Liposarcoma or invasive lipomatosis in flower horn fish, hybrid cichlid: clinical, radiological, ultrasonographical and histopathological study

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Abstract

Liposarcoma or invasive lipomatosis affecting three indoor aquarium fish (flower horn fish, hybrid cichlid) is characterized by the presence of mature adipocytes of variable sizes and by an invasive behaviour, which affected internal organs and eyes of all cases. Detailed macroscopic, radiological, ultrasonographical and histopathological features are presented. All fish had bilateral exophthalmia with some masses around the eyes. Ultrasonography confirmed the presence of hyperechoic masses in the eyes. Histopathology of all cases described the presence of variable-sized adipose cells in the eyes. The suggested diagnosis is well-differentiated liposarcoma or invasive lipomatosis. This is the first report of liposarcoma or invasive lipomatosis in flower horn fish, hybrid cichlid.

Keywords: flower horn fish, invasive lipomatosis, liposarcoma.

Introduction

The flower horn fish belongs to the Cichlid family. It is believed that this fish has been emerged through selective cross-breeding of three spot cichlid, Cichlasoma trimaculatum (Günther), guayas cichlid, Cichlasoma festae (Boulenger) and Jingang Blood Parrot, hybrid cichlid, in 1996 (Lin et al. 2008; Rahmati-holasoo et al. 2010, 2014b). This fish is becoming increasingly popular as a pet, especially in South-East Asia and Iran (Lin et al. 2008; Rahmati-holasoo et al. 2010, 2015).

Tumours arising from adipose tissue are classified as either benign (lipoma, infiltrative-invasive lipoma and angiolipoma) or malignant (liposarcoma) (Hendrick et al. 1998). Lipomas arise from mature adipocytes. Lipomas have been reported from a number of fish species including bream, Abramis brama (L.) (Mawdesley-Thomas & Bucke 1968), largemouth bass, Micropterus salmoides (Lacepède) (Mawdesley-Thomas 1972), black crappie, Pomoxis nigromaculatus (Lesueur) (Harshbarger 1972), southern bluefin tuna, Thunnus maccoyii (Castelnau) (Lester & Kelly 1983; Johnston et al. 2008), channel catfish, Ictalurus punctatus (Rafinesque) (McCoy et al. 1985), European eel, Anguilla anguilla (L.) (Easa et al. 1989a), striped mullet, Mugil cephalus (L.) (Easa, Harshbarger & Hetrick 1989b), common dab, Limanda limanda (L.) (Bruno, McVicar & Fraser 1991), striped seabream, Lithognathus mormyrus (L.) (Volpatti et al. 1998; Gómez 2009), northern bluefin tuna, Thunnus thynnus (L.) (Marino et al. 2006), European seabass, Dicentrarchus labrax (L.), (Marino et al. 2011), and molly, Poecilia velifera (Regan), (De Stefano, Bonfiglio & Montalbano 2012). Angiolipoma has been described in a male Siamese fighting fish, Betta splendens, Regan (Rahmati-Holasoo et al. 2014a). Liposarcoma is a malignant...
tumour originating from adipose cells. In humans, this tumour is one of the most common malignant mesenchymal tumours (Dei Tos 2000). Among domestic animals, liposarcoma has been most frequently described in dogs (Baez et al. 2004). Liposarcomas have rarely been diagnosed in fish (Harshbarger 2001). Liposarcomas in fish have been reported in dragonet, Callionymus lyra L. (Haddow & Blake 1933), striped bream, Lithognathus mormyrus (L.) (Volpatti et al. 1998), juvenile halibut, Hippoglossus hippoglossus (L.) (Bambir et al. 2000), and clownfish, Amphiprion ocellaris Cuvier (Sharon et al. 2014). This is the first report of liposarcoma or invasive lipomatosis in flower horn fish, hybrid cichlid.

Materials and methods

In August, November and December of 2013, three male flower horn fish, hybrid cichlid, were referred to the Faculty of Veterinary Medicine, University of Tehran. All of these fish had been obtained as a fry and maintained individually in an aquarium. Case 1 and Case 2 were referred with progressive bilateral exophthalmia, anorexia and abnormal buoyancy. Bilateral exophthalmia started from 2 months before referral time and changed from slight form to severe exophthalmia with some masses around the base of eyes (Figs 1a & 2a). Case 3 was referred with progressive anorexia, progressive bilateral exophthalmia, large holes in the head and a small mass on the base of left eye (Fig. 3a) and increased respiratory rate. Because of aggressive behaviour of flower horn fish, all cases had been kept lonely in a separated aquarium. Water parameters of all cases were in normal range (Temperature 28–30 °C, pH 6.8–7.4 and O₂ 7.7–8.1 mg L⁻¹) at the referral time. These cases had been fed with different commercial fish feed. Fish were anaesthetized in 100 mg L⁻¹ tricaine methane sulphonate (MS-222, Finquel®; Argent Chemical Laboratories) buffered with 200 mg L⁻¹ sodium bicarbonate. Wet smears of gills and skin were prepared. Standard digital radiographs (DirectView Classic CR System; Kodak) were taken in right lateral and dorsoventral (DV) projections. Ultrasonography (diagnostic ultrasound machine Voluson 730 Pro; GE Medical Systems) was performed from the left side of all fish. Because Case 1 and Case 2 had
severe exophthalmia (Figs 1a & 2a), we were able to prepare dorsal and transverse ultrasonographical view of eyes. Case 1 and Case 2 were killed, while 3 died after 1 h, and necropsy was performed under sterile conditions. For histological purposes, samples of eye, liver, kidney, spleen, gills, heart, urinary bladder and gastrointestinal tract were dissected and preserved in 10% buffer formalin, dehydrated and embedded in paraffin with 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).

Results

No parasites were observed in wet smears of skin and gills of all cases. Right lateral radiographs showed soft tissue mass in the abdominal cavity of all cases (Figs 1b, 2b & 3b), and based on the topography of the abdominal cavity, they were inferred to be liver. This was assumed that in the light of the presence of fat density around the liver we could see the liver in lateral radiographs. DV radiographs showed severe bilateral exophthalmia in Case 1 (Fig. 1c) and Case 2 (not shown), and no significant change was observed in Case 3. Ultrasonography of visceral organs showed that the liver of all cases had heterogeneous echotexture with hyperechoic parts (Figs 1d, 2c & 3c). These hyperechoic parts were more obvious in the hypoechoic liver of Case 2 (Fig. 2c). Also ultrasonography of visceral organs showed that the kidney of Case 1 (Fig. 1e) and spleen of Case 3 (Fig. 3c) were hyperechoic compared to the liver and kidney (Fig. 1e). The spleen (Fig. 3c) had small hyperechoic dots. In ultrasonographical images of eyes (Case 1 and Case 2), large hyperechoic structures (especially in right eye of Case 1 and left eye of Case 2) (Figs 1f, 2d & 4a) were observed in dorsal and transverse views. Bacterial cultures from multiple internal organs incubated at 25 °C resulted in no growth.

At necropsy, small to large multifocal and diffuse changes (Figs 3d & 4b) were observed in the liver of all cases and livers felt greasy to the touch. Post-mortem examination revealed a soft, white to yellowish fatty mass filling the abdominal cavity of all cases (Fig. 3d). In Case 1, white small...
Greasy wax-like areas were found on the ventral surface of posterior kidney (Fig. 4c). On the heart of Case 1 and Case 3 and spleen of Case 3, small white to yellowish spots were observed (Fig. 3d). Also, fatty mass were seen on the base of eyes of all cases. No internal parasites were observed in Case 1 and Case 2. *Hexamita sp.* in the intestine of Case 3 was found.

Histologically, lipocytes surrounded the internal organs and invaded the liver, eye and subcapsule of kidney.
kidney. In the liver of all cases, lipocytes were present in large areas (Fig. 5a) associated with localized hepatocellular atrophy and necrosis. These cells were also surrounding and within regions of pancreatic cells (Fig. 3e & 5a). Microscopically, lipocytes surrounded the kidney (Fig. 5b) and heart (Fig. 5c) of Case 1 and Case 3. Aggregation of atypical lipocytes in spleen of Case 3 (not shown) and subcapsule of kidney of Case 1 and Case 3 were observed (Fig. 3f & 5b). Related to the finding of exophthalmia, similar adipose cells surrounded the orbit and optic nerve (Fig. 4d) and invaded the adjacent muscular tissue (not shown). Also lipocytes were observed around the choroid, hyaline cartilage of sclera (not shown) and behind the retina (Fig. 2f). The lipocytes were characterized by variability in size; most were well-differentiated adipocyte-type cells. Some of the cells exhibited a single, large, clear and intracytoplasmic vacuole that displaced the nucleus to the periphery. There was no evidence of mitotic figures.

Discussion

Lipomas have been reported in a large number of fish species (Easa et al. 1989a,b; Marino et al. 2006; Gómez 2009; De Stefano et al. 2012). Dermis-associated lipomas have been reported to infiltrate muscle tissue (Hard, Williams & Lee 1979; McCoy et al. 1985; Johnston et al. 2008; Marino et al. 2011) without invading internal organs, and the occurrences were interpreted as benign but, in our study, the lipocytes invaded some internal organs and eye tissues. Lipomatosis, an overgrowth of mature fat, has been reported in freshwater ornamental fish, associated with mycobacterial infection (Novotny et al. 2010). Lipomatosis involves subcutaneous tissue and muscle, but has not been reported to involve nerves (Sharon et al. 2014). In humans, well-differentiated liposarcomas account for about 40–45% of all liposarcomas (Laurino et al. 2001). Mitotic figures are scarce in well-differentiated liposarcoma (Azumi et al. 1987; Yuri et al. 2011; Sharon et al. 2014) and were not observed in our study. We report the occurrence of what may be well-differentiated liposarcoma in three flower horn fish, hybrid cichlid. The simultaneous occurrence of the tumour in different, distant organs strongly suggests metastasis as also observed with clownfish, A. ocellaris liposarcoma (Sharon et al. 2014). Because well-defined cluster of lipoblasts with characteristic nuclear features was not evident in our case study, we cannot exclude a diagnosis of invasive lipomatosis. Nevertheless, this study is the first to suggest radiography and ultrasonography for diagnosis of liposarcoma or invasive lipomatosis in internal organs of fish. The aetiology of lipoma development is unknown. Errors in fat metabolism, endocrine or neurological disorders (Easa et al. 1989b), dysraphic status of bone (when lipomas arise close to the bone) (Marino et al. 2006) and exposure to N-methyl-N’-nitro-N-nitrosoguanidine (Chen et al. 1996) have been proposed as possible causes. In veterinary literature, vaccine-associated liposarcoma is a significant problem in veterinary medicine (Kabak et al. 2011).
The prognosis of liposarcoma is reserved, depending on the characteristics of local growth, histological structure and the risk of post-operative recurrence (Hendrick et al. 1998). In all of our cases, bilateral metastasis in eyes was seen and made it difficult to treat the fish.

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Conflict of interest
No conflict of interest declared.

References


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