

Biochemical and haematological parameters in dogs with Cushing's syndrome



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Abstract

The exposure of the body to stress, regardless of whether it comes from physical, chemical or emotional stimuli from the environment, causes an inadequate adaptation of the organisms which can contribute to the development of various diseases. Abnormally high blood concentrations of cortisol, known as stress hormone, lead to the development of a hormonal disorder called hyperadrenocorticism or Cushing's syndrome. In the majority of cases, Cushing's syndrome is diagnosed when symptoms are apparent, and screening endocrinological test confirms the existence either of increased cortisol production or decreased sensitivity of the hypothalamic-pituitary-adrenal axis to negative glucocorticoid feedback. In our research, we examined a total of 23 male and 7 female dogs that were suspected to have Cushing's syndrome, based on history and clinical signs. A total of 15 male and 5 female dogs were positive for Cushing's syndrome (HAC group), whereas the remaining dogs were used to form non-HAC group. Using the apparatus IDEXX

"Vet Test 8008", the following biochemical parameters were determined: glucose, urea, creatinine, phosphorus, calcium, total protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, bilirubin, cholesterol, and amylase. Regarding haematological parameters, the following parameters were investigated: erythrocytes, leukocytes, platelets, erythrocyte indices (MCV, MCH, MCHC, RDW), white blood cell count, haemoglobin and haematocrit, using "Laser cite vet lab Station" (IDEXX). No significant differences in haematological and biochemical blood parameters were noticed between the HAC and the non-HAC group of dogs. However, dogs suffering from Cushing's syndrome had a higher value in the number of erythrocytes compared to the control group. The finding that has to be payed attention to is the difference in platelet count between the control group of dogs and dogs suffering from Cushing's syndrome.

Key words: *dogs; Cushing's syndrome; blood parameters*

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Introduction

Hyperadrenocorticism (HAC) is a multisystemic disorder that occurs due to chronically elevated blood levels of cortisol (Ouschan et al., 2013). This disorder is often referred to as Cushing's syndrome, according to Harvey Cushing, a Boston neurosurgeon who first described hyperadrenocorticism in humans in 1932 (Galac, 2010). Cushing's syndrome is one of the most common endocrinopathies observed in small animal practice. Cushing's syndrome usually occurs in middle-aged and older dogs (Carotenuto et al., 2019). Excessive levels of glucocorticoids cause a combination of physical and biochemical changes that have a significant impact on the quality of life of the animal. HAC may be the result of pathological excessive adrenocorticotrophic hormone (ACTH) secretion by a functional pituitary tumour (PDH, pituitary dependent hyperadrenocorticism), or due to primary adrenal disorder (ADH, adrenal dependent HAC) (Behrend, 2010; Carotenuto et al., 2019). Oversecretion of ACTH results in bilateral adrenocortical hyperplasia and overproduction of glucocorticoids (especially cortisol). A less common cause of HAC, accounting for about 15 percent of cases, is the overproduction of cortisol by adrenal tumours (Ramsey and Ristić, 2007). Recently, HAC due to other causes has also been described in dogs, but these appear to be rare (Carotenuto et al., 2019). In humans, HAC is considered a rare disease, with an incidence ranging from 0.7 to 2.4 individuals per million per year (Ambrosi et al., 1990; Lindholm et al., 2001). A recent study conducted in the United Kingdom reported an estimated prevalence of 0.28% for HAC (O'Neill et al., 2016). A significant predisposition to HAC has been observed in older dogs and some breeds, such as miniature poodles, boxers and dachshunds (Carotenuto et al., 2019). Changes in the serum of

dogs with Cushing's syndrome include increased alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activity, hypercholesterolemia, and hyperglycaemia. The hemogram is often characterized by the constellation of changes called „stress leukogram“. Proteinuria due to glomerulosclerosis is also a very common finding (Hodžić and Hamamdžić, 2012). Increased ALP activity is not a specific parameter for Cushing's syndrome, because statistically, about 10% of dogs with Cushing's syndrome have normal ALP activity. Furthermore, any disease or injury that causes stress can lead to elevated ALP activity if it causes an increase in serum cortisol concentration. Increased ALP activity may also be a manifestation of ductile liver hyperplasia, a common finding in older dogs. High blood cortisol concentrations most commonly affect white blood cells causing lymphopenia, neutrophilia, monocytosis, and eosinopenia. Although none of the abnormalities in the aforementioned parameters is specific to the diagnosis of Cushing's syndrome, such laboratory findings should direct the clinician to suspect Cushing's syndrome (Gilor, 2011). Cushing's syndrome is associated with blood clotting disorders in dogs and humans. One study by a group of scientists showed that levels of coagulation factors II, V, VII, IX, X, XI, and fibrinogen were significantly increased in dogs with Cushing's syndrome (Jacoby et al., 2001). Low serum phosphorus, mild hypernatremia, and mild hypokalaemia can sometimes be found in dogs with Cushing's syndrome, but the clinical significance of this abnormality is minimal. Endocrine tests should be used to further confirm suspected HAC. Tests for hypercortisolism in dogs with high levels of clinical suspicion are recommended to reduce the likelihood of false-positive results (Sanders et al.,

2018). The aim of this study was to expand our knowledge of HAC through analysis of the canine population in Bosnia and Herzegovina that was diagnosed with HAC and changes in their routine laboratory findings.

Materials and methods

The study was conducted on 30 dogs suspected of having Cushing's syndrome based on history and clinical signs. Blood sampling for

haematological and biochemical blood analyses was performed at the Faculty of Veterinary Medicine, University of Sarajevo. Blood was sampled from each dog at the first examination and then biochemical and haematological parameters were determined. A comprehensive history was taken from the dog owner to find out as much information about the dog as possible, as well as the animals' whereabouts, stress exposure, etc. The LC 320 centrifuge at 3000 rpm/10 min was used to separate

Table 1. Biochemical parameters of the non-HAC and HAC groups of dogs

Biochemical parameters	Non-HAC group (N=10)				HAC group (N=20)				P=
	Median*	IQR [♀]	Min [♂]	Max [#]	Median*	IQR [♀]	Min [♂]	Max [#]	
Glucose (mmol/L)	4.21	5.15	1.12	8.05	5.55	1.81	1.33	30.58	0.113
Urea (mmol/L)	5.60	3.65	2.50	11.90	5.45	3.18	1.90	17.00	0.774
Creatinin (µmol/L)	85.00	18.25	47.00	133.00	74.50	41.00	35.00	103.00	0.234
Phosphorus (mmol/L)	1.52	0.62	1.17	2.55	1.28	0.25	0.78	2.77	0.134
Calcium (mmol/L)	2.55	0.50	1.95	3.08	2.52	0.48	2.00	3.00	0.842
Total protein (g/L)	74.50	22.00	60.00	102.00	68.00	9.50	59.00	77.00	0.133
Albumin (g/L)	35.00	5.00	23.00	50.00	31.50	6.25	24.00	39.00	0.122
Globulin (g/L)	41.50	15.50	31.00	55.00	36.00	5.75	30.00	44.00	0.151
ALT (U/L)	66.50	54.30	48.00	594.00	103.50	101.50	36.00	913.00	0.495
ALP (U/L)	80.00	115.80	32.00	594.00	95.00	392.00	29.00	2000.00	0.675
Bilirubin (µmol/L)	7.00	3.50	2.00	48.00	4.50	4.50	2.00	21.00	0.127
Cholesterol (mmol/L)	4.98	3.96	3.42	13.42	6.26	3.05	2.98	12.46	0.843
Amilase (U/L)	765.50	437.30	286.00	1187.00	643.00	337.00	435.00	2500.00	0.628
Sodium (mmol/L)	150.50	3.25	147.00	152.00	149.00	2.75	146.00	152.00	0.217
Potassium (mmol/L)	4.10	0.45	3.80	5.10	4.05	0.58	3.50	5.10	0.929
Chloride (mmol/L)	111.00	2.00	104.00	115.00	110.00	2.00	106.00	113.00	0.127

*Median – median value; [♀]IQR – interquartile range; [♂]Min – minimum value; [#]Max – maximum value

the serum. Using IDEXX's Vet test 8008 apparatus, the following biochemical parameters were determined: glucose, urea, creatinine, phosphorus, calcium, total protein, albumin, globulins, alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, cholesterol, and amylase. The following haematological parameters were determined: erythrocyte count, leukocyte count, platelet count, erythrocyte indices (MCV, MCH, MCHC, RDW), white blood cell count (WBC), haemoglobin and haematocrit, using a LaserCyte Vetlab Station (IDEXX). The test that were used for diagnosing Cushing's syndrome were the ACTH Stimulation Test and the Dexamethasone Suppression Test.

Statistical analysis of the results was performed in Minitab 17 (Minitab, Inc. 2014). Initial analysis showed that the value distributions of most of the parameters tested were significantly

different from the normal (Ryan-Joiner normality test) in the presence of more atypical (extreme) values. Therefore, non-parametric statistical tests were applied in further processing, and the results in tables are presented as median values with interquartile range (IQR), indicating the minimum and maximum values determined, or as the number of established cases. The Mann Whitney test was used to detect significance of differences between the two groups.

Results

Tables 1 and 2 show the biochemical and haematological parameters of the non-HAC and the HAC group of dogs. These parameters cannot determine Cushing's syndrome, but certainly, provide relevant information that can guide the clinician to make specific tests for the diagnosis and treatment of the animal.

Table 2. Haematological parameters of the non-HAC and HA groups of dogs

Haematological parameters	Non-HAC group (N=10)				HAC group (N=20)				P=
	Median*	IQR [‡]	Min [‡]	Max [#]	Median*	IQR [‡]	Min [‡]	Max [#]	
RBC (x10 ¹² /L)	6.38	2.41	4.79	7.45	6.56	1.42	4.39	7.79	0.290
HCT (%)	45.65	19.25	31.20	56.00	47.30	7.20	30.80	60.70	0.523
HGB (g/dL)	14.75	3.65	12.10	19.60	15.45	1.78	9.40	21.60	0.311
MCV (fL)	70.20	5.88	64.10	75.30	71.50	7.35	63.60	79.10	0.332
MCH (pg)	24.50	4.00	20.50	27.90	23.80	313	20.50	28.30	0.261
MCHC (g/dL)	31.85	3.13	29.10	37.40	33.25	2.93	27.50	38.90	0.218
Retic(K/ μ L)	84.50	71.10	48.70	218.80	88.05	36.55	36.20	182.40	0.982
WBC (x10 ⁹ /L)	9.22	5.19	5.57	22.96	8.21	5.78	5.34	15.32	0.441
NEU (x10 ⁹ /L)	6.69	5.40	3.81	18.28	5.42	4.29	3.37	12.81	0.415
LYM (x10 ⁹ /L)	1.01	1.05	0.67	3.12	1.15	0.63	0.26	2.16	0.947
MONO (x10 ⁹ /L)	1.01	0.67	0.53	2.87	0.95	0.51	0.37	1.71	0.415
EOS (x10 ⁹ /L)	0.35	0.40	0.08	1.16	0.22	0.15	0.12	0.59	0.128
BASO (x10 ⁹ /L)	0.07	0.02	0.05	0.16	0.05	0.04	0.01	0.11	0.091
PLT (K/ μ L)	210.00	195.00	71.00	367.00	349.50	240.00	88.00	1082.00	0.006

*Median – median value; [‡]IQR – interquartile range; [‡]Min – minimum value; [#]Max – maximum value

Discussion

Based on the available literature, no relevant data on the occurrence of Cushing's syndrome in dogs in the territory of Bosnia and Herzegovina has been reported to date, although the disease is evident in the area. Looking at surrounding countries, Kiš et al. (2016) reported the presence of Cushing's syndrome in dogs in Croatia.

Based on history and clinical symptoms, our study investigated dogs with visible changes that possibly suggested Cushing's disease. A total of 30 dogs were analysed. Of these, 24 dogs resided indoors and 6 dogs were held in yards. Purebred dogs were predominantly represented, of which 7 dogs of the breed Bichon frise and 4 Maltese dogs, whereas there were 7 mixed breed dogs. The clinical symptoms of the disease are a consequence of excess glucocorticoids, causing atrophy of hair follicles and sebaceous glands, calcium mobilization from bones, thinning of blood vessel walls, intensification of hepatic gluconeogenesis from amino acids and immunodepression (Boghian et al., 2008).

In our study, haematological and biochemical blood parameters showed no significant differences between the non-HAC and the HAC dogs (Table 1 and Table 2). Behrend et al. (2013) found that blood analyses are not specific to the determination of Cushing's syndrome, but that they may further assist in the easier diagnosis of the disease. Gilor and Graves (2011) reported that erythrocytosis and thrombocytosis are common in dogs with Cushing's syndrome. In our study, no statistically significant difference in the number of erythrocytes and platelets was observed between the groups (Table 2).

However, dogs with Cushing's syndrome had higher erythrocyte counts than controls. A fact that has to be considered is the difference in platelet

counts between the non-HAC group and the HAC group. The platelet count was within the physiological range in both groups; however, in dogs with Cushing's syndrome, there was a tendency toward thrombocytosis, at the borderline of statistical significance concerning the non-HAC group of dogs (Table 2).

Increased ALP activity is a common finding in dogs with Cushing's syndrome (Galac, 2010). Other common abnormalities include increased ALT activity and hyperlipidaemia, together with an increased amount of bile acid that may be present in dogs suffering from Cushing's syndrome, but also in some liver diseases (Gilor and Graves, 2011). Also, glucose, ALT, and ALP concentrations are often higher in Cushing's syndrome than the reference values (Cho et al., 2013). This was also present in our study, where increased glucose concentrations were found in three, ALT in nine, and ALP in eight dogs positive for Cushing's syndrome. The glucose, ALT, and ALP levels were almost twice as high in dogs within the HAC group when compared to the non-HAC group (Table 1). Likely, the differences in the values of these parameters would also be statistically significant between HAC and non-HAC dogs if there were no large individual variation in the values of these parameters.

Hypercholesterolemia and moderate hyperglycaemia are directly influenced by the level of cortisol secreted by the adrenal glands (Boghian et al., 2008). In the case of Cushing's syndrome, cortisol intensifies the hepatic form of gluconeogenesis, starting with amino acids, which explains hepatic congestion, hepatomegaly and increased serum liver transaminase activity (Carlotti et al., 1998; Chapman et al., 2004).

Mild hypercholesterolemia and hyperglycaemia are also found in the HAC group of dogs in our study. Lower values of proteins, albumins,

and globulins in dogs with Cushing's syndrome (Table 1) are probably due to the catabolism of structural proteins and amino acid utilization in the process of gluconeogenesis, as indicated by the aforementioned finding of mild hyperglycaemia and abdominal distension with muscle hypotension in this group of dogs.

Although the clinical significance of electrolyte abnormalities in diseased dogs is minimal, low serum phosphorus, mild hyponatremia, and mild hypokalaemia are sometimes present in dogs with Cushing's syndrome (Gilor and Graves, 2011). In our study, electrolyte values were within physiological limits in both groups, with no significant differences between the non-HAC and HAC group of dogs (Table 1).

Conclusion

To the authors' knowledge there has been no relevant information on the occurrence of Cushing's syndrome in dogs in the territory of Bosnia and Herzegovina. The definitive diagnosis of HAC cannot be based on the biochemical and haematological parameters, and specific tests need to be performed. More than one test is usually required to conclusively confirm HAC. The most reliable tests for diagnosing Cushing's syndrome are the ACTH stimulation test and Low-dose dexamethasone suppression test. One of the tests, whose reliability is still at the stage of intensive research, is to determine the concentration of cortisol by analysing its content in the hair.

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Biokemijski i hematološki pokazatelji u krvi za dijagnostiku Cushingovog sindroma u pasa

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Visoke koncentracije kortizola u krvi, poznatog kao hormon stresa, dovode do razvoja hormonalnog poremećaja koji se naziva hiperadrenokorticism ili Cushingov sindrom. U većini slučajeva Cushingov sindrom dijagnosticira se kada su simptomi vidljivi, a endokrinološki testovi potvrde ili postojanje povećane proizvodnje kortizola ili snižene osjetljivosti osovine hipotalamus-hipofiza-nadbubrežna žlijezda na inhibirajući učinak glukokortikoida. Naše je istraživanje obuhvatilo ukupno 23 muška i 7 ženskih pasa za koje se na temelju anamneze i kliničkih znakova sumnjalo da imaju Cushingov sindrom. Ukupno 15 muških i 5 ženskih pasa bilo je pozitivno na Cushingov sindrom (HAC skupina), dok su preostali psi grupirani u skupinu pasa bez hiperadrenokorticisma (non-HAC skupina). Pomoću uređaja IDEXX "Vet Test 8008", određeni su sljedeći biokemijski parametri: glukoza, ureja,

kreatinin, fosfor, kalcij, ukupni proteini, albumin, globulin, alanin aminotransferaza, alkalna fosfataza, bilirubin, kolesterol i amilaza. Od hematoloških pokazatelja, ispitivani su sljedeći pokazatelji: eritrociti, leukociti, trombociti, hematološki indeksi (MCV, MCH, MCHC, RDW), diferencijalna krvna slika, hemoglobin i hematokrit, korištenjem "Lasercyte Vetlab Station" (IDEXX). Nisu ustanovljene značajne razlike u hematološkim i biokemijskim pokazateljima u krvi između skupine pasa s dijagnosticiranim hiperadrenokorticismom i bez njega. Međutim, psi oboljeli od Cushingovog sindroma imali su više vrijednosti broja eritrocita u odnosu na kontrolnu grupu, a ustanovljena je i razlika u broju trombocita između skupina koja nije dosegla statističku značajnost, ali bi na nju trebalo obratiti pozornost.

Ključne riječi: psi, Cushingov sindrom, krvni pokazatelji