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Anaplastic lymphoma kinase protein positive diffuse large B cell lymphoma; A developing world experience

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ABSTRACT

Anaplastic lymphoma kinase (ALK) positive diffuse large B-cell lymphoma (ALK + DLBCL) is a rare, distinct and aggressive subtype of non-Hodgkin's lymphoma (NHL). These tumors are considered to be derived from post-germinal center B cells but peculiarly their distinction is based on the fact that they are ALK-positive neoplastic B cells but lack expression of B cell markers (CD19,CD20, CD79a), T cell markers (CD3, CD5) and CD30. Its broad differential diagnosis and similarities to plasmablastic lymphoma, immunoblastic DLBCL, Anaplastic large-cell lymphoma (ALCL) of T-null cell lineage, and poorly differentiated/anaplastic carcinoma pose a grave challenge to physicians with conventional costly treatment for DLBCL failing to yield any clinical or prognostic significance in ALK + DLBCL. In this article we present 7 cases which were reported at Aga Khan University Hospital, Department of Pathology and Laboratory Medicine from 2009 to 2015 and a review of literature on ALK+ DLBCL, which according to the best of our knowledge is the second largest reported series and the first from South Asian subcontinent.

1. Introduction

Anaplastic lymphoma kinase (ALK) positive diffuse large B-cell lymphoma (ALK + DLBCL) is a rare, distinct and aggressive subtype of non-Hodgkin's lymphoma (NHL) first reported in a 7 case study by Delsol et al. in 1997 [1] and was recently included in the WHO classification of tumors [2]. These tumor cells are considered to be derived from post-germinal center B cells but peculiarly their distinction is based on the fact that they are ALK-positive neoplastic B cells but lack expression of B cell markers (CD19,CD20, CD79a), T cell markers (CD3, CD5) and CD30. [3] Due to its distinct morphologic and immunohistochemical features it is often misdiagnosed and often labeled as ALK+ Anaplastic large cell lymphoma (ALK + ALCL) which usually stains CD30 positive but is less aggressive and has a significantly better prognosis than ALK + DLBCL. Keeping in mind these unique features of ALK + DLBCL, conventional costly treatment for DLBCL (Rituximab + Cyclophosphamide, Hydroxydaunomycin, Oncovin, Prednisone) fails to yield any clinical or prognostic significance in ALK + DLBCL [3]. Therefore, it is of paramount importance that the clinician and histopathologist reflect broadly so that the clinical diagnosis is made efficiently and the proper treatment modality is used to improve patient morbidity. Hence forth, we present 7 cases which presented to Aga Khan University Hospital from 2009 to 2015 and a review of literature on ALK+ DLBCL, which according to the best of our knowledge is the second largest reported series and the first from the South Asian subcontinent.

2. Methods

We retrieved 7 cases of ALK Protein positive DLBCL from the surgical pathology files at Aga Khan University Hospital, Karachi, Pakistan. Patient consent was obtained from the patients in whom follow up was obtained. All specimens were fixed in 10% buffered formalin and processed routinely for paraffin wax embedding for light microscopy. Five-micron thick sections were stained with haematoxylin and eosin. Clinical data were noted from the surgical pathology reports of 5 referral cases and from patient files of two in patient cases. Follow-up was obtained by telephone communication with the patient's family of the referral cases.

For immunohistochemistry, the sections were dewaxed, rehydrated, and moistened with running tap water. The sections were pretreated in a microwave oven at 450W for 20 min with target retrieval solution of high pH; they were then incubated with antibodies on an automated immunostaining system (Dako autostainer plus Produkionsvej 42 DK-2600 Glostrup Denmark) for 25 min at

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Table 1
Clinical characteristics of reported cases (n = 7).

Case	Age/Sex	Primary site of lesion and biopsy site	Survival (months)
1	70/M	Cervical LN	1
2	26/M	Generalized LN	1
3	21/M	Generalized LN	4
4	40/M	Para-aortic LN + CSF	6
5	33/M	Parotid LN	Loss to FUP
6	49/M	Generalized LN and Hepatomegaly	2
7	60/M	Hepatomegaly and Cervical LN	4

LN lymph node; CSF cerebrospinal fluid; FUP follow up.

room temperature. Immunohistochemical studies were performed with the Flex technique using the following antibodies: Leukocyte common antigen (LCA), CD20, CD3, CD79a, CD30, CD138, CD56, CD117, Pax 5, Epithelial membrane antigen (EMA), Mum 1, Placental alkaline phosphatase (PLAP), MPO (myeloid peroxidase), Melan A, HMB 45, and ALK Protein.

Detection of ALK gene rearrangement was done by Fluorescence In Situ Hybridization (FISH) according to manufacturer's guidelines. A 4–5 μm thick paraffin section from patients' tumor block was deparafinized in xylene and then dehydrated with graded ethanol. Following incubation in 0.2N HCl for 20 min, slides were washed and then immersed in protease solution for 30 min and finally fixed in 10% buffered formalin. ALK probe cocktail (Vysis, Abbott, USA) and tissue section were co-denatured at 73 °C for 7 min in Thermobrite. The slides were incubated overnight at 37 °C and next day post washed in 2X SSC/0.3% NP-40 solution twice and counterstained with DAPI. The slides were observed under a fluorescent microscope.

3. Results

All 7 patients were male and age ranged from 21 to 70 years (mean 42.7 years). Almost half of the patients presented with generalized lymphadenopathy and weight loss (Table 1). Three patients also complained of abdominal pain. Cervical lymph node excision biopsy was received in 5 patients for primary diagnosis and lymph node core biopsy in 2 patients. The size of lymph nodes biopsies ranged from 1.5 to 4 cm (mean 2.1 cm). The core biopsy from para-aortic lymph node was initially misinterpreted as plasma cell neoplasm due to plasmablastic morphology and positive immunohistochemical stains for CD138 and Mum 1(Table 2). The diagnosis of plasma cell neoplasm was retained later on resection of intussuscepted bowel containing a fungating tumor of 4.5 × 4 cm. The corrected diagnosis was made 6 months later on a cervical lymph node biopsy.

Histologically, the lymph nodes showed effacement of nodal architecture by sheets of large monomorphic immunoblast-like cells filling the sinuses. These cells showed round pale nucleus containing large central eosinophilic nucleoli and abundant pink cytoplasm (Fig. 1A–B). Mitotic figures were easily appreciated within the tumor.Occasional binucleated cells and focal anaplastic cells were noted in few cases. A plasmablastic morphology with large cells exhibiting eccentric round clock face nucleus, and abundant pink cytoplasm is seen in one case (Fig. 1C). This case also showed focal emperipolesis (Fig. 1D). Extranodal extension was seen in two cases. The bowel wall involvement was full thickness, multinodular in configuration (Fig. 2A). Tumor cells were plasmablastic (Fig. 2B). Interestingly, the tumor cells had immunoblastic morphology in para-aortic lymph nodes involved by the tumor. Focal therapy related changes were noted in the bowel tumor showing sheets of histiocytes and necrosis (Fig. 2C,D). CSF involvement was also seen in this patient on cytology.

The tumor cells were negative for CD20 (0/7; Fig. 3A), CD79a (0/6), Pax 5 (0/2), CD3 (0/7; Fig 3B), CD56 (0/7),CD30 (0/7; Fig. 3C), CD117 (0/4), MPO (0/4),PLAP(0/1),Melan A and HMB45 (0/1) immunohistochemical stains. The tumor cells were positive for LCA (6/6; Fig 4A), EMA (6/6; Fig 4B), CD138 (6/6; Fig 4C) and Mum-1 (5/5; Fig. 4D). All 7 cases demonstrated diffuse granular cytoplasmic positivity for Alk protein (Fig. 5A).

ALK protein gene rearrangement was detected in 4/5cases by FISH (Fig. 5B). The test was failed in two cases. Follow up was available for 5 patients. All five patients died of disease within 1–6 months. Only two patients received chemotherapy.

4. Discussion

The 2008 edition of World Health Organization(WHO) Classification of Lymphoid Neoplasms has recognized ALK+ LBCL as a distinct entity of mature B-cell neoplasms. It is very rare and accounts for <1% of diffuse large B-cell lymphoma (DLBCL).[4] ALK + DLBCL is a relatively rare clinical entity with more than 134 cases cited in literature so far [5]. Unlike any other B cell Lymphoma, ALK + DLBCL derives its uniqueness based on the fact that they are ALK-positive neoplastic B cells, but lack B cell markers (CD19,CD20, CD79a), T cell markers (CD3, CD5) and CD30 markers. There has been no consensus on the age and sex predilection due to lack of major studies, but it is proposed that it has a male-to-female ratio of approximately 4:1 [6,7] and a mean age of 64 which is in agreement with our study in which all were male with an age range 21–70 years (mean 42.7 years). Among the lymphomas, ALK + DLBCL is one of the most aggressive subtypes and as seen in our cases, all of them had a mortality period within 6 months of diagnosis which is a dangerous sign and which makes the prompt diagnosis and correct management crucial in improving patient prognosis.

This ectopic ALK expression is expressed in two ways. The gene encodes a tyrosine kinase receptor located on chromosome 2p23 which translocates to one of two gene loci to result in fusion products with either Clathrin t (2; 17) (p23; q23) with CLTC/ALK rearrangement or Nucleophosmin t (2;5) (p23;q35) (7–9). This rearrangement is important as they both have distinguished appearances, with the former having a cytoplasmic and granular ALK staining pattern and the latter showing cytoplasmic and nuclear staining [8,9] which may aid in the early identification, however exceptions may arise and have been cited in literature [10–12].

The most common tumor location in our study was lymph node enlargement (4/7 cases). We also had 2 cases presenting as splenomegaly/hepatomegaly with one case diagnosed post CNS

Table 2
Immunohistochemical (IHC) profile of cases (n = 7).

Case	IHC markers (positive)	IHC markers (negative)
1	LCA, CD138,EMA, Alk Protein	CD20,CD79a,CD3,CD30,CD56
2	CD138,EMA, Alk Protein	CD20, Pax 5, CD3,CD30, CD56
3	LCA,CD138, EMA,Mum 1, Alk Protein	CD20,CD79a,CD3,CD30,CD56
4	LCA, CD138, Mum 1, Alk Protein	CD20,CD79a, Pax 5, CD10, CD3,CD30,CD117, MPO, CD56
5	LCA, CD138, EMA, Mum 1, Alk Protein	CD20,CD79a,CD3,CD30,MPO,CD117, CD56
6	LCA, Mum 1, EMA, Alk Protein	CD20,CD79a,CD3,CD30,MPO,CD117, CD56, Melan A, HMB45, PLAP, CD138
7	LCA,CD138, EMA, Mum 1, Alk Protein	CD20,CD79a,CD3,CD30,MPO,CD117, CD56