



Short Communication

Histomorphology and immunohistochemistry of adenocarcinoma and fibroma in Indian oil sardine (*Sardinella longiceps* Valenciennes, 1847) from Palk Bay, South-east coast of India

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A single specimen of Indian oil sardine *Sardinella longiceps* was observed with wart-like appearances in three different regions of the fish: one on the premaxillary part, and other two on the skin and at the vent region respectively. Mucosal tissues were expunged and preserved in 10 % neutral phosphate-buffered formalin for histological examination. Based on the histopathological analysis, lesions were identified as adenocarcinoma and fibroma on the premaxillary part and skin, respectively. Based on the immunohistochemistry, vimentin and cytokeratin-like proteins were demonstrated, suggesting that the wart-like extrusions were fibroma and adenocarcinoma respectively.

[**Keywords:** Cytokeratin, Histopathology, Premaxilla, Tumour, Vimentin]

Introduction

The Indian oil sardine, *Sardinella longiceps* is one of the major pelagic resources contributing to about 6.6 % of the total marine fish landings of India during the year 2016. This species is a small pelagic, migratory, and planktivorous species found along the coastal waters¹. In general, tumours may be caused by various agents like viruses, bacteria, chemical carcinogens, inherent genetic factors and some biological toxins⁸. Over the past two decades, the reported incidences of tumours have increased in teleost fishes (51 %) compared to cartilaginous fishes (7 %)³. The cause-relationship of tumour development in fish is unknown⁴. Globally, the number of scientific reports on fish tumours showed an increasing trend during the past two decades. In India, researchers have observed and reported diverse type of tumors in fish e.g. epidermal papilloma in *Anabas testudineus*⁵, and

osteogenic fibroma in Indian cat fish *Wallagoattu*⁶, and osteoma in marine cat fish⁷ and epidermal tumours on a sciaenid species, *Johnius aneus*⁸. Various types of tumours have been reported in Indian oil sardine *S. longiceps* like neoplasia in the skin⁹, bronchioblastoma¹⁰, gastric tumours¹¹ and lymphocystis¹². Tumour tissues are characterized by a specific pattern of intermediate filaments (IFs), polypeptides, where cytokeratins are such intermediate filament-forming proteins that provide mechanical support and fulfil a variety of additional functions in epithelial cells. Cytokeratins are found in almost all epithelial cells. Vimentin has been suggested for the identification of myoepithelial cells in salivary gland tumours¹³. To the best of our knowledge, limited numbers of reports are present on immunohistochemical analysis using antibodies against vimentin and cytokeratin to study tumour tissues.

Materials and Methods

During November 2016 to February 2017, a single specimen of Indian oil sardine *S. longiceps* measuring 160 mm in total length (TL) and weighing 116 g was collected from shrimp trawl catches at Palk Bay, southeast coast of India (Lat. 9.286143° N, Long. 79.156079° E). The specimen was observed with wart-like neoplastic extrusions in three different regions of the fish: one on the premaxillary part, and other two on the skin and at the vent region, respectively. The specimen was identified as female and the stomach was in empty condition. The growth structures was dissected and preserved in 10 % neutral phosphate-buffered formalin. Later on, the tissues were, dehydrated and embedded in paraffin wax and sectioned at the size (5 µm thickness), stained with hematoxylin and eosin (H&E) for histopathological examination¹⁴.

Immunohistochemical analysis was performed on 3 to 4 micron paraffin embedded tissue sections on PolyL-lysin coated glass slides. After heat drying, sections were deparaffinized in xylene and subsequently rehydrated in gradients of ethanol. Antigen retrieval to unmask antigenic sites was done using 10 mM citric acid solution (pH 6) followed by a washing step with Tris buffered saline (TBS pH 7.6)

and incubated with primary mouse monoclonal antibodies (1:100 dilutions) against luminal cytokeratin (CK 7) and vimentin (V9 clone). The slides were washed with PBS and incubated with horseradish peroxidase polymer based secondary antibody (M/s Biogenex USA). The brown colour immunoprecipitate was visualized by treating slides with 3,3'-diaminobenzidine. The sections were counterstained with Harris hematoxylin than mounted with DPX and examined under microscope with image analyzer system. Positive and negative controls were included in both the reactions. The cytokeratin and vimentin were expressed as the number of cells with positive cytoplasmic staining per 100 counted cells. The presence of intermediate proteins (IP) like vimentin and cytokeratin were analyzed using the IH.

Results

Out of 1500 samples screened from trawl fishery, a single specimen (0.06 %) was observed with neoplastic growth at three different regions. Grossly, a hard tumour mass was observed on the premaxilla area, close to the vent region and also on the skin below dorsal fin (Fig. 1).

Histologically, the tumour from premaxillary region showed variable sized acinar to papillary structures lined by cuboidal to pleomorphic cells. The nuclei were hyperchromatic - diagnosed as adenocarcinoma (Figs. 2a, b). The skin and vent growth tissues showed fibrocytes arranged in different directions with collagen bundles. Based on the histological features, the neoplasms were identified as a fibroma (Fig. 3a). The fibroma showed red coloured collagen networks running in different directions after the use of picosirius red staining (Fig. 3b). The immunohistochemistry revealed that in adenocarcinoma, cytokeratin was observed in the cytoplasm of cells indicating the cells were of epithelial origin (Fig. 4). In fibroma, presence of vimentin was detected by staining of the cytoplasm of spindle-shaped cells (Fig. 5). The control tissue sections revealed negative staining. In the present

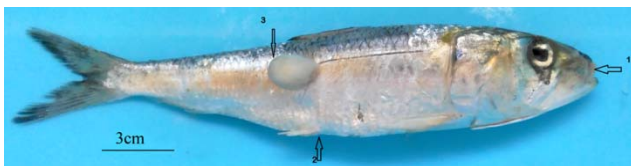


Fig. 1 — A hard round-shaped tumour mass on the premaxilla area (1), close to the vent region (2) and on the skin below dorsal fin (3)

study, two different kinds of tumours, adenocarcinoma at the premaxillary region and fibroma at the skin and near to the vent region were observed.

Discussion

In recent years, neoplasms have been described in a few specimens of marine fishes from Indian waters such as papilloma⁹; bronchioblastoma¹⁰; gastric tumours¹¹;

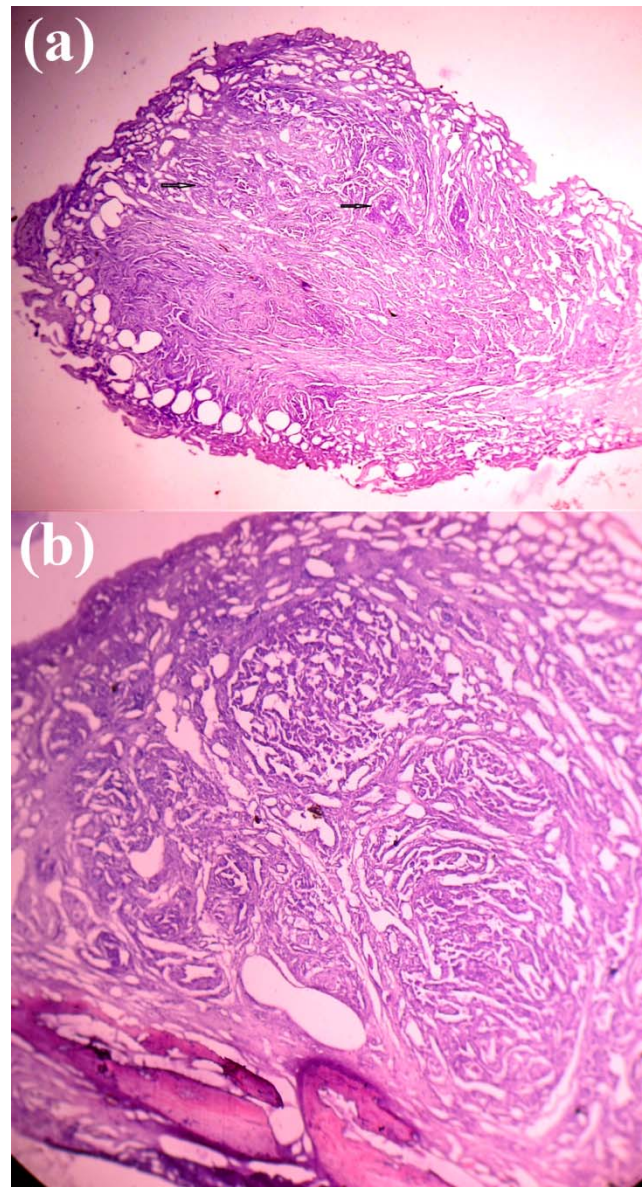


Fig. 2 — a) Variable sized acinar to papillary structures lined by cuboidal to pleomorphic cells. The nuclei are hyperchromatic Adenocarcinoma H&E x 100x; and b) Higher magnification. Variable sized acinar to papillary structures lined by cuboidal to pleomorphic cells. The nuclei are hyperchromatic H&E x400x

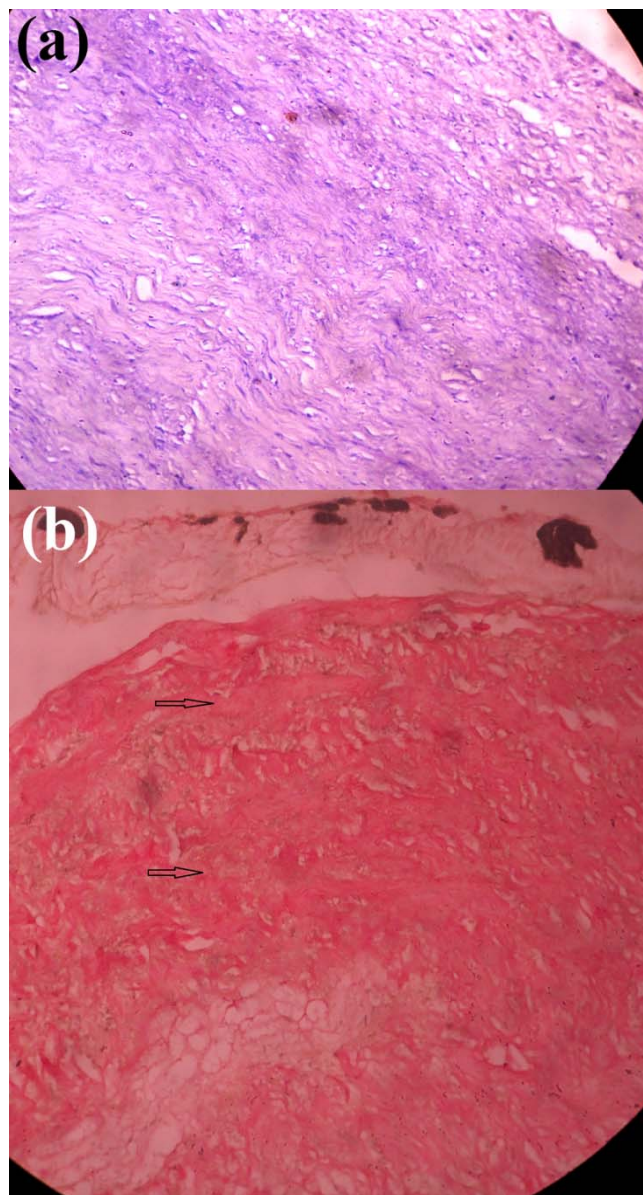


Fig. 3 — a) Fibrous connective tissue cells arranged in different directions like wavy fibres (H&E x100x); and b) The fibroma showed red coloured collagen networks running in different directions (Picrosirius red stain x 100x)

scleral fibrosarcoma and conjunctival squamous papilloma in *Sardinella longiceps*; Iridophoroma in Indian mackerel¹²; odontoma in *S. jello*¹⁵ and leiomyosarcoma in *Sphyraena jello*¹⁶ and also four types of neoplasm's reported in Asian sea bass (*Lates calcarifer*)¹⁶. Distribution in Indian oil sardine and barracuda along the south-east coast of India has been explored, and the prevalence of a skin tumour in oil sardine has previously been quantified as relatively low (between 0.35 % and 0.38 %), as compared to barracuda (between 0.42 % and 1.01 %) ³. In the

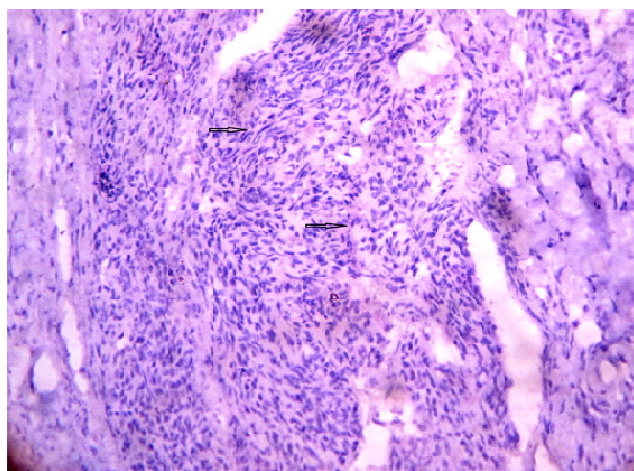


Fig. 4 — Adenocarcinoma, expression of cytokeratin in the cytoplasm of cells. Immunohistochemistry: DAB, counter stained by Harry's haematoxylin x 400x.

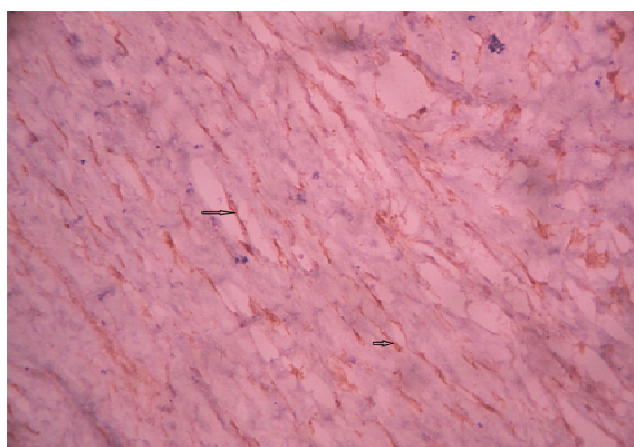


Fig. 5 — Fibroma revealed positive expression of vimentin in the cytoplasm of spindle-shaped cells. Immunohistochemistry: DAB, counter stained by Harry's haematoxylin x 400x

present survey, the incidence of a tumour was 0.06 % which was less than the previous observation. A stomach tumour has also been documented in the Indian oil sardine¹¹. The incidence of stomach tumour has been reported to be about 0.19 %, where four different types of tumours (Gastric adenoma, Myofibroblastoma, Lipoma and Fibrosarcoma) has been characterized without any local invasion and distant metastases. Benthic fish species are more prone to tumours when compared to pelagic species due to higher concentration of environmental carcinogens and/or exposure to infectious agents².

Conclusion

In the current study, histopathological analysis of the lesions, the tumors were identified as adenocarcinoma and fibroma on the premaxillary

part and skin, respectively. Based on the immunohistochemistry, the presence of vimentin and cytokeratin were demonstrated in fibroma and adenocarcinoma, respectively. However, a single observation of a single specimen with tumours may not be representative, necessitating a deeper survey of the occurrence of fish tumours. An elaborated study also requires higher numbers of a sample sizes.

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Conflicts of Interest

The author (s) declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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