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PATHOLOGY

Clinical and pathological aspects of progressive hyperphosphatasemia associated with vacuolar hepatopathy and hepatocellular carcinoma in a Scottish Terrier bitch

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ABSTRACT: *Hyperphosphatasemia refers to an increase in alkaline phosphatase serum activity, and Scottish Terriers (STs) are predisposed to develop this condition of uncertain pathogenesis. This study describes a case of progressive hyperphosphatasemia with vacuolar hepatopathy and hepatocellular carcinoma (HCC) in a ST bitch. This dog had a five-year clinical follow-up with progressive hyperphosphatasemia (up to 5503 U/L) and with ultrasound findings and histologic diagnosis of vacuolar hepatopathy, in addition to posterior onset of HCC. A steroidogenic adrenal panel revealed an increase of adrenocortical hormones, especially progesterone and androstenedione, consistent with a subdiagnosed hypercortisolism. Euthanasia was elected and at necropsy, multinodular, yellow to red masses were observed in the liver, which were histologically and immunohistochemically defined as HCC. The association of the clinical, imaging, biochemical, adrenal panel and pathologic findings allowed to characterize and confirm a progressive disorder in this ST bitch associated with elevated adrenocortical hormones.*
Key words: *adrenocortical hormones, alkaline phosphatase, dog, liver, neoplasm.*

Aspectos clínicos e patológicos da hiperfosfatasemia progressiva associada à hepatopatia vacuolar e carcinoma hepatocelular em uma cadela da raça Scottish Terrier

RESUMO: *Hiperfosfatasemia é o aumento sérico de fosfatase alcalina, sendo que Scorrish Terriers estão predispostos a desenvolverem essa condição de patogênese desconhecida. Este trabalho descreve um caso de hiperfosfatasemia progressiva com hepatopatia vacuolar e carcinoma hepatocelular em um canino da raça Scottish Terrier. Uma cadela Scottish Terrier foi acompanhada clinicamente por cinco anos devido à hiperfosfatasemia persistente (até 5503 U/L), com achados ultrassonográficos e histológicos compatíveis com hepatopatia vacuolar, além de posterior desenvolvimento de carcinoma hepatocelular. O painel esteroidogênico realizado indicou aumento dos hormônios adrenocorticais, principalmente progesterona e androstenediona, consistente com diagnóstico de hipercortisolismo subdiagnosticado "atípico". Devido ao prognóstico desfavorável, a eutanásia foi realizada e na necropsia, massas amarelas a vermelhas e multinodulares foram observadas no fígado, com diagnóstico de carcinoma hepatocelular pela análise histológica e imuno-histoquímica. A associação dos achados clínicos, de imagem, bioquímicos, do painel androgênico e patológicos permitiram caracterizar e confirmar um distúrbio progressivo no canino da raça Scottish Terrier associado ao aumento dos hormônios adrenocorticais.*
Palavras-chave: *hormônios adrenocorticais, fosfatase alcalina, cão, fígado, neoplasia.*

INTRODUCTION

Hyperphosphatasemia refers to an increase in alkaline phosphatase (ALP) serum activity and may be caused by hepatic and non-hepatic disorders, such as neoplasms, endocrine diseases, use of anticonvulsants, exogenous glucocorticoids, and chronic illness (SEPESY et al., 2006; FERNANDEZ & KIDNEY, 2007). Scottish Terriers (STs) have been regarded as predisposed to have hyperphosphatasemia, but the pathogenesis is not yet fully understood (NESTOR et al., 2006; ZIMMERMAN et al., 2010; ZIMMERMAN et al., 2018). Additionally, this condition may be a breed-related progressive disorder, which is mainly associated with altered synthesis and metabolism of adrenocortical hormones (CORTRIGHT et al., 2014; ZIMMERMAN et al., 2010; ZIMMERMAN et al., 2018), and it may culminate in the development of hepatocellular carcinoma (HCC) and liver failure (CORTRIGHT et al., 2014). Thus, this study aimed to describe the clinical and pathological findings of a case of progressive hyperphosphatasemia associated with vacuolar hepatopathy and HCC in a ST bitch.

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An eight-year-old ST bitch was first referred to the Veterinary Teaching Hospital at the Universidade Federal do Rio Grande do Sul with a history of altered hepatic enzymes activity and other non-specific clinical signs, and had a clinical follow-up of five years until the age of thirteen. Initially, the animal was already receiving vitamin E and Silimarin for antioxidant and cytoprotective actions, and over the follow-up period ursodiol and S-adenosyl methionine (SAMe) were also prescribed. Laboratory exams initially revealed severe increase of serum ALP (605.3 U/L; reference value (RV): < 156 U/L) and moderate increase of alanine aminotransferase (ALT) (127.42 U/L; RV: < 102 U/L), which were the hepatic enzymes with the most evident abnormalities along all clinical course. The ALP serum levels ranged from 79.8 U/L to 5503 U/L, with values up to 35-fold the RV, while ALT varied from 92 U/L to 866 U/L, with values of 8-fold the RV.

Even though hypercortisolism was suspected, the bitch did not have any signs compatible with Cushing´s syndrome (CS), except a discrete potbelly abdomen attributable to hepatomegaly. An adrenal panel was conducted to evaluate adrenal function and four of the five measured hormones were elevated after administration of synthetic adrenocorticotropic hormone (ACTH): 17-OH-progesterone (2.37 ng/mL; RV: < 1.6 ng/mL), androstenedione (12.19 ng/mL; RV: 0.27-3.97 ng/mL), cortisol (212.8 ng/mL; RV: 60-170 ng/mL), and progesterone (5.49 ng/mL; RV: < 1.3 ng/mL). The only parameter within normal range was aldosterone (243.54 pg/mL; RV: 70-750 pg/mL). Therefore, an adrenal dysfunction compatible with hypercortisolism was considered, and treatment with trilostane (0.3 mg/kg), PO, q24h, was employed despite the absence of main clinical signs of CS.

A series of abdominal ultrasounds was performed for five years, at least once a year, which revealed that the adrenal glands were normal sized in all exams. Additionally, the liver had a heterogeneous echotexture and the parenchyma was hypoechoic with a nodular pattern in all US evaluations during this period (Figure 1A). In the fifth year of follow-up, a small fragment of liver was removed for histologic analysis, which was macroscopically pale tan with mild white nodules within the parenchyma. Histologically, there was severe and diffuse hepatocellular cytoplasmic vacuolation, which was characterized by swollen hepatocytes with irregular and variably sized cytoplasmic clearings and vacuoles, in addition to multifocal areas of hemosiderosis (Figure 1B). The formalin-fixed paraffin-embedded liver biopsy section was latter stained with Periodic Acid-Schiff (PAS), which revealed a marked, diffuse, and stippled to granular cytoplasmic staining. Thus, a diagnosis of degenerative and chronic vacuolar hepatopathy compatible with hepatic glycogenosis was achieved.

In the last year of follow-up, the dog had worsening of the clinical condition and euthanasia was elected by the owner. At the necropsy, the liver was enlarged and had multiple nodular, yellow to red masses up to 7.0 cm in diameter, which were firm with cystic areas (Figure 1C). On the cut surface, a multinodular and solid pattern was observed, in addition to multifocal dark-red areas (Figure 1D). Histologically, the masses corresponded to a moderately well-demarcated and partially encapsulated neoplasm of hepatocytes, which were arranged in variably thick trabeculae and with occasional solid areas. These cells were polygonal, with abundant cytoplasm and round nuclei with prominent nucleoli. Moderate anisocytosis and anisokaryosis, occasional binucleated cells and rare mitotic figures were also noted (Figure 1E). The adjacent hepatic parenchyma had moderate to severe hepatocellular degeneration and multifocal areas of hemosiderosis, which were similar to that observed in the liver biopsy. Also, multifocal and moderate periportal fibrosis was also observed, which may indicate a chronic progression of the hepatic lesions throughout the years. Additionally, scattered neoplastic cells were observed within the medullary sinus of the pancreaticduodenal lymph node. Cut sections of the hepatic tumor were submitted to immunohistochemistry (IHC) with the monoclonal Hep-Par-1 antibody (code M7158, Dako), as previously described (ARGENTA et al., 2020). At the IHC, the neoplastic cells had diffuse and moderate granular intracytoplasmic immunolabeling (Figure 1F).

The association of the clinical, imaging, biochemical, adrenal panel, and the pathological findings allowed us to reach a final diagnosis of a progressive disorder in this ST bitch, which apparently was unresponsive, or at least poorly responsive, to the medical treatment with cytoprotective drugs (WEBSTER & COOPER, 2009). STs are more predisposed to have hyperphosphatasemia than other breeds, and are 2.4 times as likely to have a related clinical disease (NESTOR et al., 2006), such as VH (CORTRIGHT et al., 2014; PEYRON et al., 2015). Elevated ALP is the most frequent abnormality observed in dogs with CS (FERNANDEZ & KIDNEY, 2007), and the adrenal panel performed herein enabled us to detect elevated

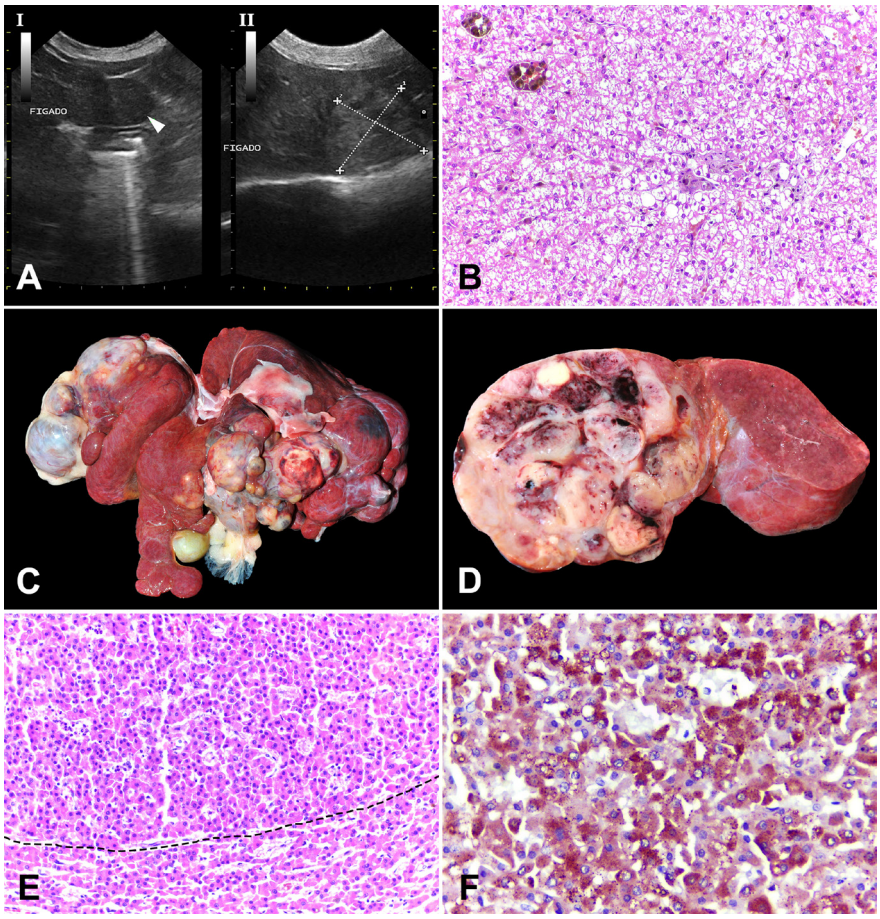

Figure 1 - Progressive hyperphosphatasemia associated with vacuolar hepatopathy and hepatocellular carcinoma in a Scottish Terrier bitch. (A) Selected ultrasound features. I- hepatomegaly with rounded edges and moderately heterogeneous and hypoechoic parenchyma (arrowhead). II- Heterogeneous and hyperechoic hepatic nodule with poorly delimited edges measuring 2.68 x 2.46 cm (dotted lines); (B) Histologic features of hepatic biopsy. The hepatocytes are severely swollen and with variably sized cytoplasmic clearings and vacuoles. There is also mild hemosiderosis. Hematoxylin and eosin (HE), 200× (C-F) Macroscopic and histologic findings of necropsy. (C) The liver is severely enlarged by multifocal to coalescent, yellow to red, and firm to cystic masses affecting multiple hepatic lobes. (D) On the cut surface, these masses have a multinodular pattern and are white to yellow with red areas. (E) Liver: there is a moderately well-demarcated and unencapsulated hepatocellular carcinoma above the dotted line, which is characterized by hepatocytes arranged in a trabecular pattern with occasional solid areas 200×. (F) The neoplastic hepatocytes have moderate to marked, granular, intracytoplasmic immunolabeling for anti-Hep-Par-1. Immunohistochemistry anti-Hep-Par-1, 400×.

adrenocortical steroid hormones and confirm the diagnosis of subclinical hypercortisolism, since the bitch did not have any clinical signs compatible with hypercortisolism and the adrenal glands were normal sized. These findings have been described in STs with hyperphosphatasemia and clinical signs of hypercortisolism may be absent or reported in only 40% of the affected dogs, indicating a subclinical disease (ZIMMERMAN et al., 2010; CORTRIGHT et al., 2014).

In our research, a progressive hepatic disease associated with persistently elevated ALP was also observed, and it was initially characterized by VH with posterior onset of HCC. VH in dogs is usually

associated with increased glucocorticoids, and even though amost half of affected dogs may not have been exposed to glucocorticoids (SEPESY et al., 2006), in the present case the adrenal panel results allowed us to determine it as the cause of the VH. The diagnosis of hepatic lesions in these cases should not be solely obtained via US, but also confirmed by histologic analysis (CORTRIGHT et al., 2014), as performed in the current study. HCC is the most common primary liver neoplasm (PATNAIK et al., 1981), and in STs, an association with elevated ALP and VH have been described, with neoplastic rates ranging from 12.5% to 34% (CORTRIGHT et al., 2014; PEYRON et al., 2014; 2015). CS has also been reported as a potential risk factor for HCC (LEELA-ARPORN et al., 2019), and since in the current case the bitch had both diagnosis (VH and hypercortisolism), there was a higher predisposition to develop HCC.

At necropsy, there was no evidence of enlargement of either the pituitary or the adrenal glands, suggesting that the pituitary-adrenal axis was intact, which has been described in STs with hyperphosphatasemia and VH (ZIMMERMAN et al., 2010; CORTRIGHT et al., 2014; ZIMMERMAN et al., 2018). Areas of adrenal hyperplasia were not observed either at macroscopic or histologic evaluation, hence it was not considered the cause of excessive sex hormones secretion. Aberrant receptors on adrenocortical cells are a possible cause of this syndrome in ST as well as reduced expression of the HSD17B2 enzyme, which is involved in the metabolism of adrenal steroids and would allow greater intracellular exposure to corticosteroids in the liver (ZIMMERMAN et al., 2018). However, neither hypothesis could be checked in the case herein described, even though these remain possible causes. Thus, in the current case, the authors believe that subclinical hypercortisolism represented a breed-related disorder that affected the adrenal steroidogenesis, which has been proposed as a possible cause of increased ALP, VH and development of HCC in STs (CORTRIGHT et al., 2014).

CONCLUSION

The bitch in the current research had adrenal dysfunction of undetermined cause with oversecretion of sex hormones, especially progesterone and androstenedione. Moreover, US and necropsy exams revealed that there were no abnormalities in the adrenal and pituitary glands, which emphasizes the possibility of adrenal dysfunction without lesions in these organs. The excess of corticosteroid hormones in the bitch of the current work led to persistently elevated ALP and progressive VH, as observed during the five-year follow-up with laboratory exams, imaging results and histologic analysis. VH in ST may represent a progressive condition, with primary development of vacuolated hepatocytes, followed by regenerative nodules and, finally, onset of massive HCC. Therefore, this disorder may happen in dogs of this breed in Brazil and requires frequent monitoring.

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DECLARATION OF CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

We declared, for all due purposes, that the project that gave rise to the present data has not been submitted for evaluation of the Ethics Committee of the Universidade Federal do Rio Grande do Sul (UFRGS), but we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) if it involves animals. Thus, the authors assumed full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

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