CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF ANAPLASTIC LARGE CELL LYMPHOMA

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ABSTRACT

Objective: To study the clinicopathological and immunohistochemical features of Anaplastic Large Cell Lymphoma (ALCL).

Study Design: The study design was cross sectional descriptive study.

Place and Duration of Study: The study was conducted in Armed forces institute of pathology. The duration of study was two years from 1st Jan 2010 to 31st Dec 2011.

Material and Methods: A total of twenty five consecutive biopsy proven cases of anaplastic large cell lymphoma (ALCL) were selected through non probability, consecutive sampling. The inclusion criteria was, all newly diagnosed patients of ALCL having sufficient tumour material in paraffin embedded tissue blocks with appropriate clinical information regarding age, gender and anatomic location. The exclusion criteria included all poorly fixed specimen. The clinical information regarding age, gender and location was noted. All the cases were evaluated on Haematoxylin and Eosin (H & E). Cases were subjected to Immunohistochemistry (IHC) using CD45 (LCA), CD3, CD 45 RO, CD 15, CD20, CD 30, ALK, EMA, Cytokeratin and classified according to WHO classification of lymphoid neoplasm.

Results: Twenty five cases of anaplastic large cell lymphoma were reported during this time period. Out of 25 cases, 22(88%) were ALCL ALK positive, 2(8%) were ALCL ALK negative and 1(4%) case was cutaneous ALCL. The male to female ratio was 2.5:1. The age range was between 6 years and 70 years with majority of cases in third decade. Seventy six percent were nodal and rest were extranodal. The cervical lymph nodes were the commonest nodal group involved making 15(60 %) cases followed by 3 (12%) cases of axillary lymph nodes. The histopathological appearance showed complete effacement of architecture in 17 (68 %) of cases followed by sinusoidal distribution in 6(24 %) cases while partial effacement of architecture in 2 (8%) of cases. All the cases were positive for CD30 while 23 (92%) cases for CD3, 22 (88 %) cases for ALK and 19 (76%) cases positive for EMA. ALK negative lymphomas were 3(12%) cases.

Conclusion: Anaplastic large cell lymphoma is more common in males and young adults. Nodal involvement is more common. Majority of cases show complete effacement of architecture. All cases are CD 30 positive. Most of cases are anaplastic lymphoma kinase antigen positive.

Keywords: Anaplastic large cell lymphoma (ALCL), Anaplastic lymphoma kinase (ALK), Non-Hodgkin lymphoma.

INTRODUCTION

Anaplastic large cell lymphoma (ALCL) was first described in 1985 by Stein et al as a unique lymphoma characterized by large pleomorphic lymphoid cells, often with horseshoe-shaped nuclei and a sinusoidal growth pattern[1]. It was defined originally by its expression of CD30 (Ki-1) antigen[2]. Therefore, ALCL was defined as a disease with a T cell or null-cell phenotype in the revised European-American lymphoma classification scheme[3]. Most of the cases of null cell type identified by immunohistochemical analysis or flow cytometric immunophenotyping (FCI) display clonal T-cell receptor gene rearrangements by molecular techniques, more definitively defining ALCL as a T-cell lymphoma[4]. The recent World Health Organization classification defines exclusively ALCL as a subtype of peripheral T-cell lymphoma[5]. Anaplastic large cell lymphoma (ALCL) showed strong and uniform expression of CD30[6-8] .The CD 30 is expressed by a wide spectrum of neoplasms ranging from benign cutaneous lymphoproliferative disorders to systemic lymphoma[9,10]. In a worldwide survey primary systemic ALCL accounted for 2.4% of non-Hodgkin lymphoma diagnoses but represents a greater fraction of non-Hodgkin lymphomas in children[11,12].

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Based on the presence or absence of aberrant expression of the anaplastic lymphoma kinase (ALK) gene located on chromosome 2, two forms of ALCL are recognized. Anaplastic lymphoma kinase (ALK) protein is expressed by approximately 70% of ALCLs as a result of chromosomal abnormalities involving the ALK gene. The t (2;5)(p23; q35) is the most frequent genetic abnormality.

Involving the nucleophosmin (NPM) gene on chromosome 5 and the ALK gene on chromosome[2]. The ALK gene encodes a tyrosine kinase receptor that is not expressed in normal lymphoid cells but expressed normally in the small intestine, testis, and central nervous system[13]. Anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL) is recognized as a distinct entity in the 2008 World Health Organization (WHO) classification of malignant lymphomas[14].

With the help of immunohistochemistry ALCL can be differentiated to ALCL ALK positive, ALCL ALK negative and Cutaneous ALCL as it is not possible on morphology only. The expression of ALK predicts good clinical outcome in ALCL. The overall 5-year survival in ALK-positive ALCL is better than for ALK negative cases. In contrast, anaplastic large cell lymphoma ALK negative patients have been reported to have poor clinical outcome[14,15]. Unlike ALCL ALK positive, the peak incidence of ALCL ALK negative is in adults (40-65 years) without a clear male or female preponderance. Patients present with peripheral and/or abdominal lymphadenopathy and/or extranodal tumor, although extranodal involvement is less common than in ALCL ALK positive[16]. The expression of ALK can be seen in both nodal and extranodal ALCLs, with the exception of the primary cutaneous subtype in which ALK expression is rarely seen. In most cases, virtually all neoplastic cells show strong CD30 staining on the cell membrane and in the Golgi region[17]. The majority of ALCLs are positive for EMA[14]. The great majority of ALCLs express one or more T-cell antigens and/or NK cell antigens[18,19]. However, due to loss of several pan T-cell antigens, some cases may have an apparent "null cell" phenotype. CD3, the most widely used pan T-cell marker, is negative in more than 75% of cases[18].This tendency for loss of CD3 is also seen in ALCL, ALK-negative. As there is no study regarding ALCL, in our population. The purpose of this study is to determine the frequency of ALCL subtypes on the basis of immunohistochemistry with clinicopathological presentation. This will also help us to know the prognosis of disease, as ALK positive have good prognosis.

MATERIAL AND METHODS

This cross-sectional descriptive study was conducted at Armed Forces Institute of Pathology (AFIP) from Jan 2010 to Dec 2011. The inclusion criteria was, all newly diagnosed patients of ALCL having sufficient tumour material in paraffin embedded tissue blocks with appropriate clinical information regarding age, gender and anatomic location. The exclusion criteria included all poorly fixed specimens. A total of twenty five consecutive biopsy proven cases of anaplastic large cell lymphoma were selected through non-probability after approval of ethical review board of AFIP, consecutive sampling. The pertinent clinical information of all the patients was noted for age, gender, site of the tumour which was available with the patient's request at the time of registration of biopsy samples. The cases were classified according to WHO classification. All the biopsied samples taken from nodal or extranodal sites were fixed in 10% neutral buffered formalin and processed for paraffin embedding, sectioning and staining by H and E and special stains. Immunohistochemistry (IHC) was done on 5Um thick sections of representative tumour areas, of all the cases. Histological slides were deparaffinized in xylene followed by target retrieval of histological sections with target retrieval solution in water bath at 95ºC for 40 minutes. Background quenching in all specimens was performed by 3% H_2O_2 for 10 minutes. The antibodies used were CD45/ LCA, CD 3, CD 15, CD 20, CD 30, Alk, EMA and CD 45 RO. The panel of primary antibody was decided on histomorphology. Primary antibody was incubated for 1 hour in optimized dilution at room temperature. IHC detection

was performed. Positive control slides were included with each batch. Slides were examined for the presence of nuclear/ cytoplasmic/ membranous staining (depending on the location of the positivity) within the tumour itself. Each case for IHC was evaluated by three histopathologists separately. A disagreement was resolved by joint review on multi-head microscope and a final consensus was established in each case. Data was analyzed on SPSS (version 17.0). Gender, immunohisto-chemical evaluation (types of ALCL) and anatomic location (site of ALCL) were computed in terms of frequency and percentages. Age (years) was presented as mean and standard deviation (SD).

RESULTS

Twenty five cases of anaplastic large cell lymphoma were reported during the study period. Out of 25 cases, 18 (72%) were males and 7 (28%) females. The male to female ratio was 2.5:1. The age range was 6 to 70 years. Thirteen cases (52%) were in second and third decades. The mean age of all ALCL patients was 34 ± 17.2 years, with median being 30 years. The site of ALCL in 19 (76%) cases was nodal and 6 (24%) were extranodal. ALCL with nodal presentation showed cervical lymph node involvement in 15 (60%) cases followed by 3 (12%) cases in axillary lymph node. The site distribution of extranodal origin was observed as, 2 (8%) cases from bone. Moreover, skin and nose involvement was also noted in 1 (4%) case each. The histomorphological features including architecture, necrosis, individual cell morphology, background and their frequency are given in table-1. (Fig-1).

Table-1: Histomorphological features of ALCL.

Histological pattern	Features	Cases frequency (%)
Architecture	Sinusoidal pattern	6 (24)
	Partial effacement	2 (8)
	Diffuse effacement	17 (68)
Necrosis	Necrosis seen	15 (60)
	Necrosis not seen	10 (40)
Morphology	Large cells with vesicular nuclei	10 (40)
	Large cells with vesicular and horse shoe shape nuclei	13 (52)
	Large cells with multinucleated nuclei	2 (8)
Background	Mature lymphocytes, plasma cells and eosinophils	17 (68)
	Mature lymphocytes, plasma cells and karyorrhexis	8 (32)

The subtype of ALCL included 22(88%) cases of ALCL ALK positive (fig-2 & 3), 2 (8%) cases were ALCL ALK negative and 1(4%) case was cutaneous ALCL. ALCL ALK positive cases were mostly in young adults in second and third decade while ALCL ALK negative and cutaneous ALCL presented in old age. ALCL ALK positive presenting in nodal site were 77% and 23% of cases were in extranodal sites. The most common nodal site for ALCL ALK positive was cervical lymph node in 59% cases. ALCL ALK negative cases presented in cervical lymph node only. The ALCL ALK positive cases were more in males (73%). ALCL ALK negative cases were seen in older males and cutaneous ALCL was seen in older female. According to immunophenotypic profile, LCA and CD 30 were positive in all cases and there was no case of null cell phenotype. (Table-2).

Table-2 : Immunoprofile of ALCL (n=25).

Immuno Marker	Positive Frequency(%)	Negative Frequency (%)
LCA	25 (100)	0 (0)
CD 3	23 (92)	2 (8)
CD30	25 (100)	0 (0)
EMA	19 (76)	6 (24)
ALK	22 (88)	3 (12)

DISCUSSION

The recent World Health Organization classification defines ALCL exclusively as a subtype of peripheral T-cell lymphoma. The ALCL can be either ALK positive, ALK negative or cutaneous ALCL[5]. Immunohistochemical techniques are the mainstay in the final diagnosis and immunophenotypic characterization of ALCL. The characteristic CD30 (Ki-1) marker can help differentiate ALCL from most diffuse large B-cell lymphomas and peripheral T-cell lymphomas. The ALK expression is an important prognostic indicator, warranting immunophenotypic, cytogenetic, or molecular testing for the ALK antigen in all cases of ALCL[15]. Immunohistochemistry has key role to label ALCL as ALK positive and ALK negative because it is not possible to differentiate these lymphomas on the basis of morphology alone.

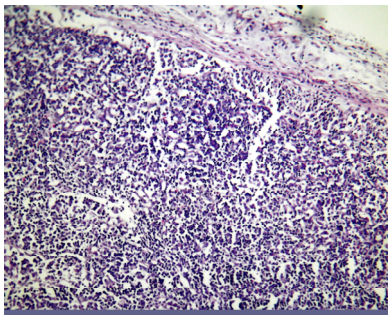

Figure-1: ALCL in Lymph node with sinusoidal pattern.

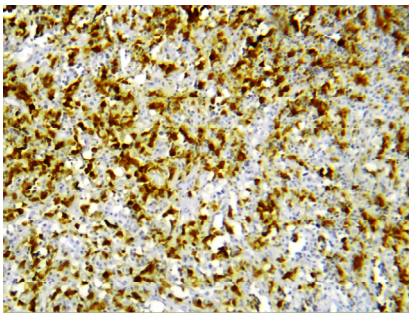

Figure-2: CD 30 positivity in ALCL.

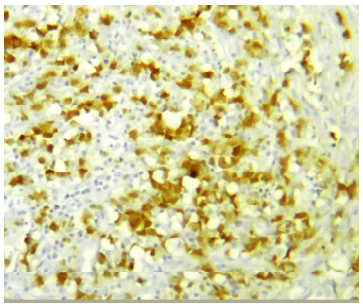

Figure-3: ALK positivity in ALCL.

In a study Mushtaq et al in 2008[21], ALCL was 2.5% of all nonHodgkin's lymphomas. In another study by Mushtaq et al[2] in 1994 and Noor et al in 2004[8], ALCL was more common in young age and males. The study also showed that cervical lymph node was the most common site and CD 30 was positive in all cases and these results were similar to present study. In a study by Delsol et al in 2002, ALCL ALK positive represent 50% to 80% of all ALCL with the relative frequency highest in young patients[5] . Present study showed 88% of ALCL were ALCL ALK positive with relative frequency is highest in young patients. The EMA expression in the present study was also slightly more than already reported[7].

Falini et al in his study showed that ALCL ALK positive cases were mostly present in second and third decades and male to female ratio was 6.1:1.6. In our study ALCL ALK positive were also more common in second and third decades. The male to female ratio was 2.6:1. In a study by Benharroch et al, CD 3 was negative in more than 75% of ALCL ALK positive[19], with great majority of ALCLs express one or more T-cell antigens and/or NK cell antigens but due to loss of several pan T-cell antigens, some cases may had an apparent "null cell" phenotype. This tendency for loss of CD3 was also seen in ALCL, ALK-negative, while in present study 92% cases were CD 3 positive and rest of cases were CD 45RO positive with no case of null cell phenotype.

A study by Stein et al demonstrated that ALCL ALK negative type was more common in old age and male to female ratio was equal. Nodal sites were more common than extarnodal[17]. In the present analysis, only a small percentage were ALCL-ALK negative and were in old age males at nodal sites. Cutaneous ALCL was more in old age males (present study had female patient) and are mostly ALK negative[10].

CONCLUSION

Anaplastic large cell lymphoma is more common in males and young adults. Nodal involvement is more common. Majority of cases show complete effacement of architecture. All cases are CD 30 positive. Anaplastic large cell lymphoma ALK positive is the most common subtype of Anaplastic large cell lymphoma.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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