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NOTE

Clinical Pathology

Atypical hypoadrenocorticism with intact zona glomerulosa of the adrenal cortex after long-term observation: a case report of a dog

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ABSTRACT. An 8-year-old intact male pointer presented with lethargy and hypoalbuminemia. On abdominal ultrasonography, both adrenal glands were reduced in thickness. Based on the ACTH stimulation test results and the absence of electrolyte abnormalities, the dog was diagnosed with atypical hypoadrenocorticism. After treatment with low-dose prednisolone, his general condition improved, and blood tests normalized. The dog died 818 days later, and a complete autopsy was performed. Histologically, the architecture of the zonae fasciculata and reticularis was disrupted in both adrenal glands; however, the zona glomerulosa remained relatively normal. In summary, in this study, we detailed the pathological presentation of atypical hypoadrenocorticism without electrolyte abnormalities.

KEYWORDS: Addison's disease, adrenal gland, atypical hypoadrenocorticism, dog, glucocorticoid-deficient hypoadrenocorticism

Hypoadrenocorticism (Addison's disease) is an endocrine disorder caused by impaired secretion of adrenocortical hormones [16]. Primary Hypoadrenocorticism in dogs most commonly results from immune-mediated destruction of both the zona fasciculata and zona glomerulosa, resulting in deficiencies of both mineralocorticoid and glucocorticoid hormones. Usually, electrolyte abnormalities such as hyponatremia and hyperkalemia occur.

Alternatively, hypoadrenocorticism may occur without hyperkalemia and hyponatremia. This is thought to be a disease in which only secretion of the glucocorticoids is affected and is referred to as atypical hypoadrenocorticism, atypical Addison's disease, or glucocorticoid-deficient hypoadrenocorticism [8, 18]. With this pathogenesis, destruction of the zona fasciculata but not the zona glomerulosa is expected, thus preserving mineralocorticoid secretion. However, there are no reports of a case of atypical hypoadrenocorticism in a dog that accurately correlates the clinical course and clinicopathological results with adrenal pathology.

In this report, we describe the case of a dog diagnosed with atypical hypoadrenocorticism that progressed without electrolyte abnormalities and was eventually autopsied to confirm destruction of the zonae fasciculata and reticularis while the glomerular area retained normal architecture.

An 8-year-old intact male pointer was referred to the Veterinary Medical Center of Osaka Prefecture University because it had gradually become lethargic over the past 2 years, and a blood test showed hypoalbuminemia. The dog had no known history of glucocorticoid therapy, weighed 28.0 kg, and had a body condition score of 3/5. Hair loss, redness at the ends of both hind limbs, and peripheral lymphadenopathy were observed. A complete blood count showed normal leukocyte (10,700 cell/μL), neutrophil, and lymphocyte counts. Blood test results showed mild non-regenerative anemia (35.3%), hypoglycemia (72 mg/dL), low cholesterol

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Table 1. Blood test results during the treatment period

Analyte	1 day	14 day	28 day	155 day	793 day	Reference range
White blood cell count (/μL)	10,700	9,700	9,700	8,500	12,400	(6,000–17,000)
Neutrophils (/μL)	5,564	6,305	5,238	5,610	9,052	(3,000–11,500)
Lymphocytes (/μL)	3,852	2,425	3,395	1,870	1,860	(1,000–4,800)
Moncytes (/μL)	428	485	380	595	744	(150–1,350)
Eosinophils (/μL)	0	582	0	425	744	(100–1,250)
Red blood cell count ($\times 10^6$/μL)	6.25	6.28	6.79	6.52	5.03	(5.50–8.50)
Hemoglobin (g/dL)	11.8	12.8	14.6	15.7	12.1	(12–18)
Hematocrit (%)	35.3	39.1	43.7	44.9	34.2	(37–55)
Mean corpuscular volume (fL)	56.5	62.5	64.4	68.9	68.0	(60–77)
Mean corpuscular hemoglobin (pg)	18.9	20.4	21.5	24.1	24.1	(19.5–26.0)
Mean corpuscular hemoglobin concentration (%)	33.4	32.7	33.4	35.0	35.4	(32.0–36.0)
Platelet count ($\times 10^3$/μL)	301	298	327	257	220	(175–500)
Total protein (g/dL)	8.3	8.7	7.6	6.9	6.9	(5.3–7.9)
Albumin (g/dL)	1.5	2.3	2.6	2.7	2.6	(2.3–3.6)
Blood Urea Nitrogen (mg/dL)	13	9	12	14	13	(6–33)
Creatinine (mg/dL)	0.6	0.5	0.5	0.5	0.5	(0.6–1.6)
Calcium (mg/dL)	10.6	11.0	11.4	10.8	12.5	(10.2–13.7)
Asparate aminotransferase (U/L)	20	12	12	10	20	(<41)
Alanine aminotransferase (U/L)	18	25	39	29	27	(<123)
Total bilirubin (mg/dL)	0.2	0.2	0.2	0.2	0.2	(<0.3)
Total cholesterol (mg/dL)	92	188	300	323	267	(84–287)
Glucose (mg/dL)	72	85	96	91	92	(75–117)
Sodium (mEq/L)	143	145	146	145	144	(139–154)
Potassium (mEq/L)	4.9	4.8	4.3	4.8	4.7	(3.5–5.0)
Chloride (mEq/L)	106	104	104	113	106	(114–136)
CRP (mg/dL)	2.35	0.30	0.00	0.00	0.75	<1
T4 (μg/dL)	1.04			2.35		1.00–2.90
FT4 (ng/dL)	0.5			1.8		0.5–3.0
TSH (ng/mL)	1.98			1.75		0.00–0.50

(92 mg/dL), high globulin (6.8 g/dL), low albumin (1.5 g/dL), and mildly elevated CRP (2.35 mg/dL). Electrolytes were within reference values. Increased α-2 and γ fractions were identified via serum electrophoresis. Levels of NH_3 (55 μg/dL, reference range: 16–75 μg/dL) and Total bile acid (<1.0 μmol/L, reference range: <7.9 μmol/L) were within reference values. Urinalysis was negative for proteinuria, and the protein/creatinine ratio was 0.15. Chest and abdominal radiography showed no obvious abnormalities. On abdominal ultrasonography, although there were no abnormalities in the liver or gastrointestinal tract, both adrenal glands were reduced in thickness (right 3.1 mm, left 2.9 mm). Fine-needle aspiration examination of the lymph nodes showed an increased number of small lymphocytes, probably indicating reactive hyperplasia.

A gram stain showed cocci and bacilli on skin examination but no neutrophilic infiltration or phagocytosis of bacteria. Adrenocorticotropic hormone (ACTH) stimulation test showed low serum cortisol concentration (0.2 μg/dL) before and after stimulation. Endogenous ACTH was 245 pg/mL (reference value 5–36 pg/mL). Based on the ACTH stimulation test results and the absence of electrolyte abnormalities, the dog was diagnosed with atypical hypoadrenocorticism. Levels of T4 and FT4 were within the reference values (at 1.04 μg/dL and 0.5 ng/dL, respectively), and levels of TSH were elevated at 1.98 ng/mL (Table 1).

On the 14th day post presentation, prednisolone was orally administered at 0.18 mg/kg every 24 hr, and the patient showed no lethargy. The blood test values improved on the 28th sick day (Table 1). However, on the 50th sick day, polyuria and hypercholesterolemia were observed; therefore, prednisolone was reduced to 0.09 mg/kg every 24 hr. Subsequently, the patient's general condition was well-maintained, skin improved, and lymph node enlargement resolved. No electrolyte abnormalities developed (Table 1). Thyroid hormones were remeasured on day 155, and T4 was 2.35 μg/dL, FT4 was 1.8 ng/dL, and TSH was 1.75 ng/mL (Table 1).

On the 525th day of the illness, the dog began coughing. Radiography and echocardiography confirmed atelectasis near the caudal segments of the left cranial lung lobe. On the 532nd sick day, computed tomography and tru-cut biopsy of the lungs were performed, and the pathological examination results indicated histiocytic sarcoma. The patient was treated with nimustine (Nidran; Daiichi Sankyo, Tokyo, Japan, 25 mg/m^2 IV) and prednisolone (0.18 mg/kg PO every 24 hr) on day 546. Thereafter, anticancer therapy was discontinued; however, prednisolone was continued at the same dose.

The dog eventually died 818 days after the first presentation. A complete autopsy was performed, revealing that histiocytic sarcoma had metastasized to organs throughout the body. Both adrenal cortices had atrophied. Histologically, the architecture of the zonae fasciculata and reticularis was absent in both adrenal glands, while the zona glomerulosa was relatively normal (Fig. 1A). The approximate normal ratio of adrenal cortex:medulla:cortex is considered to be 1:1:1 [14], whereas the ratio in this case was approximately 1:3:1. Additionally, there was mild infiltration of lymphocytes and plasma cells with accumulation of ceroid-laden

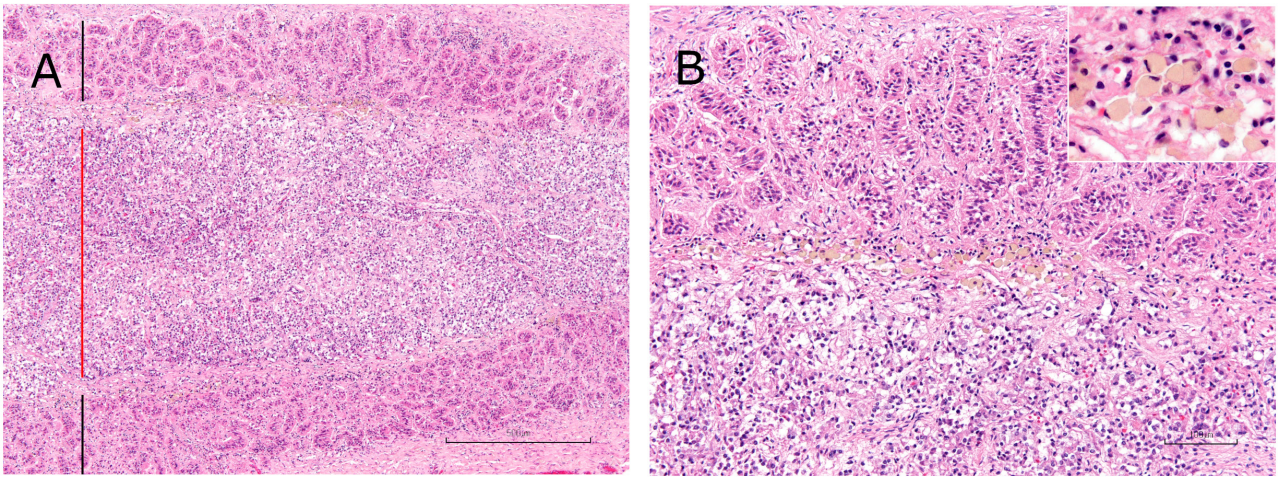

Fig. 1. Adrenal histology at autopsy; the adrenal cortex shows a marked, diffuse and circumferential atrophy. (**A**) The zona reticularis and zona fasciculata are unobservable while the zona glomerulosa is relatively well-retained. The black bars indicate the cortex, and the red bar indicates the medulla. Hematoxylin and eosin stain (HE). Bar, 500 µm. (**B**) Higher magnification of Fig. 1A. There is mild infiltration of lymphocytes and plasmacytes with accumulation of ceroid-laden macrophages in the border between the cortex and medulla. HE. Bar, 100 µm. Inset: High magnification of infiltrating cells.

macrophages and mild fibrosis at the border between the adrenal cortex and medulla (Fig. 1B). The capsule of the adrenal gland was thickened because of the proliferation of fibrous connective tissue. Follicles of the thyroid glands were slightly small in size, and some follicles contain lightly eosinophilic colloid. The pituitary gland showed no significant changes in parenchymal cells (pituitary cells). Chronic liver damage was suspected owing to mild liver fibrosis. The kidneys showed tumor metastasis but no glomerular abnormalities. The gastrointestinal tract showed no significant changes.

Lifton *et al.* [8] listed three diagnostic criteria for atypical hypoadrenocorticism: 1) low serum cortisol response on ACTH stimulation test (<5.0 µg/dL after stimulation); 2) normal serum Na/K ratio (Na/K >27); and 3) no history of steroid administration for 6 weeks prior to onset. In addition, many recent reports have used a post-stimulation <2.0 µg/dL as a criterion for serum cortisol response after ACTH stimulation test [18]. The present case fulfilled these criteria, and thus was diagnosed as atypical hypoadrenocorticism. Additionally, when glucocorticoid deficiency is observed, it is necessary to consider whether the patient has secondary hypoadrenocorticism, in which ACTH secretion is impaired [11, 17], or primary atypical hypoadrenocorticism, in which the zona fasciculata of the adrenal gland is dysfunctional. In the present case, we concluded that the patient had primary atypical hypoadrenocorticism because ACTH was increased above the reference level. Mild non-regenerative anemia, hypoglycemia, hypoalbuminemia, hypocholesterolemia, and mildly elevated CRP were observed. In addition, electrolytes met the reference values up to 793 days. This is consistent with the clinicopathological features of primary atypical hypoadrenocorticism previously reported [8, 15].

It is especially important to distinguish among protein-losing enteropathy, protein-losing nephropathy, and liver failure as causes of hypoalbuminemia. Various clinical examinations ruled out these diseases in the present case. Furthermore, the autopsy revealed no liver, kidneys, or gastrointestinal tract abnormalities that would cause hypoalbuminemia. Distinguishing between atypical hypoadrenocorticism and protein-losing enteropathy is very difficult. Hauck *et al.* [5] reported that adrenal function tests should be performed as a standard screening test in dogs with the chronic gastrointestinal disease to differentiate hypoadrenocorticism from chronic gastrointestinal disease. Lyngby *et al.* [9] reported hypoadrenocorticism mimicking protein-losing enteropathy in four dogs. The clinical signs in these four cases were all attributed to untreated hypoadrenocorticism, and not intestinal disease, as both clinical signs and laboratory abnormalities of hypoalbuminemia resolved after the administration of physiologic replacement doses of prednisolone. This was similar to the present case.

This report is the first to describe a case clearly demonstrating atypical hypoadrenocorticism in which long-term observation showed no electrolyte abnormalities and maintenance of the zona glomerulosa in a dog. Most cases of hypoadrenocorticism in dogs occur due to the destruction of the adrenal cortex by an immune mechanism [16]. However, the pathology of atypical hypoadrenocorticism has not been elucidated.

Some previous reports of so-called atypical Addison's disease in dogs have reported the development of electrolyte abnormalities during follow-up [8, 10, 13, 18]. Therefore, the observation that most dogs develop electrolyte changes sooner or later has led to the hypothesis that immune-mediated destruction of the adrenal cortex is first limited to zona reticularis/fasciculata but spreads to the zona glomerulosa in the later stages of the disease [2].

Frank *et al.* retrospectively studied 33 dogs that presented with adrenal inflammation at autopsy [4]. In this report, lymphoplasmacytic infiltration of the canine adrenal cortex was typical of immune-mediated reactions, usually accompanied by severe and often diffuse cortical atrophy. However, in three dogs suspected of having hypoadrenocorticism with lymphoplasmacytic adrenal inflammation and severe cortical atrophy, the glomerular zone was partially preserved. In one of these dogs, serum sodium and potassium levels

were normal, which may be consistent with adequate mineralocorticoid production by the zona glomerulosa. However, all three dogs with preserved zona glomerulosa had severe lymphoplasmacytic adrenal inflammation, indicating that the zona glomerulosa might become compromised in the future.

However, there are reported cases of atypical hypoadrenocorticism in dogs that do not show electrolyte abnormalities after long-term observation [8, 13, 18]. In the present case, the patient was maintained on physiological-dose prednisolone alone through 818 days of clinical observation, during which no electrolyte abnormalities were observed. Furthermore, histopathology showed inflammation and diffuse atrophy of the zonae fasciculata and reticularis, possibly as a result of degeneration, and preservation of the zona glomerulosa without inflammation, which is expected to have maintained aldosterone secretion and electrolyte levels. In summary, the clinicopathological and clinical behavior of this case over a long period was consistent with the pathological findings. This case proves the existence of atypical hypoadrenocorticism with at least a residual glomerular phase.

Histological findings in the present case were observed after-term prednisolone treatment and not at diagnosis. Therefore, the effect of long-term prednisolone administration on adrenal tissue should be considered. Previous reports have shown adrenal atrophy even at physiologic doses of prednisone [3]. However, although the zonae fasciculata and reticularis are atrophic, they are not completely disrupted as was observed in the current case. Furthermore, in that report, cortisol levels were elevated after an ACTH stimulation test, even after long-term administration of physiologic prednisolone. In the present case, cortisol levels were not elevated in the ACTH stimulation test at the initial presentation. In addition, the clinical symptoms were recognized for quite some time. Based on these facts, we speculate that the adrenal atrophy in the current case was present at the time of the initial visit and that the primary cause of the atrophy was atypical hypoadrenocorticism rather than the effects of physiologic prednisolone administration.

In addition, there are several reports of pathologically suspected atypical hypoadrenocorticism [1, 7]. These were a combination of atypical hypoadrenocorticism and hypothyroidism. Autopsy revealed adrenal inflammation and thyroid atrophy. These conditions have been diagnosed as autoimmune polyendocrine syndrome type II or Schmidt-like syndrome. In the present case, T4 and FT4 were within the reference values but slightly low, and TSH was elevated at the initial examination. However, the decrease in T4 and FT4 could be euthyroid sick syndrome. Elevated TSH is known to occur in human and canine hypoadrenocorticism before adrenal replacement [12], where it can be attributed to an enhancement of TSH release due to the chronic cortisol deficiency. In the present case, the thyroid gland was also measured again after treatment with physiological prednisolone. Results showed T4 and FT4 were elevated even though thyroid hormones had not been replaced. Furthermore, autopsy showed no gross atrophy of the thyroid gland, and the colloid presence was observed histologically. These findings suggest that, in contrast to previous reports, the thyroid gland function was maintained in this case, and only the zonae fasciculata and reticularis of the adrenal gland was destroyed.

The thickness of the left adrenal gland is the most useful parameter in supporting the diagnosis of hypoadrenocorticism. Ultrasonographic assessment of left adrenal thickness may be a good tool for clinicians to determine if a dog has adrenal insufficiency (cutoff value <3.2 mm) [20]. It is assumed that the echogenic findings in typical hypoadrenocorticism are due to atrophy of the adrenal cortex [6]. Additionally, dogs with atypical hypoadrenocorticism have been reported to have reduced thickness of the adrenal glands on ultrasonographic evaluation [19]. In the present case, ultrasonography of the adrenal glands showed that the thickness of the right and left adrenal glands was 2.9 mm and 3.1 mm, respectively. Autopsy revealed that the zonae fasciculata and reticularis were also atrophic. The small size of the adrenal glands on ultrasonography in atypical hypoadrenocorticism would be expected to reflect atrophy of the cingulate and reticular cortices. Thus, confirming the thickness of the adrenal glands using echography is a useful diagnostic method even in atypical hypoadrenocorticism.

In summary, we have detailed the pathological picture of atypical hypoadrenocorticism without electrolyte abnormalities in this study. In this case, we found atrophy of the zonae fasciculata and reticularis, and ultrasonography showed atrophy of the adrenal glands. Previous reports have shown that aldosterone levels may be decreased even in hypoadrenocorticism disease, where electrolyte levels are not decreased [2]. However, the relationship between these pathology results and aldosterone levels has not been investigated. In the present study, aldosterone levels may have been maintained because the (mineralocorticoid-producing) zona glomerulosa was maintained, and electrolyte levels were maintained during treatment. The relationship between aldosterone levels and pathology needs to be investigated in the future. Additionally, the causes of hypoadrenocorticism have not been well studied in dogs. To clarify the causal differences between typical and atypical hypoadrenocorticism, it will be necessary to study these differences through genetic studies and molecular immunological approaches.

CONFLICT OF INTEREST. The authors have no conflicts of interest directly relevant to the content of this article.

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REFERENCES

1. Adissu HA, Hamel-Jolette A, Foster RA. 2010. Lymphocytic adenohypophysitis and adrenalitis in a dog with adrenal and thyroid atrophy. *Vet Pathol* **47**: 1082–1085. [Medline] [CrossRef]
2. Baumstark ME, Sieber-Ruckstuhl NS, Müller C, Wenger M, Boretti FS, Reusch CE. 2014. Evaluation of aldosterone concentrations in dogs with hypoadrenocorticism. *J Vet Intern Med* **28**: 154–159. [Medline] [CrossRef]
3. Chastain CB, Graham CL. 1979. Adrenocortical suppression in dogs on daily and alternate-day prednisone administration. *Am J Vet Res* **40**: 936–941. [Medline]

4. Frank CB, Valentin SY, Scott-Moncrieff JC, Miller MA. 2013. Correlation of inflammation with adrenocortical atrophy in canine adrenalitis. *J Comp Pathol* **149**: 268–279. [Medline] [CrossRef]
5. Hoerauf A, Reusch C. 1999. Ultrasonographic evaluation of the adrenal glands in six dogs with hypoadrenocorticism. *J Am Anim Hosp Assoc* **35**: 214–218. [Medline] [CrossRef]
6. Hauck C, Schmitz SS, Burgener IA, Wehner A, Neiger R, Kohn B, Rieker T, Reese S, Unterer S. 2020. Prevalence and characterization of hypoadrenocorticism in dogs with signs of chronic gastrointestinal disease: A multicenter study. *J Vet Intern Med* **34**: 1399–1405. [Medline] [CrossRef]
7. Kooistra HS, Rijnberk A, van den Ingh TS. 1995. Polyglandular deficiency syndrome in a boxer dog: thyroid hormone and glucocorticoid deficiency. *Vet Q* **17**: 59–63. [Medline] [CrossRef]
8. Lifton SJ, King LG, Zerbe CA. 1996. Glucocorticoid deficient hypoadrenocorticism in dogs: 18 cases (1986–1995). *J Am Vet Med Assoc* **209**: 2076–2081. [Medline]
9. Lyngby JG, Sellon RK. 2016. Hypoadrenocorticism mimicking protein-losing enteropathy in 4 dogs. *Can Vet J* **57**: 757–760. [Medline]
10. Mansfield CS. 2000. Glucocorticoid deficiency (atypical hypoadrenocorticism) and exocrine pancreatic insufficiency in a German shepherd dog. *Aust Vet Pract* **30**: 2–5.
11. Platt SR, Chrisman CL, Graham J, Clemmons RM. 1999. Secondary hypoadrenocorticism associated with craniocerebral trauma in a dog. *J Am Anim Hosp Assoc* **35**: 117–122. [Medline] [CrossRef]
12. Reusch CE, Fracassi F, Sieber-Ruckstuhl NS, Burkhardt WA, Hofer-Inteeworn N, Schuppisser C, Stirn M, Hofmann-Lehmann R, Boretti FS. 2017. Altered serum thyrotropin concentrations in dogs with primary hypoadrenocorticism before and during treatment. *J Vet Intern Med* **31**: 1643–1648. [Medline] [CrossRef]
13. Rogers W, Straus J, Chew D. 1981. Atypical hypoadrenocorticism in three dogs. *J Am Vet Med Assoc* **179**: 155–158. [Medline]
14. Rosol TJ, Meuten DJ. 2017. Tumors of the adrenal cortex: adenoma, carcinoma, myelolipoma. pp. 782–787. In: Tumors in Domestic Animals, 5th ed. (Meuten DJ. ed), Wiley, Hoboken.
15. Sadek D, Schaer M. 1996. Atypical Addison's disease in the dog: a retrospective survey of 14 cases. *J Am Anim Hosp Assoc* **32**: 159–163. [Medline] [CrossRef]
16. Schaer M, Riley WJ, Buergelt CD, Bowen DJ, Senior DF, Burrows CF, Campbell GA. 1986. Autoimmunity and Addison's disease in the dog. *J Am Anim Hosp Assoc* **22**: 789–794.
17. Syme HM, Scott-Moncrieff JC. 1998. Chronic hypoglycaemia in a hunting dog due to secondary hypoadrenocorticism. *J Small Anim Pract* **39**: 348–351. [Medline] [CrossRef]
18. Thompson AL, Scott-Moncrieff JC, Anderson JD. 2007. Comparison of classic hypoadrenocorticism with glucocorticoid-deficient hypoadrenocorticism in dogs: 46 cases (1985–2005). *J Am Vet Med Assoc* **230**: 1190–1194. [Medline] [CrossRef]
19. Wakayama JA, Furrow E, Merkel LK, Armstrong PJ. 2017. A retrospective study of dogs with atypical hypoadrenocorticism: a diagnostic cut-off or continuum? *J Small Anim Pract* **58**: 365–371. [Medline] [CrossRef]
20. Wenger M, Mueller C, Kook PH, Reusch CE. 2010. Ultrasonographic evaluation of adrenal glands in dogs with primary hypoadrenocorticism or mimicking diseases. *Vet Rec* **167**: 207–210. [Medline] [CrossRef]