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CASE REPORT

Pathological and immunohistochemical characterization of ovarian papillary adenocarcinoma in a spitz dog

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ABSTRACT

A case of ovarian papillary adenocarcinoma in a Spitz was reported. Twelve years old female spitz dog was presented to the Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal with the history of progressive abdominal distension for the past one month. Haematology and serum biochemistry revealed leucocytosis and mild hypercalcemia respectively. Lateral abdominal radiography and transabdominal ultrasonography showed homogenous intra-abdominal mass and irregular mass adjacent to spleen respectively. The computed tomography of abdomen exhibited presence of mass beneath both kidneys extending up to bladder with HU index 60 to 75 suggestive of tumor. The exploratory laparotomy revealed large cauliflower like mass in right ovary and was removed surgically by ovariohysterectomy. The impression smears from the mass showed clusters of cells with indistinct cell border and significant anisokaryosis. Histopathologically, glandular cells were arranged in papillary pattern with irregular branches and the neoplastic cells showed cellular atypia, scanty eosinophilic cytoplasm with hyperchromatic ovoid nuclei. Furthermore, the immunohistochemistry showed moderate to strong expression for pan-cytokeratin and estrogen receptor (ER). Based on histopathology and immunohistochemistry the tumor was confirmed as ovarian papillary adenocarcinoma.

Keywords: Dog, histopathology, immunohistochemistry, ovarian adenocarcinoma

Ovarian tumors are most common in bitches and are usually arise from surface epithelium and subepithelial structures of ovaries. The most common epithelial tumors of ovary in canine are papillary adenoma and adenocarcinoma[1]. The ovarian tumors that generally appear as arboriform papillae projecting into the lumen of cystic cavities are subclassified as papillary or cystic adenoma and carcinoma[2]. The clinical features associated with ovarian papillary adenocarcinoma in dogs include ascites, pleural effusion and presence of palpable abdominal mass. Bitches with papillary adenocarcinoma show hormonal imbalances resulting in pyometra and vaginal bleeding[3]. Canine papillary ovarian adenocarcinoma is often associated with extensive peritoneal implantation and formation of malignant effusion[4]. Histopathologically, the ovarian adenocarcinoma is characterized by arrangement of neoplastic epithelial cells in papillary pattern[5]. Immunohistochemical staining provides a more definitive description of morphologically overlapping entities like ovarian epithelial tumors, sex cord-stromal tumours and germ cell tumours[1,6].

The cytokeratin belongs to the family of intermediate filament and are used as the most important marker for the differentiation of various epithelial tumours by immunohistochemistry. Both benign and malignant epithelial tumors show positive immunoreaction for cytokeratin[7,8]. Estrogen from ovaries play a critical role in the regulation of growth and differentiation of normal ovarian follicles and its actions are mediated by estrogen receptor (ER). It is nuclear transcription factors that bind to the estrogen responsive elements present in the target genes and deliver signalling systems for cell division and differentiation. ER expression is seen in 67% of ovarian epithelial tumor cases[9,10,11]. Progesterone is a steroid hormone works together with estrogen and promote follicle maturation, ovulation and corpus luteum formation[10]. Progesterone receptor (PRs) mediates the functions of progesterone and its expression in ovarian tumors, is a good prognostic marker correlated with longer survival period of affected patients[12,13]. The present study was undertaken to unveil the type of tumor and to elucidate the pathology of ovarian adenocarcinoma. Furthermore, the origin of tumor and its hormone dependence

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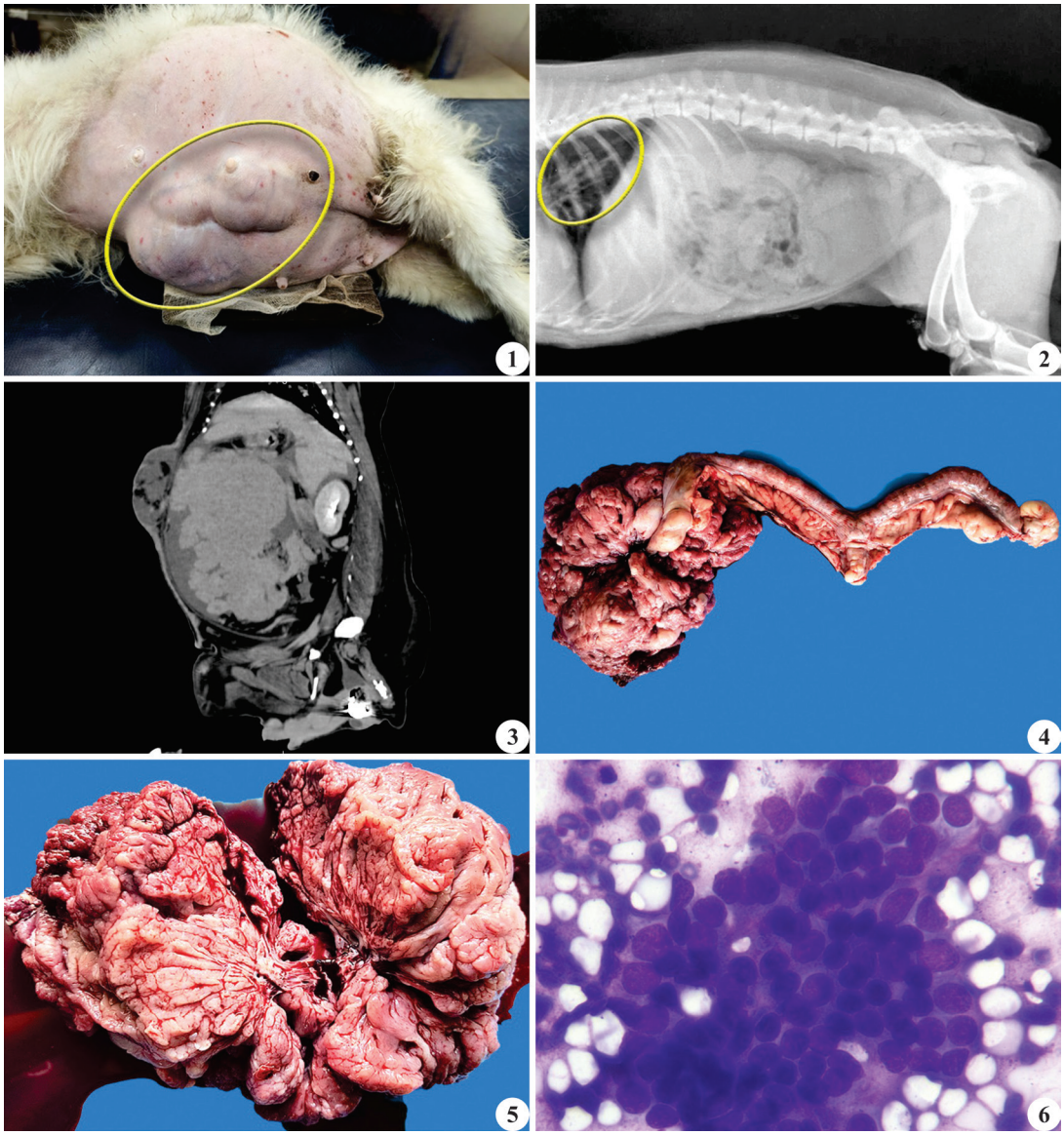

Fig. 1. The female spitz dog showing severe abdominal distension; **Fig. 2.** The dog showing radio dense mass caudal to kidney and pulmonary metastases (circle); **Fig. 3.** Computed tomography: Mass beneath both the kidneys extending up to bladder; **Fig. 4.** A huge cauliflower like mass on the right ovary; **Fig. 5.** Cut-section of the tumor mass showing papillary appearance with haemorrhage; **Fig. 6.** Impression smear showing radial arrangement of nuclei with numerous secretory vacuoles and granules in the cytoplasm. (May Grunwald Geimsa x400).

were studied by immunohistochemistry.

A 12 years-old female spitz was presented to the Veterinary clinical complex, Veterinary College & Research Institute, Namakkal with the history of progressive enlargement of ventral abdomen for one month. Detailed clinical examination including abdominal palpation was carried out. Lateral abdominal radiography, abdominal ultrasonography and computed tomography were done to assess the location of the mass and to detect metastasis if any. The blood sample was collected in EDTA coated vacutainer by venipuncture and analysis of blood was done using M/s Rayto RT-7600 haematology analyser, China. Clot activator vials were used for serum samples and biochemical analysis was done using M/s Biosystems A50, India.

Exploratory laparotomy was carried out which revealed presence of huge mass in the right ovary. Ovariohysterectomy was performed to remove the mass surgically and gross morphometry was recorded. Impression smears were taken from the cut surface of the mass and fixed with methanol and stained with Giemsa, May-Grunwald Giemsa and Wright-Giemsa combination for cytological examination. The representative tissue samples from the mass were collected in 10 per cent neutral buffered formalin for histopathology. The tissue samples were processed, embedded in paraffin,

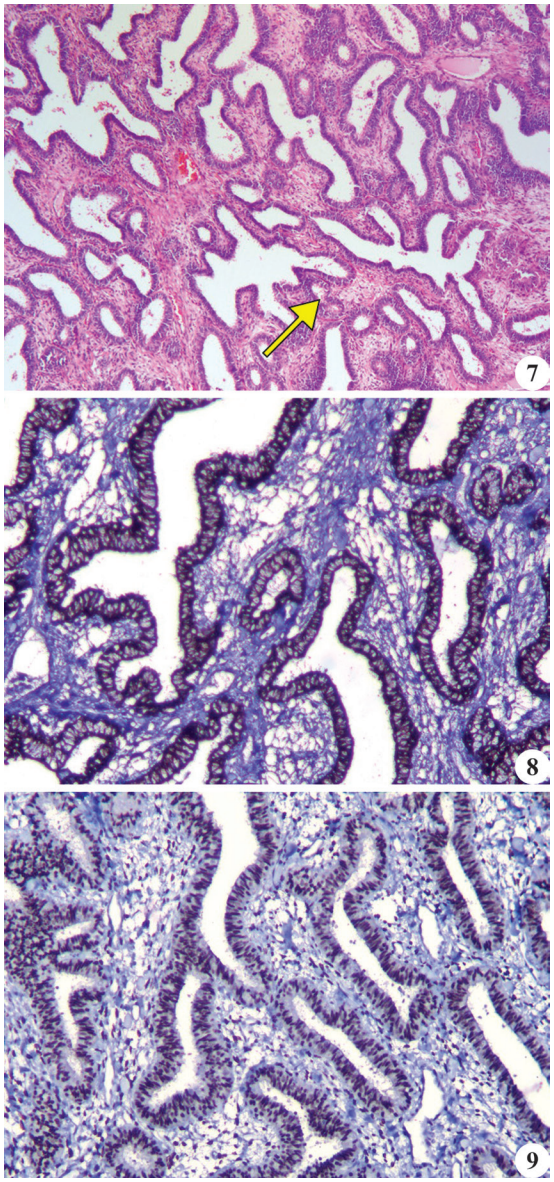

Fig. 7. Ovarian mass showing numerous glandular structures with slit like opening and micropapillary growth (arrow) (H&E x40); **Fig. 8.** Papillary ovarian adenocarcinoma showing strong cytoplasmic expression to cytokeratin (IHC AE1/AE3 x100); **Fig. 9.** Papillary ovarian adenocarcinoma showing moderate to strong nuclear expression to estrogen receptor alpha (IHC ER x100).

sectioned at 3-5µm thickness and stained with haematoxylin and eosin (H&E) for histopathological examination.

For immunohistochemistry, the tissue sections were mounted on Poly-L lysine coated slides for demonstration of pan-cytokeratin (CK), estrogen receptor alpha (ER) and progesterone (PR) receptors. Sections were incubated with primary antibodies followed by HRP conjugated secondary antibody and then counter stained with Mayer's haematoxylin.

Clinically, the animal was dull and showed severe abdominal distension with bilateral abduction of forelimbs (Fig. 1). In transabdominal ultrasonography, an irregular mass varying echogenicity adjacent to spleen with high vascularization was observed. Lateral abdominal radiography revealed the radiodense mass caudal to kidney and pulmonary metastases were also noticed in x-ray (Fig. 2). Computed tomography showed a mass beneath the kidneys and extending up to bladder with HU index 60 to 75, suggestive of tumor (Fig. 3). Complete blood count revealed leucocytosis (25.08 x 10^3 cells/µl) and serum biochemical values showed mild hypercalcemia (15.7 mg/dl).

Grossly, cauliflower like hard mass was present on the right ovary (Fig. 4) measuring about 71.2 mm x 112 mm (length & breadth), weighing around 1.2 kg and the cut surface of the mass showed papillary pattern with haemorrhage (Fig. 5). But the left ovary was normal. The cytological smears prepared from the mass revealed large unified cluster of proliferating epithelial cells were arranged in papillary pattern. The neoplastic epithelial cells exhibited significant anisocytosis with indistinct cell border and anisokaryosis. The cytoplasm was pale blue, contained secretory vacuoles and granules (Fig. 6). The nuclei were uniformly ovoid with hyperchromatia and open chromatin.

On histopathological examination, the tumor mass revealed irregular branching of proliferating epithelial cells in papillary pattern, numerous glandular structures with slit like opening and micropapillary growth (Fig. 7). The numerous papillary projections of surface epithelium appeared as arboriform pattern with micropapillary growth. The multiple layers of pleomorphic cuboidal cells lined the walls of cystic cavities and invasion of neoplastic cells into large stromal core. The neoplastic cells were characterized by scanty eosinophilic cytoplasm, round to ovoid hyperchromatic nucleus with dense chromatin. In addition, the neoplastic cells were invading into the supporting stroma tissue. The immunohistochemical analysis of the ovarian mass demonstrated the positive immunoreaction for pan-cytokeratin (Fig. 8) and estrogen receptor alpha (ER) (Fig. 9). The surface epithelium of ovarian mass revealed moderate to strong cytoplasmic expression of AE1/AE3 and nuclear expression of ER. However, the ovarian mass did not show any expression for progesterone receptor (PR).

The mild hypercalcaemia observed in this study was in accordance with previous workers who also reported the elevated serum calcium in 10-year old Golden Retriever with ovarian serous papillary adenocarcinoma[3]. The gross pathology and cytology observed in the present study were in accordance with the earlier reports[7]. Histopathology of the mass in the present study revealed more micropapillary growth and this observation was in concurrence with the earlier findings[2,6].

AE1/AE3 is a pan cytokeratin marker used to identify the proliferations of epithelial origin. AE1/AE3 expression in this study agreed with the earlier authors[8]. The strong immunoreaction of ER in this study was more evident in this study compared with previous reports. ER expressions in neoplastic cells suggest that

estrogen secreted from the ovaries might have contributed to the proliferation of ovarian papillary adenocarcinoma which clearly indicates the ovarian tumors are estrogen dependent. The negative IHC expression of PR in this study was contrary to the finding of previous workers who encountered positive expression in the subsurface epithelial structures[14]. The negative response to PR in the present study might be of progesterone independent nature of ovarian papillary adenocarcinoma in this bitch.

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