Malignant iridophoroma in a male Siamese fighting fish (*Betta splendens* Regan): A clinical, surgical and histopathological study

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Abstract

A male Siamese fighting fish (*Betta Splendens* Regan) was referred with a raised mass at the basis of the dorsal fin. Most of dorsal fin rays were missing. Surgical biopsy of the mass was performed in less than 2 min and macroscopically the mass appeared to invade the underlying musculature. The fish died after 29 days. Transverse sections showed that the primary mass had expansive growth and invaded the underlying muscles. Internal examination revealed the abdominal cavity to be filled with a secondary large mass. Also white spots were observed on the liver. Squash preparations of small section of the masses showed olive to green coloured pigments that were birefringent under polarized light. Histologically, the mass consisted of neoplastic iridophores arranged in bundles with moderate amounts of cytoplasm containing olive to green pigments that were birefringent with polarized light. In most areas, the neoplastic cells invaded the underlying muscles and bone trabeculae. Microscopic metastasis, with similar morphologic features to the primary tumour, were observed in the kidney, liver, spleen, and intestine. Nuclei of metastatic cells in the kidney and liver were round to ovoid with one to three nucleoli. These metastatic cells had birefringent intracytoplasmic olive to green pigments. A diagnosis of malignant iridophoroma was made.

Introduction

Dermal chromatophoromas or pigment cell tumours are common in fish and occurrences have been reported in both marine and freshwater species (Okihiro et al., 1992, 1993; Ramos et al., 2013). Melanoma is the most commonly reported pigment cell tumour in fishes (Camus et al., 2011). Guanophoroma in the wide-banded hardyhead silverside *Atherinomorus lacunosus* (Colorni, 1997) has also been described. The chromatophore tumours found in the pebbled butterflyfish *Chaetodon multicinctus* were predominantly iridophoromas (Okihiro, 1988). Iridophoromas have been reported in grayling *Thymallus thymallus* (Schmidt-Posthaus et al., 2005), rainbow trout *Oncorhynchus mykiss*

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(Bruno et al., 2013), goldring surgeonfish Ctenochaetus strigosus (Work and Aeby, 2014), Indian oil sardine Sardinella longiceps (Singaravel et al., 2016), and Indian mackerel Rastrelliger kanagurta (Singaravel et al., 2017). An iridophoric osteoma has been identified in a goldlined seabream Rhabdosargus sarba (Singaravel et al., 2018).

The Siamese fighting fish or betta (Betta splendens Regan) an anabantid fish, is popular worldwide due to its vibrant colours. Tumours in B. splendens have rarely been reported. Nephroblastoma in two male Siamese fighting fish (Lombardini et al., 2010) and invasion of melanoma to angioli-poma in a male Siamese fighting fish (Rahmati-Holasoo et al., 2015) are the only 2 reports of tumours described in Siamese fighting fish. This report is the first report of malignant iridophoroma in B. splendens.

Materials and Methods
In December 2016, a male B. splendens was referred to the Ornamental Fish Clinic, Faculty of Veterinary Medicine, University of Tehran. The fish was referred with a raised mass at the base of the dorsal fin. Most of the dorsal fin rays

Figure 1. Gross appearance of the lesion in Betta splendens. (a) Soft, whitish and raised mass (arrow) on the base of the dorsal fin; (b) Post-operative lesion on day 18; (c) Progressive bilateral abdominal distention and protrusion of scales between 20 and 28 days after removal of the tumour (arrows); (d) Necropsy showing secondary mass (SM) is seen in the kidney and white spots (arrow) are seen on the liver. Splenomegaly with pale color (arrowheads).
were missing (Figure 1a). Based on the owners’ information the fish had progressive anorexia. The fish was 2 years old at the time of referral and the mass had appeared 2 months prior to presentation.

The raised soft oval mass was well demarcated and whitish in color (Figure 1a). For surgery the fish was anaesthetised in 100 ppm PI222 (Pars Imen Daru, Iran). The fish was positioned in lateral recumbency on sterile wet gauze sponges. Surgical biopsy of the mass was performed in less than 2 min and macroscopically the mass appeared to invade the underlying musculature. Due to the size and invasive nature of the mass complete surgical excision was not possible. An electrosurgical device (Martin ME 50, intensity 1-10) with a bipolar active electrode was used for hemostasis. The electrosurgical device was set to intensity 3 to safeguard the underlying tissues, margins were delaminated following the scale shape then intensity was increased to 6.

For histological purposes, samples of the mass were preserved in 10% buffered formalin. Post-operatively, tetracycline (5 mg/L) was added to the tank water and retreated on day 3 after a 50% water change. After the initial treatment, 50% of tank water was changed on days 6 and 9. Following the excision of the mass, the wound was monitored until day 28.

After surgery the fish was anorexic but remained clinically normal until day 18 after surgery (Figure 1b). Bilateral abdominal distention and protruding scales were observed day 20 after surgery. These clinical signs were progressive until day 28 after surgery (Figure 1c). On day 29 the moribund fish with a prolapsed rectum was referred to the clinic. The fish died after 20 min and necropsy was performed under sterile conditions. For histological purposes, samples of the mass, underlying muscles, skin, liver, spleen, gills, heart, and gastrointestinal tract were dissected and preserved in 10% buffered formalin, dehydrated and embedded in paraffin with a paraffin tissue processor (DS 2080/H; Did Sabz Co.) and paraffin dispenser (DS 4LM; Did Sabz Co.), sectioned at 5 µm (Rotary Microtome RM2145; Leica) and stained with haematoxylin-eosin (H&E). Sections were observed by light microscopy (E600; Nikon) and representative images were taken using a microscope camera (uEye; UI-2250).

Results
Aerobic and anaerobic bacterial cultures, incubated at 25 °C from the liver and kidney mass, resulted in no growth. At necropsy, transverse sections showed that the primary mass had expansive growth and invaded the underlying muscles adjacent to the caudal part of the swim bladder. Internal examination revealed that the abdominal cavity was filled with a secondary large mass that appeared to encompass most of the visceral organs, including the cranial part of the swim bladder. It appeared that the secondary mass primarily involved the kidney, but suspicious white spots were observed on the liver (Figure 1d). Splenomegaly exhibiting a pale appearance was also observed (Figure 1d).

The microscopic study of the surgical biopsy showed the epidermis was normally structured at the sides of the mass and completely eroded on the surface of the neoplasm. The tumour consisted of neoplastic iridophores arranged in bundles with moderate amounts of cytoplasm containing olive to green pigments that were crystalline and birefringent with polarized light.
Figure 2. Histologic appearance of the iridophoroma in *Betta splendens*. (a-c) Histological sections of the primary mass shows tumour cells containing olive to green pigment, crystalline and birefringent with polarized light (arrowheads) invading between underlying muscles layers (M) and bone (B); Muscle fiber necrosis is also observed (N); (d) Metastatic lesion in the kidney. Note tumour cells with one to three basophilic nucleoli (white arrowheads) containing intracytoplasmic olive to green pigments (black arrowheads). Mitotic figures (black arrows). Hematoxylin and eosin (H&E).

Figure 3 (a-b) Diffuse metastasis of the neoplastic cells in the kidney of *Betta splendens*. Intracytoplasmic olive-green pigments are birefringent under polarized light. Hematoxylin and eosin (H&E).
Their nuclei were round to ovoid with one to three nucleoli. Histologically, the mass originated from the dermal pigment layer. Anisocytosis and anisokaryosis were moderate and mitotic figures were rare (0-2 per high-power field). In most areas, the neoplastic cells invaded the underlying muscles and bone trabeculae (Figures 2a, b and c).

Histological examination of transverse sections of the primary mass (after necropsy) showed that tumour cells had invaded the underlying layers adjacent to the spinal cord. Microscopic study showed that the secondary mass originated from the main lesion and penetrated into the kidney. Nuclei of metastatic cells in the kidney were round to ovoid with one to three nucleoli. These metastatic cells had birefringent intracytoplasmic olive to green pigments and mitotic figures (Figures 2d, 3a and b). Microscopic metastasis, with similar morphologic features to the primary tumour, were observed in the liver (Figures 4a and b), spleen, and intestine (Figures 4c and d). A diagnosis of

Figure 4. (a-b) Microscopic metastatic nodule in the liver of Betta splendens. Focal microscopic metastasis (M) is seen in the liver (L); Mitotic figure in metastatic cells (arrow) are seen. In the metastatic area, heavily pigmented cells containing olive to green granules (iridophores) (black arrowheads) are present. Neoplastic cells contain abundant amounts of birefringent, anisotropic crystalline material (white arrowheads) are observed under polarized light; (c-d) Intestinal epithelial cell necrosis (N). The metastatic area (arrowheads) in the intestine with intracytoplasmic clumps of olive-green pigments (arrowheads). Hematoxylin and eosin (H&E).
chromatophoroma with sub-classification as a malignant iridophoroma was made.

**Discussion**

Fish, like mammals, are frequently affected by neoplastic proliferations (Knüsel et al., 2007; Rahmati-Holasoo et al., 2018) and cutaneous neoplasms have been commonly reported in fish (Shokrpoor et al., 2018). The majority originate from chromatophors, several of which do not occur in mammals (Masahito et al., 1989; Okihiro et al., 1993). Unlike mammals, in which only the melanocyte imparts coloration to the skin, the skin of fishes, amphibians, and reptiles (lower vertebrates) contain multiple different pigment cells referred to collectively as chromatophores (Bagnara et al., 1968).

The skin of lower vertebrates is characterised by a wide pattern of colours. These cells are derived from the neural crest during embryonic development. Chromatophores are grouped into two distinct sub-classes, light-absorbing and light-reflecting, that reside in the dermis (Amiri and Shaheen, 2012; Lewis et al., 2015; Muñoz-Gutiérrez et al., 2016). In reptiles and fishes, chromatophores include xanthophores, erythrophores, melanophores and iridophores (containing birefringent purine crystals) (Schmidt-Posthaus et al., 2005, Muñoz-Gutiérrez et al., 2016). Iridophores are responsible for the silver or blue coloration and iridescence of the skin via diffraction of light using stacked plates. They contain large crystalline platelets that are composed of guanine and contain purines (Schmidt-Posthaus et al., 2005, Lewis et al., 2015). Iridophoromas are characterised at the light microscopic level by the presence of olive-green pigments which are birefringent with polarized light (Schmidt-Posthaus et al., 2005). Iridophoromas are whitish or metallic silver in color (Okihiro, 1988; Singaravel et al., 2016), which is consistent with the current case. Gross pathological examinations of the distended, invasive, metastatic and exophytic neoplastic growth led to the suggestion of the malignant character of this neoplasm. The histopathological findings of the present tumour are similar to those observed in a grayling *T. thymallus* (Schmidt-Posthaus et al., 2005), rainbow trout *O. mykiss* (Bruno et al., 2013) and Indian mackerel *R. kanagurta* (Singaravel et al., 2017) in having olive-green pigments that are birefringent with polarized light. Histologically, fish iridophoromas are usually locally invasive neoplasms and only rarely infiltrate adjacent skeletal musculature. Metastatic lesions have not been found in any internal organs (Okihiro, 1988). Slight infiltration of the neoplastic cells into the surrounding tissue was observed in the grayling *T. thymallus* iridophoroma without metastatic spread (Schmidt-Posthaus et al., 2005). However, malignant iridophoromas with metastases to visceral organs have been described in reptiles (Muñoz-Gutiérrez et al., 2016).

From the available reports, curative surgical excision has been most commonly used for treatment of chromatophoromas in reptiles (Irizarry-Rovira et al., 2006; Heckers et al., 2012), however, recurrence and metastasis to the skin and viscera has been reported (Heckers et al., 2012; Lewis et al., 2015). In fish, cryosurgery was effective in treating chromatophoroma in largemouth bass (Yaw et al., 2016).

Predisposing factors such as carcinogenic compounds, viruses, irritants, oncogenes and
parasites have all been suggested in teleosts and should be considered potential sources for tumour induction in teleosts (Stoskopf, 1993). Most of the chromatophoromas are of unknown etiology (Okihiro 1988; Camus et al., 2011). However, a combination of hereditary, carcinogenic, and age-related factors are postulated in fishes (Masahito et al., 1989). The correlation between environmental chemicals and neoplasia of the pigmented cells has been suggested in some species (Okihiro et al., 1993) and induced by chemical agents such as N-nitroso-N-methylurea in *Xiphophorus* hybrid fish (Kazianis et al., 2001). On the basis of the history, macroscopic and microscopic findings in this case, the cause of this condition remains unknown.

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**References**


Okihiro MS, Whipple JA, Groff JM and Hinton DE (1992). Chromatophoromas and related hyperplastic lesions in Pacific rockfish...
(Sebastes spp.). Marine environmental research 34, 53-57.


