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CASE REPORT

A widely metastatic hepatocellular carcinoma and intrahepatic bile duct cystadenocarcinoma in Spitz dog

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ABSTRACT

A ten-year-old male Spitz dog was brought for post mortem examination with a history of emaciation, weakness, difficulty in breathing and death. At necropsy, multiple tumour nodules were noticed on the liver surface. The metastatic tumour growths were also noticed in the lungs, lymph nodes and rectum. Morbid changes were observed in the spleen, heart, kidneys, urinary bladder and stomach. Microscopically, the tumour nodules were characterised by the presence of hepatocellular carcinoma (HCC) and intrahepatic bile duct cystadenocarcinoma (ICC). The pleomorphic neoplastic cells in HCC contained large, hyperchromatic, vesiculated nuclei, prominent nucleoli and multiple mitotic figures. The hepatic tissues around the tumour nodules were invaded with neoplastic cells and infiltration of mononuclear cells. The ICC consists of many cysts lined by single or multiple layers of cuboidal or columnar cells. The cystic lumen contained eosinophilic secretions and exfoliated cells. The metastatic neoplastic cells were noticed in the lungs, lymph nodes, spleen, heart, kidneys, urinary bladder, stomach and rectum. The immunoreactivity of neoplastic cells showed a mild expression of *arginase-1*, *Hep Par-1*, *glypican-3*, *AFP* and *Bcl-2*. The normal hepatic cells showed a marked expression of *arginase-1*, strong expression of *Hep Par-1* and mild expressions of *glypican-3* and *AFP*. Spleen showed marked expression of *PCNA* and *CD3* in the lymphoid cells. Mesenteric lymph node showed a moderate expression of *CD3* in the lymphoid cells.

Keywords: Dog, HCC, histopathology, ICC, immunohistochemistry

Hepatocellular carcinoma (HCC) is rare in dogs and most common in older animals with higher incidence in males. The malignant variant of HCC usually occurs as massive, nodular and diffuse forms[1,2]. The metastasis of HCC occurs mostly within liver by local invasion or extrahepatic organs like lungs, lymph nodes and peritoneum[1]. The other metastatic organs include kidney, heart, urinary bladder, adrenal glands, pancreas and bone marrow in dogs[1-3]. The intrahepatic invasion of neoplastic cells arranged as tubular carcinomas (cholangiocarcinoma) and intrahepatic bile duct cystadenocarcinomas (ICC). The prognosis of mixed variants of HCC and ICC are generally determined by histology and morphology. The pathophysiology, diagnosis and treatments of this tumour is highly challenging in advancing veterinary oncology. Hence, the present report describes the pathological characteristics of a rare variant of HCC and ICC in Spitz dog.

A ten-year-old male Spitz dog was brought for post mortem examination with a history of emaciation, weakness, difficulty in breathing, congested visible mucous membrane and death. After necropsy, representative tissue pieces from liver along with tumour nodules and visceral organs were collected in 10% neutral buffered formalin. Tissue samples were trimmed, processed, embedded, sectioned at 3-5 µm thickness and stained with routine hematoxylin and eosin (H&E) for histopathology[4]. Few sections were taken on positively charged slides for immunohistochemical (IHC) staining. Sections were incubated with primary antibodies (Table 1) followed by Poly Excel HRP / DAB detection system conjugated secondary antibody (PEH002) and counter stained with Mayer's haematoxylin[5,6]. Both primary and secondary antibodies were purchased from M/s PathnSitu Biotechnologies, Hyderabad, India.

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Grossly, multiple tumour nodules of varying sizes (8x9x6 cm) were noticed on the surface of liver. Nodules were pinkish-white in colour, well-delineated and had central depressions (Fig. 1). The metastatic tumour nodules were noticed in all lobes of the lungs. Mesenteric and mediastinal lymph nodes were enlarged and grayish-white in colour. Spleen was mildly enlarged and moderately congested. Heart revealed few

Table 1. Immunohistochemical expression of various primary antibodies in tumour tissues and lymphoid organs.

S.No.	Primary antibody	Host	Clone	Dilution	Location of expression	IHC scoring / expressions			
						Normal hepatic cells	Neoplastic hepatic cells	Stromal cells	Lymphoid cells
1.	Arginase-1	Mouse	Monoclonal (ARG1)	1:25	Cytoplasmic	4+	2+	1+	Negative
2.	Hep Par-1	Mouse	Monoclonal (EP265)	1:50	Cytoplasmic	5+	2+	1+	Negative
3.	Glypican-3	Mouse	Monoclonal (GPC3)	1:50	Cytoplasmic	2+	2+	Negative	Negative
4.	Alpha Fetoprotein (AFP)	Rabbit	Monoclonal (EP209)	1:50	Cytoplasmic	2+	2+	Negative	Negative
5.	Bcl-2	Rabbit	Monoclonal (EP36)	1:50	Cytoplasmic	Negative	2+	Negative	Negative
6.	Proliferating cell nuclear antigen (PCNA)	Rabbit	Monoclonal (EP091)	1:50	Nuclear	Negative	Negative	Negative	Spleen - 4+
7.	CD3	Rabbit	Polyclonal (PP160)	1:25	Membranous, cytoplasmic	Negative	Negative	Negative	Spleen - 4+, Lymph node - 3+

1+ minimal; 2+ mild; 3+ moderate; 4+ marked; 5+ strong

whitish raised areas in the cardiac muscles and valves. The kidneys were smaller in size and capsules peeled-off easily. On incision, thinning of cortex and widened medulla were noticed. The urinary bladder contained numerous yellow coloured crystals of various sizes and its mucosa revealed thickening with mild congestion. The mucosal folds of stomach covered with mucous shreds and underlying mucosa revealed congestion. The rectal (8 cm) muscles were thickened due to whitish coloured growth and congestion in the underlying mucosa that causes narrowing of the lumen.

Microscopically, the tumour nodules were mostly encapsulated with few ill-defined borders and infiltration of neoplastic cells. The neoplastic cells were characterized by a mixed variant of HCC and ICC. The HCC characterized by the trabecular pattern of neoplastic cells with eosinophilic and vacuolated cytoplasm intertwined with fibrous tissues (Fig. 2). The pleomorphic neoplastic cells contained large, hyperchromatic and vesiculated nuclei, prominent nucleoli and multiple mitotic figures (25-30 mitoses per 10 high-power fields) (Fig. 3). Angiogenesis, invasion of neoplastic cells in the adjacent hepatic tissues and infiltration of mononuclear cells were noticed. The cystadenocarcinoma developing from ICC consists of many cysts lined by single or multiple layers of cuboidal or columnar cells (Fig. 4). The cystic lumen contained eosinophilic secretions with exfoliated cells and debris. The nuclei were spherical, hyperchromatic and had few mitoses.

The lungs revealed metastatic neoplastic cells in most of the alveoli, atelectasis in the adjacent alveoli and mononuclear cells infiltration in interstitial areas (Fig. 5). The mesenteric lymph node showed a diffuse area of metastatic neoplastic cells replacing the lymphoid cells in the medullary region (Fig. 6). The neoplastic cells infiltration noticed in spleen, heart, kidneys, urinary bladder, stomach and rectum were morphologically similar to the tumour cells found in the liver.

The IHC scoring in tumour tissues and lymphoid organs against various primary antibodies are presented in Table 1. The immunoreactivity of HCC showed mild cytoplasmic expression of *arginase-1* (Fig. 7), *Hep Par-1* (Fig. 8), *glypican-3* (Fig. 9), *AFP* (Fig. 10) and *Bcl-2* (Fig. 11) in the neoplastic cells. The normal hepatic cells showed a marked cytoplasmic expression of arginase-1, strong cytoplasmic expression of *Hep Par-1* and mild cytoplasmic expressions of *glypican-3* and *AFP*. The stromal tissues intertwined with the neoplastic cells showed minimal cytoplasmic expression of *arginase-1* and *Hep Par-1*. The spleen showed marked nuclear expression of proliferating cell nuclear antigen (PCNA) in the lymphoid cells (Fig. 12), and marked nuclear and membranous expressions of CD3 in the lymphoid cells of periarterial lymphoid sheath (Fig. 13). The mesenteric lymph node revealed moderate nuclear and membranous expressions of CD3 in the lymphoid cells (Fig. 14).

A rare variant of HCC and ICC was observed in a ten-year-old male Spitz dog. Available literature stated that the average age of the dogs affected with HCC is more than 10 years and frequency of occurrence is high in males[1]. According to previous workers, the most commonly reported clinical signs in affected dogs were hepatomegaly, anorexia, weakness, emaciation, difficulty in breathing and congested mucous membranes[1]. These unusual signs were attributed to the paraneoplastic syndromes and cause for cancer related death.

Grossly, multiple tumour nodules noticed on the surface of liver is

agreed to the findings of earlier workers[1,2,7]. The nodular variants were most commonly occurs as multifocal in single or all liver lobes. The nodules were coalescing together and efface to hepatic parenchyma[2,8]. The nodules were greyish-white, well-delineated, had central depressions and solid or cystic with increased stromal tissues[9].

Histopathologically, tumour nodules were characterised by the mixed variants of HCC and ICC. The nodules were encapsulated and invaded in few areas of hepatic tissues[1]. The neoplastic cells were organized into trabecular, cystic, adenoid, pleomorphic and mixed patterns indicate malignancy and aggressiveness[1]. The intrahepatic invasion into bile duct commonly manifested as tubular carcinoma[10] and bile duct cystadenocarcinoma[3]. The occurrence of ICC is less common, more malignant and has high frequency of metastasis in dogs[9]. The extra hepatic metastases to distant organs of this study is agreed to the findings of previous workers[1,2,7].

The immunoreactivity of neoplastic cells showed mild expression of *arginase-1*, *Hep Par-1*, *glypican-3*, *AFP* and *Bcl-2* are in agreement with earlier workers who used these markers for the diagnosis of well-differentiated HCC in humans and animals[5,6,11-13]. The normal hepatic cells showed marked expression of *arginase-1*, strong expression of *Hep Par-1* and mild expression of *glypican-3* and *AFP* are similar to the findings of previous workers[5,6,11-13]. The stromal tissues intertwined with neoplastic cells showed minimal expression of *arginase-1* and *Hep Par-1* is similar to the findings of earlier workers[5,6,11-13]. A mild expression in the neoplastic cells, marked expression in the normal hepatic cells and

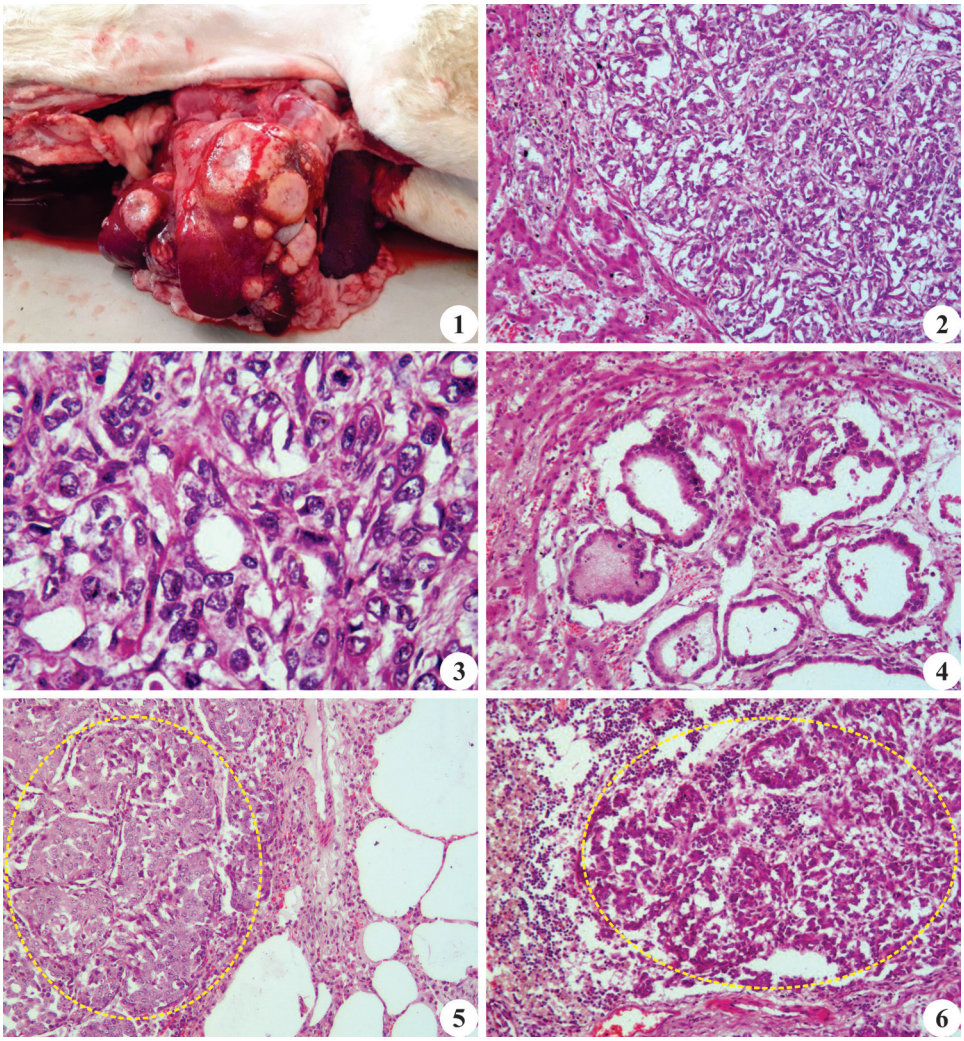

Fig. 1. Spitz: Hepatic tumour nodules showing pinkish-white in colour, well-delineated and central depressions; **Fig. 2.** Hepatocellular carcinoma showing encapsulation, ill-defined areas and invasion of neoplastic cells (H&E x100); **Fig. 3.** Hepatocellular carcinoma showing pleomorphic neoplastic cells with eosinophilic cytoplasm, vesiculated nuclei, prominent nucleoli and mitotic figures (H&E x400); **Fig. 4.** Intrahepatic bile duct cystadenocarcinoma showing many cysts lined by single or multiple layers of cuboidal or columnar cells (H&E x100); **Fig. 5.** Lung showing metastatic neoplastic cells (circle) in most of the alveoli and atelectasis in the adjacent alveoli (H&E x100); **Fig. 6.** Mesenteric lymph node showing metastatic neoplastic cells (circle) replacing the lymphoid cells in the medullary region (H&E x100).

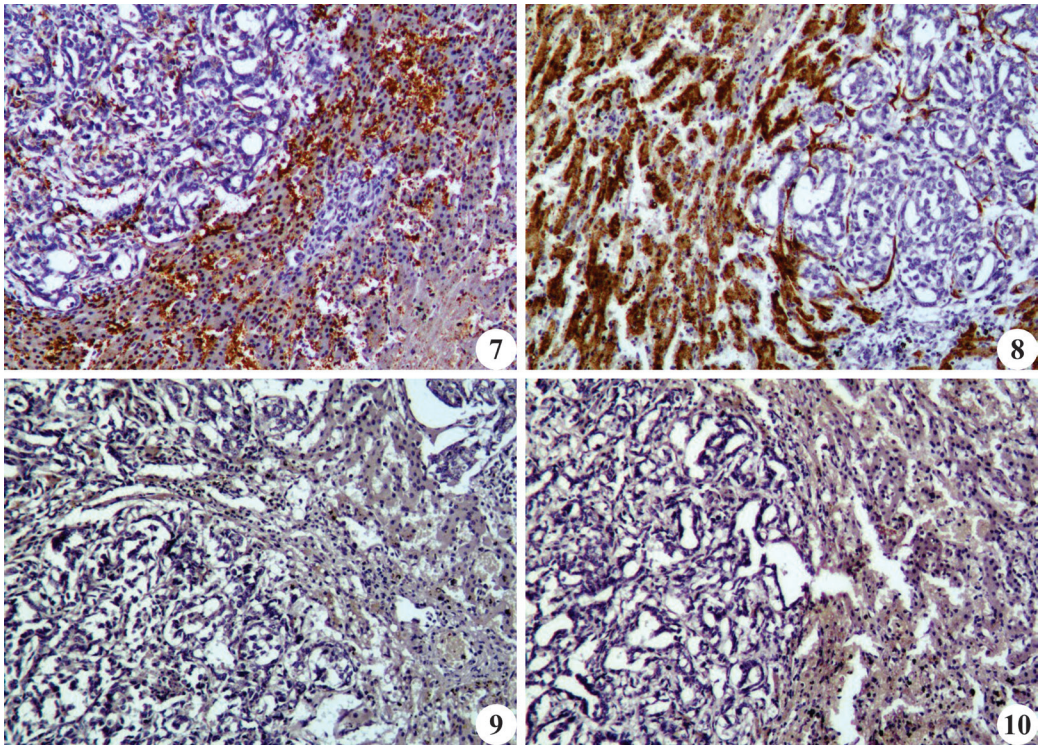

Fig. 7. Tumour section showing marked cytoplasmic expression in normal hepatic cells, mild expression in neoplastic cells and minimal expression in stromal cells to *arginase-1* (IHC, Arginase-1 x100); **Fig. 8.** Tumour section showing strong cytoplasmic expression in normal hepatic cells, mild expression in neoplastic cells and minimal expression in stromal cells to *Hep Par-1* (IHC, Hep par-1 x100); **Fig. 9.** Tumour section showing mild cytoplasmic expression to glypican-3 in the normal hepatic cells and neoplastic cells (IHC, Glypican-3 x100); **Fig. 10.** Tumour section showing mild cytoplasmic expression to AFP in the normal hepatic cells and neoplastic cells (IHC, AFP x100).

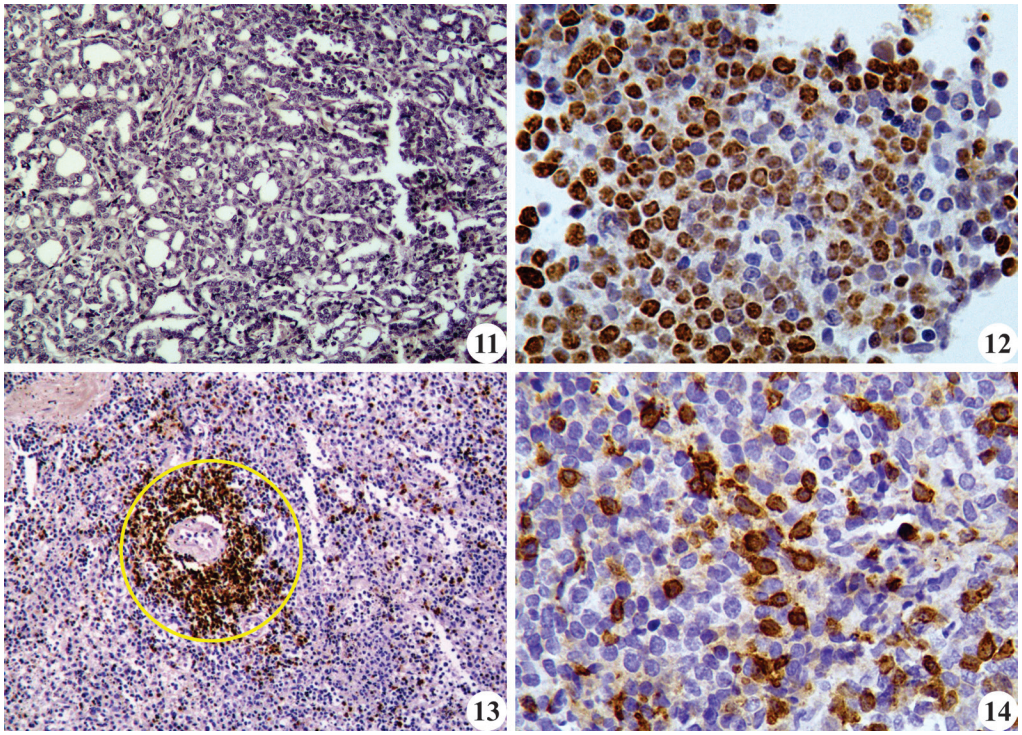

Fig. 11. Tumour section showing mild cytoplasmic expression to Bcl-2 in the neoplastic cells (IHC, Bcl-2 x100); **Fig. 12.** Spleen showing marked nuclear expression to PCNA in the lymphoid cells (IHC, PCNA x400); **Fig. 13.** Spleen showing marked nuclear and membranous expressions to CD3 in the lymphoid cells of periarterial lymphoid sheath (IHC, CD3 x100); **Fig. 14.** Mesenteric lymph node showing moderate nuclear and membranous expressions to CD3 in the lymphoid cells (IHC, CD3 x400).

minimal expression in the stromal cells revealed a mixed variant of HCC and ICC.

Spleen and lymph nodes showed marked expression of PCNA and CD3 in the lymphoid cells of this study are in agreement with earlier reports[14]. PCNA is a nuclear marker usually expressed in proliferating neoplastic and lymphoid cells. CD3 is a nuclear and membranous marker generally expressed in rapidly proliferating lymphoid cells. Expression of both markers in lymphoid organs shows an increased turnover as well as phagocytic activity of lymphoid cells against metastatic neoplastic cells. The intrahepatic invasion and extrahepatic metastases to distant organs shows the malignancy potentials of HCC. The pathological features of HCC and ICC will be useful for the early diagnosis and prognosis, hence the case is reported.

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