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IMMUNOEXPRESSION OF MASPIN AND KI-67 AS PROGNOSTIC MARKERS FOR BENIGN CHARACTERIZATION OF A CANINE MAMMARY FIBROEPITHELIAL HYPERPLASIA: CASE REPORT

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ABSTRACT

Mammary hyperplasia is characterized by an increase in the whole mammary gland, considered benign and with a good prognosis. A two-year-old dog showed a volume increase in the entire mammary chain after hormone stimulation. To characterize the process prognosis, a immunohistochemical procedure was used to evaluate the Maspin staining, considered good prognosis marker, and the Ki-67, a proliferation marker, whose high expression indicates malignancy. As a result, we obtained a low expression of Ki-67 and a high expression of Maspin, suggesting an excellent prognosis for the animal.

KEYWORDS: Female dog, imunohistochemistry, neoplasia, prognosis.

RESUMO

IMUNOEXPRESSÃO DE MASPIN E KI-67 COMO MARCADORES PROGNÓSTICOS DA CARACTERIZAÇÃO BENIGNA DA HIPERPLASIA FIBROEPITELIAL MAMÁRIA CANINA

A hiperplasia mamária é caracterizada por um aumento de toda a glândula mamária, considerada benigna e de bom prognóstico. Uma cadela de dois anos apresentou aumento de volume de toda a cadeia mamária após estímulo hormonal. Para caracterizar o prognóstico do processo foi utilizada a técnica de imunohistoquímica com avaliação da marcação do Maspin, considerado um marcador de bom prognóstico e o Ki-67, marcador de proliferação celular e que, portanto, mostraria alta expressão na malignidade. Como resultado obteve-se uma baixa expressão do Ki-67 e uma alta expressão do Maspin, sugerindo um excelente prognóstico para o animal.

PALAVRAS- CHAVES: Cadela, imunohistoquímica, neoplasia, prognóstico.

INTRODUCTION

The mammary gland hyperplasia (fibroepithelial hyperplasia) is characterized by fast and abnormal growth of the mammary gland, and it is considered a benign non-neoplastic proliferation (NELSON & COUTO, 1998).

This alteration usually occurs in female cats, under 2 years old, 1 or 2 weeks after the oestrus or at the beginning of the pregnancy. It occurs when they are exposed to (endogenous or exogenous) progesterone for a long time. Therefore, old female cats also develop this condition when exposed to megestrol acetate (WITHROW & MacEWEN, 1989) or in re-

currence of pseudo pregnancy cases (LORETTI et al., 2005). It has also been described in spayed male cats or in those that are not exposed to progesterone. There is sufficient evidence to conclude that this is a hormone-dependent condition. Nevertheless, it has already been described in ovariohysterectomized female cats (NELSON & COUTO, 1998; SOUZA et al., 2002; VASCONCELLOS, 2003). This disorder is very uncommon in dogs (SOUZA et al., 2002).

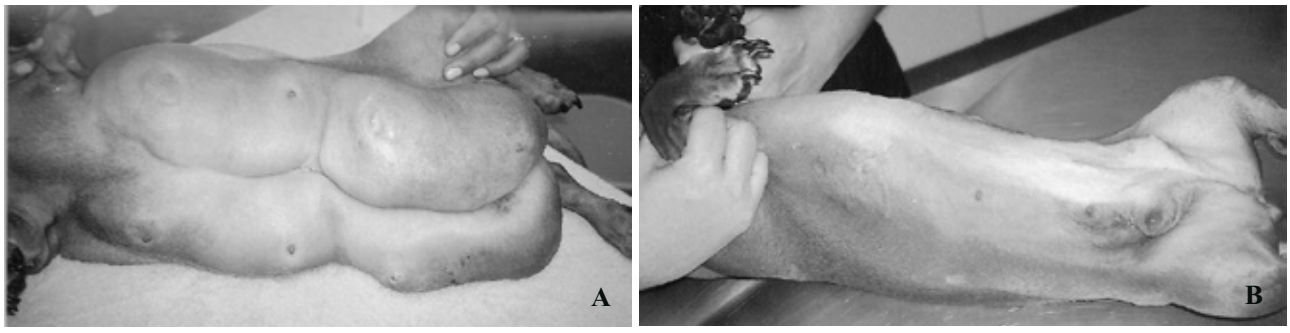

FIGURE 1. A. Clinical appearance of the fibroepithelial hyperplasia in a female dog; B. The female dog after treatment.

Immunohistochemistry has been used as a diagnostic method for prognostic markers of mammary gland cancer in dogs (THOMAS & BERNER, 2000; KANDIOLER-ECKERSBERGER et al., 2000; KIM et al., 2010). However, there is no report relative to the use of Ki-67 and Maspin as immunohistochemical markers to characterize fibroepithelial hyperplasia of the mammary gland of dogs, renal cell carcinoma (TURUNC et al., 2010), nasopharyngeal carcinoma (FENG et al., 2010), lung cancer (NAM & PARK, 2010), and cell proliferation (CHANO et al., 2010). The aim of the present study is to use the immunohistochemical markers – Maspin and Ki-67 – as possible molecular markers in the prognosis of fibroepithelial hyperplasia of mammary glands in female dogs.

MATERIALS AND METHODS

A 2-year-old female Dachshund dog was evaluated. Two months prior to examination, the bitch was reported to have developed an enlargement of all mammary glands and had a diagnosis of pseudo pregnancy (Figure 1A). The dog was then medicated with progesterone (unknown dosage), which resulted in enlargement of the mammary glands. The skin at these areas was very irritated, there was edema at the lower extremities, and she had gained over 4 kg of weight. The bitch was brought to the veterinary care service and submitted to ovariohysterectomy with regression of more than a half of the glands after 60 days. The biopsy exam confirmed mammary hyperplasia (Figure 2A) and the dog was also prescribed metergolina, at doses of 0.1 mg / kg orally BID for 7 days, returning to normal within 3 months (Figure 1B). Anti-Maspin and anti-Ki67 antibodies were used to predict the clinical outcome and were considered prognostic markers in mammary neoplasia. The immunohistochemical procedure had the blockage of the endogenous peroxidase carried out with hydrogen peroxide (10%), and its antigenic retrieval was performed in a steam pan with a citrate buffer solution (pH = 7.0). The incubation was performed with primary antibody (diluited in BSA+PBS) in an overnight process at – 4 ° C with anti-Maspin (Novocastra™) 1:50; the antibody anti-Ki-67 (MIB-11 Dako, 1:50). After the incubation with a secondary antibody (Solution A-Kit Dako LSAB+, Universal- Peroxidase), the slides were incubated with Streptavidina/Peroxidase complex (Solution B-Kit Dako LSAB+, Universal-Peroxidase) for 30 minutes. The development was performed with a DAB chromogenic substrate (chromogen 20 mG + 1 mL Buffered, Dako™) and counterstained with Harris Hematoxylin (Figure 2B and 2C). A negative control was utilized deprived of primary antibody (Figure 2).

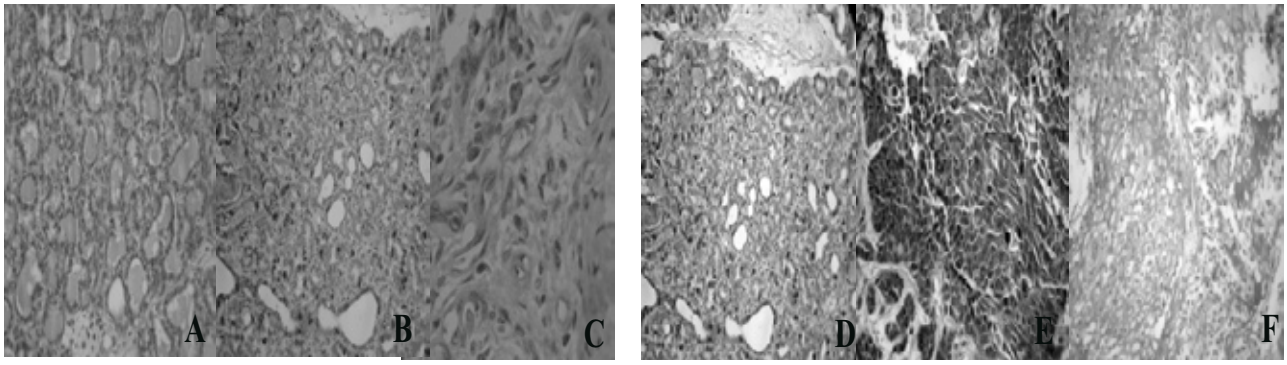

FIGURE 2. A. Histopathologic diagnosis of fibroepithelial hyperplasia; B. Expression of anti- Maspin antibody (40x); C. Expression of anti-ki-67 antibody (40x). D. Positive control of ki-67 (40x), E. Positive control of Maspin (40x) and F. Negative control (40x).

RESULTS AND DISCUSSION

The present study highlights the perspective of new molecular markers that can be used for the differential diagnosis of neoplasia and benign diseases, such as this unusual hyperplasia case of the mammary gland in dog. The study warns for the aggravation of hyperplasia with the use of progesterone and shows that the ovariohisterectomy was effective in controlling the size of the mammary gland.

The use of molecular markers by the immunohistochemistry has been described in the literature as predictive of the diagnosis and prognosis of neoplasias (KANDIOLER-ECKERSBERGER et al., 2000). The present study used these markers in a benign disease and showed that their expressions were consistent with their proposal. The outcomes showed a strong expression of the anti-Maspin antibody (Erviegas) and a weak staining of the anti-Ki-67 antibody (Dako), which was normally expected in the benign processes corroborating a good prognostic for the female dog. According to the literature (ZUCCARI et al., 2004), Ki-67 is a good cell proliferation marker; thus, the prognosis is considered good when its expression is low. On the other hand, it has been considered that Maspin, as a cell adhesion molecule, has a strong expression when the adhesion is preserved, a characteristic of benign tumors. Experiments have demonstrated an inverse correlation between loss of Maspin expression and increased malignancy of mammary gland tumor and the worst disease outcome (MAASS et al., 2001; STARK et al., 2010; PRASAD et al., 2010). Studies have indicated that Ki-67 is a marker to characterize malignant tumors due to intensive cell proliferation in mammary gland tumors (ZUCCARI et al., 2004). In this report we can see an agreement with the literature in relation to these markers in accordance with its expression by immunohistochemistry.

CONCLUSION

During the present study, the low expression of Ki-67 and the strong expression of Maspin suggest a benign process, indicating their possible use to confirm it as a benign mammary disease. Because it was favorable to this case, a greater number of real tests are necessary for the confirmation of the potential markers ki-67 and Maspin.

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