treatment

Parameter (unit)	Pre-treatment	Post-treatment	*p* value
SBP (mmHg)	161.27 ± 19.75	156.93 ± 26.14	0.354
HR (bpm)	110 (80–185)	115 (75–170)	0.345
Troponin I (ng/mL)	0.052 (0.006–0.547)	0.031 (0.02–0.306)	0.221
Ao (cm)	1.60 ± 0.35	1.55 ± 0.34	0.516
LA (cm)	1.89 ± 0.38	1.98 ± 0.47	0.353
LA/Ao	1.19 ± 0.11	1.27 ± 0.08	0.028*
RVIDd (cm)	0.56 (0.38–0.98)	0.76 (0.39–1.08)	0.020*
IVSd (cm)	0.72 ± 0.17	0.69 ± 0.13	0.335
LVIDd (cm)	2.43 ± 0.48	2.40 ± 0.42	0.710
LVWd (cm)	0.67 ± 0.14	0.66 ± 0.12	0.628
LVIDs (cm)	1.38 ± 0.34	1.40 ± 0.34	0.698
FS (%)	43.5 ± 4.7	41.8 ± 6.8	0.331
EF (%)	76.7 ± 5.3	74.5 ± 7.4	0.237
E wave (cm/sec)	70.54 ± 15.67	70.70 ± 9.46	0.975
A wave (cm/sec)	70.1 (41.3–83.7)	62.0 (39.5–104.7)	0.917
E/A ratio	1.11 (0.77–1.52)	1.27 (0.7–2.0)	0.916
AoFV (cm/sec)	114.5 (78.0–234.0)	114.0 (96.1–152.0)	0.594
AoFG (mmHg)	5.24 (2.43–21.9)	5.7 (3.7–9.24)	0.594
PFV (cm/sec)	113.87 ± 23.88	94.38 ± 22.25	0.024*
PFG (mmHg)	4.75 (2.65–9.88)	3.23 (1.65–8.09)	0.025*

*Indicates statistically significant difference between patients before and after treatment ($p < 0.05$). SBP, systolic blood pressure; HR, heart rate; Ao, aorta; LA, left atrium; RVIDd, right ventricle internal diameter in diastole; IVSd, interventricular septum thickness in diastole; LVIDd, left ventricular internal diameter in diastole; LVWd, left ventricular free wall thickness in diastole; FS, fractional shortening; EF, ejection fraction; AoFV, aortic flow peak velocity; AoFG, aortic flow gradient; PFV, pulmonary flow peak velocity; PFG, pulmonary flow gradient.

cardiogram (TEB ECGPC; TEB, Brazil). The ECG tracings were recorded for 2 min. Both cardiac rhythm and heart rate were considered for statistical analysis. Cardiac arrhythmias were divided into disturbances of frequency, formation, or conduction of electrical cardiac impulse.

Blood samples were collected for complete blood count (Procyte Dx; IDEXX Laboratories, USA), platelet count, plasma fibrinogen levels, prothrombin time (PT), activated partial thromboplastin time (aPTT), biochemistry profile, and measurement of cTnI. The samples placed in 3.2% buffered sodium citrate tubes were centrifuged immediately after collection and the plasma was frozen for later analysis, in duplicate, of PT and aPTT within 2 h after collection, using HemoStat Tromboplastin-SI and HemoStat aPTT-EL kits (*in vitro* diagnosis). The biochemistry profile (colorimetric assay, CM-200; Wiener lab, Argentina) was consisted of the measurement of albumin, alanine aminotransferase (ALT), total calcium, total cholesterol, creatinine, alkaline phosphatase (ALP), phosphorus, fructosamine, glucose, urea, and triglycerides. Serum was stored at −20°C for up to 6 months for analysis of cTnI concentrations, measured using an immunometric technique previously validated for dogs [29, 30]. The inter-assay and intra-assay coefficient of variation were less than 10%. Measured concentrations of cTnI below the limits of detection of the assay (0.006 ng/mL) were assigned the value of detection limits.

The SPSS software (ver. 18.0; SPSS, USA) was used for the statistical analysis. The categorical variables were described as frequencies and percentage rates. The quantitative variables were described as mean and SD (when the distribution was symmetric) or as median, minimum, and maximum (if asymmetric). The categorical variables were estimated by the 95% confidence interval for proportions, and the quantitative ones were calculated by the 95% confidence interval for means. The quantitative variables were compared before and after treatment by Student's *t*-test for paired samples. The asymmetric variables were compared by the Wilcoxon's test. The McNemar's test was used to compare categorical variables before and after treatment. The significance level was set at 5%. The Pearson's correlation coefficient was used to compare cardiocirculatory parameters between themselves and with the biochemical and hematological parameters.

Results

Fifteen dogs (6 males; 1 castrated male; 3 females; 5 spayed females) with mean age of 9.4 ± 2.1 years and mean weight of 8.1 ± 4.5 kg were included in the study. Of these, 10 were pure breeds (2 Malteses, 2 Miniature Poodles, 1 Bichon Frise, 1 Dachshund, 1 Beagle, 1 Yorkshire, 1 Lhasa Apso, and 1 Brazilian Terrier) and five were mongrel dogs. The 15 dogs

Table 3. Cardiorespiratory clinical signs (number of events and %) of dogs with HAC before and after treatment

Clinical sign	Pre-treatment	Post-treatment	*p* value
Dyspnea	8 (53.3)	5 (33.3)	0.375
Coughing	2 (13.3)	2 (13.3)	1.0
Exercise intolerance	3 (20)	4 (26.7)	1.0
No clinical sign	5 (33.3)	10 (66.6)	0.125

$P < 0.05$ indicates statistically significant difference between patients before and after treatment.

Table 2. Biochemical and coagulation parameters [mean ± SD, or median (minimum maximum)] in 15 dogs with HAC before and after treatment

Parameter (unit)	Pre-treatment	Post-treatment	*p* value
Albumin (g/L)	32.78 ± 5.80	32.79 ± 4.23	0.995
ALT (U/L)	444.0 (47.38–1,179.0)	184.1 (72.6–1,257.0)	0.047*
Calcium (mg/dL)	9.56 ± 2.43	10.40 ± 1.5	0.266
Cholesterol (mg/dL)	392.0 (133.0–931.0)	352.0 (142.0–838.7)	0.020*
Creatinine (mg/dL)	0.58 ± 0.27	0.65 ± 0.24	0.327
ALP (U/L)	1,440.0 (121.6–4,799.0)	496.0 (29.99–3,428)	0.022*
Phosphorus (mg/dL)	5.49 (3.1–7.00)	5.5 (3.75–12.8)	0.221
Fructosamine (μmol/L)	158.0 (17.1–609.7)	178.1 (82.39 –302.5)	0.776
Glucose (mg/dL)	105.3 (69.54–315.0)	110 (71.77–371.6)	0.427
Urea (mg/dL)	25.45 (12.0–316.0)	31.0 (12.88–91.07)	0.394
Triglycerides (mg/dL)	155.5 (30.0–2,691.0)	152.0 (73.88–915)	0.925
Platelets (× 10^3/μL)	525.8 ± 179.36	582.61 ± 188.79	0.470
PT (sec)	6.08 ± 1.14	5.91 ± 0.45	0.607
aPTT (sec)	15.7 ± 1.90	16.07 ± 1.35	0.518
Fibrinogen (mg/dL)	200 (100–400)	400 (100–600)	0.168

*Indicates statistically significant difference between patients before and after treatment ($p < 0.05$). ALT, alanine aminotransferase; ALP, alkaline phosphatase; PT, prothrombin time; aPTT, activated thromboplastin time.

Table 4. Cardiac arrhythmias (number of events and %) of dogs with HAC before and after treatment

Arrhythmia	Pre-treatment	Post-treatment	*p* value
Formation (supraventricular premature complex)	1 (6.7)	0 (0)	
Conduction (sinus arrest)	3 (20)	6 (40)	
Frequency (sinus tachycardia)	2 (13.3)	3 (20)	
Total	6 (40)	9 (60)	0.453

$P < 0.05$ indicates statistically significant difference between patients before and after treatment.

included in the study had pituitary-dependent HAC diagnosis based on bilateral adrenal hyperplasia and on the low-dose dexamethasone suppression test.

The comparison between cardiocirculatory and biochemical/hemostatic parameters of the dogs assessed before and after the treatment for HAC (Tables 1 and 2, respectively) showed that abnormal biochemical parameters at diagnosis (ALT, ALP, and cholesterol) improved significantly after treatment. Moreover, the echocardiogram evidenced an increase in the LA/Ao ratio and a change in parameters related to the right ventricle and to pulmonary flow. The clinical signs related to the cardiorespiratory system (Table 3) and cardiac arrhythmias (Table 4) did not improve significantly. The cardiac arrhythmias detected to include isolated supraventricular premature complexes (one dog pre-treatment), sinus arrest (three dogs pre-treatment and six dogs post-treatment) and sinus tachycardia (two dogs pre-treatment and three dogs post-treatment). No significant correlations were found among cardiocirculatory, biochemical, and coagulation parameters.

Discussion

Reports show that 59 to 86% of dogs with HAC have systemic hypertension [11, 13, 20, 21]. While it is not clear yet how HAC leads to systemic hypertension, different mechanisms have been described: the mineralocorticoid effects of cortisol, activation of the renin-angiotensin-aldosterone system, larger angiotensinogen synthesis, and action of cortisol upon the peripheral vasculature [10, 17]. The present study did not find significant changes in systemic hypertension when comparing the time of diagnosis with a period of 3 to 4 months of trilostane therapy, as occurred in other studies that monitored the blood pressure of dogs with HAC after treatment [11, 32]. The lack of SBP control when HAC was stabilized highlights the importance of blood pressure monitoring in dogs with HAC before and after treatment of the disease. Moreover, this result suggests that the use of antihypertensive drugs can be necessary. Human studies corroborate that systemic hypertension persists in 25 to 55.6% of adult patients with HAC after treatment and surgical remission [8, 9].

In human medicine, HAC patients have a fivefold higher risk than the average population for vascular disorders [7, 37]. Hypertension, glucose intolerance, dyslipidemia, and visceral obesity represent vascular risk factors in patients with HAC [25, 31]. Elevated serum ALT, ALP, and cholesterol levels were the most common biochemical findings in the dogs assessed in the present study, as previously described [3, 24]. However, these abnormal parameters, triggered by an increase in hepatic glycogen and intrahepatic cholestasis secondary to hypercortisolism plus glucocorticoid-induced ALP isoform, demonstrated a statistically significant reduction after treatment. Hypercoagulable states are another possible risk factor associated with vascular accidents in HAC [3]. In dogs, the PT, aPTT, fibrinogen, and thromboelastographic values were not associated with HAC [15]. Unlike their biochemical counterparts, coagulation parameters did not have any significant change after treatment, but hypercoagulable states were reported in dogs before and after trilostane therapy [23].

The most common Doppler echocardiographic structural changes observed in the present study were related to the left ventricle, with findings such as reversal of the ventricular filling pattern, LVWd thickening, and mitral valve failure, similarly to what is observed in humans with HAC [1, 2, 18, 36, 39]. A recent study on the left ventricular function of dogs with HAC indicated higher prevalence of left ventricular hypertrophy compared to the control group, without any association with systemic hypertension [5]. In addition, speckle-tracking echocardiography revealed reduction of systolic function, which was not detected by conventional echocardiography [5]. Unlike the present study, in which no significant improvement was observed in echocardiographic parameters of the left ventricle within the first months of treatment, magnetic resonance of human patients with HAC indicated that systolic function and left ventricular mass parameters improved after an average period of 6 months of clinical and/or surgical treatment [14]. Even though right ventricle parameters were normal, there was a significant enlargement of the right ventricular cavity and a reduction in pulmonary flow velocity and gradient after the onset of treatment. Dehydration can be related to smaller than normal cardiac chambers; therefore, RVIDd and LA/Ao elevation after trilostane therapy may be related to stabilization of blood volume and venous return after control of polydipsia and polyuria. Most studies involving HAC patients usually evaluate left ventricular parameters, which indicate more consistent alterations. Therefore, future studies should clarify the impact of HAC on right ventricular structure and function.

Cardiac biomarkers are widely used in human medicine and their importance in veterinary medicine has been acknowledged in the past few years [22]. Cardiac troponins

are biomarkers that are highly sensitive and specific to myocardial cell damage in dogs [38]. Zero or low levels of cardiac troponin are detectable in the blood of healthy patients [22]. In the present study, the mean cardiac troponin concentration in dogs with HAC was higher than that observed in previous studies with healthy dogs [16, 38], which may be related to echocardiographic structural changes. Although not statistically significant, decreases in serum cTnI concentration after clinical treatment suggest lower cardiac damage secondary to hypercortisolism with the control of the disease. It would be interesting to measure other cardiac damage biomarkers such as NT-pro BNP, whose levels are high in atrial stretch and in ventricular hypertrophy and hypoxia [22]. However, we do not have access to this biomarker in our experimental routine.

High-risk arrhythmias related to electrical impulse formation were not observed in the evaluated dogs. Supraventricular premature complexes detected in one dog were also reported in human patients with HAC [33], and that may be related to cardiac remodeling induced by HAC or to electrolytic disorders. Sinus arrest and sinus tachycardia, the major findings related to cardiac rhythm, are low-risk arrhythmias, thereby no major conclusions can be drawn about the influence of clinical treatment on the occurrence of cardiac arrhythmias. Sinus arrest is characterized by a pause at least twice as long as the preceding R-R interval [35] and is considered a normal incidental finding in brachycephalic breeds [34]; however, no patients with sinus arrest in the present study were brachycephalic. Weakness or syncope may occur as a result of a pause of 5 or more seconds [35]; however, all of the patients with sinus arrest had short pauses in the present study. Sinus arrest could be associated with an increase in vagal tone activity [35] and evidence in human patients suggests that HAC causes a vasovagal imbalance characterized by an increase in parasympathetic activity [9] and, therefore, it can also occur in dogs.

The clinical signs of HAC can mimic cardiac disease. Despite the non-significant difference between clinical signs related to the cardiorespiratory system before and after treatment, the number of asymptomatic patients doubled after trilostane therapy. Trilostane inhibits the 3-beta-hydroxysteroid dehydrogenase, thereby reducing cortisol synthesis and release by the adrenal cortex, being a good alternative in the management of dogs with this syndrome [12, 27]. The lack of improvement in several parameters in dogs with HAC after trilostane therapy can be related to the mechanism of action of the drug, which involves modulation of steroidogenesis. Given this mechanism of action, several dogs with cortisol levels within the therapeutic target range on ACTH stimulation tests during trilostane treatment maintained the clinical signs of the disease, possibly as a result of an increase in the synthesis of other adrenal corticosteroids [19, 26]. A limitation of the present study is the absence of assessment of serum cortisol of all dogs at the time of the second evaluation, due to financial constraint and lack of ACTH on the market. Thus, it would be interesting if new studies could compare these parameters in patients subjected to different treatments, such as drug therapy with mitotane and surgical treatment by adrenalectomy or hypophysectomy. A comparison of untreated dogs with HAC would be interesting, albeit ethically questionable, to evaluate the effectiveness of therapy.

Hyperadrenocorticism can be associated with several cardiocirculatory, biochemical, and hemostatic abnormalities in dogs. Unlike biochemical parameters, hemostatic and cardiocirculatory parameters were not significantly influenced by the onset of treatment. The authors believe that clinical treatment with trilostane for 3 to 4 months might not be enough for the stabilization of cardiocirculatory abnormalities (*e.g.*, hypertension) that pose risk for the patients. Dogs with HAC must be submitted to cardiocirculatory monitoring at diagnosis and during drug treatment, with SBP measurement and Doppler echocardiographic assessment.

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