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# ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): A CLINICOPATHOLOGIC STUDY ALONG WITH IHC PROFILE.

**Pathology**

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## ABSTRACT

**INTRODUCTION**: Anaplastic large cell lymphomas (ALCLs) are T-cell lymphomas characterized by large pleomorphic cells,and expression of CD 30. Based on ALK expression can be categorised as ALK+ve and ALK -ve.

**OBJECTIVES:** To study the clinicopathological profile of all diagnosed cases of ALCL, along with morphology and immunohistochemistry displaying different patterns of ALK staining.

**STUDY DESIGN:** All cases diagnosed as ALCL on histomorphology and CD 30+, ALK +ve or ALK –ve from 2001 to 2015 were studied. The histomorphology along with IHC were assessed. The clinical details were obtained from the patients files.

**RESULTS:** The 51 cases included (ALK+ =28; ALK- =13; ALK unavailable=10).The median age of presentation in ALK+ cases was 29.5years while 46.5 years in ALK-ve. There was a male predominance in both the subgroups with M: F (ALK+=2.1:1; ALK- =1.6:1). Most common presenting complaint was lymphadenopathy (90%), cervical lymphadenopathy being most frequent. Extranodal involvement was seen in 17 cases (33.3%) which included soft tissue (5), skin (4), and one case each from lung, appendix, colon and testis. Other symptoms included fever, loss of weight, and pleural effusion. The classic variant was the most common histological subtype (39), followed by Hodgkin-like (6), Lymphohistiocytic variant (3), small cell variant (2), composite variant(1). Of the 28 ALK+ ve cases, nuclear and cytoplasmic positivity was seen in 19 cases.

**CONCLUSION:** ALK +ve ALCLs were more common in < 30yrs age group and ALK –ve ALCLs were more common in 30-60yrs age group. As the condition has a very varied histology and numerous close differentials include Hodgkins lymphoma, DLBCL, peripheral T cell lymphoma, Burkitts lymphoma, Langerhans cell histiocytosis and metastatic carcinoma . correlation of histomorphology along with an extensive panel of IHC is required to make the diagnosis.

## KEYWORDS

Anaplastic Large Cell Lymphoma, ALCL, Immunohisochemistry, ALK, CD30, EMA.

**Introduction**:

Anaplastic large cell lymphoma (ALCL) is a high grade T-cell lymphoma comprising of lymphoid cells that are large, with abundant cytoplasm and pleomorphic nuclei [1]. ALCL accounts for 2–3% of all NHLs and 12% of T-cell NHLs. ALCLs have 'hallmark cells', which are typically large with eccentric horseshoe-shaped or kidney-shaped nuclei and are seen in all variants. CD30, a member of the tumor necrosis receptor family, is believed to be universally expressed in ALK+ve ALCL in an intense membranous and Golgi pattern. There is a characteristic translocation t (2; 5) (p23; 35) wherein the anaplastic lymphoma kinase (*ALK*) gene on chromosome 2 fuses with the nucleophosmin (*NPM*) gene on chromosome 5, resulting in NPM-ALK chimeric protein with constitutive tyrosine kinase activity[2]. ALK expression can be demonstrated in 50% to 85% of all systemic ALCLs[3].

ALK+ve ALCL mainly affects young male patients and, if treated with chemotherapy, has a favorable prognosis. It constitutes 20% to 30% of NHLs in children and adolescents and usually presents as widespread disease involving lymph nodes and extranodal sites [4, 5]. However, involvement of bone marrow (BM) and peripheral blood (PB) is unusual. Though ALK+ve ALCL is of T-cell origin, CD2, CD5 and CD4 are more commonly expressed and CD 3 may be negative in 75 % cases. However, dual-negative cases also exist.[6]

ALK-ve ALCL occurs in older patients, affecting both genders equally and has an unfavorable prognosis.

The clinical presentation and the morphologic spectrum of ALCL vary according to the population studied. As there are very few case series from India [7], we undertook this study. It was done to get a better understanding of the clinical and morphologic profiles of ALCLs in a developing country like India. To our knowledge, this is one of the largest case series with 51 cases from a single centre.

**Material and Methods:**

All newly diagnosed cases of ALCL, from January 2001 to January 2015, were included in the study. This study was approved by the Institutional Ethical Committee. The histopathology slides stained with Hematoxylin and Eosin were retrieved and reviewed. The clinical data with respect to age, presentation, imaging features and follow-up details were obtained from medical records of the patients.

The histomorphology and IHC were assessed based on Morphologic variants,Presence or absence of necrosis, Nuclear features (embryonated, horse shoe bi/multinucleation), background inflammation, Perinodal extension, ALK staining patterns (nuclear , nucleolar, cytoplasmic), CD 30 staining patterns (golgi, membrane , cytoplasmic).

**Lymph node biopsy – Histological preparation:**

All tissues for histological evaluation were fixed in 10% neutral buffered formalin and routinely prepared for Hematoxylin and eosin (HE) staining. IHC with CD3, CD20, LCA, CD15, CD30, ALK, EMA, CK, and Ki67 were done to differentiate ALCL from its close mimics.

**Bone Marrow aspiration and biopsy:**

The bone marrow aspiration and biopsy slides, done as part of staging work-up, for, were reviewed. Aspiration slides were air dried, fixed in methanol and stained with Giemsa stain. Bone marrow biopsy sections were stained with Haematoxylin and Eosin stain.

**Results:**

***Demographic and clinical aspects:***

During the study period, (2001-2015), of the 51 cases of ALCL (ALK+ =28; ALK- =13; ALK results not available=10). There was a male predominance in both the subgroups with M: F (ALK+ve =2.1:1; ALK-ve =1.6:1). The median age of presentation in ALK+ cases was 29.5 (age range=2-67 years, with 81% cases < 30years of age),

whereas in the ALK-ve subgroup, the median age of presentation was 46.5years (age range =11-65 years, only 15.3% cases <30 years).

Statistical Analysis of variance (ANOVA) was performed between three different age groups for ALK positive expression and found statistical significance ($p<0.002$) with the age of < 30 years.

The most common presentation was lymphadenopathy seen in 90% of cases in both subgroups, of which the most commonly involved nodes were the cervical lymph nodes (ALK+ve=18;ALK-ve =5). The other regions involved were retroperitoneal (ALK+=5; ALK- =2), followed by axillary, supraclavicular and inguinal lymph nodes. Involvement of multiple lymph node groups were seen in 14 cases (ALK+=11; ALK-VE=3). Hepatosplenomegaly was only seen in 9 cases. Other presenting symptoms included fever (ALK+=26; ALK-=10 cases ) , loss of weight (ALK+=15;ALK-=6 cases) , pleural effusion (4 cases).

Extranodal involvement was seen in 17 cases (33.3%) of which 10 cases (ALK+=8; ALK-=2) presented as primary extranodal disease. The extranodal sites of involvement included soft tissue (5), cutaneous ALCL (4), and 1 case each involving buccal mucosa, subcutaneous nodule, vertebrae, bone marrow, lung, appendix, colon and testis.

***Histopathological aspects:***

Histopathology slides were reviewed in all the cases. The classic or common variant was the most common histological subtype noted in 39 cases (ALK+=21; ALK- =9) followed by " Hodgkin like" - 6 cases (ALK+=2; ALK- =4), "Lymphohistiocytic variant", "small cell variant", "composite variant" . (Table 1).

The classic variant showed diffuse and sinusoidal pattern .The anaplastic cells resembled the characteristic "Hallmark cells" with embryonated and reniform nuclei.The Hodgkin like variant resembled the nodular sclerosing variant however there were no RS cells and the large cells were negative for CD15. The lymphohistiocytic variant comprised of dense aggregates of histiocytes among which the anaplastic cells with characteristic nuclei were dispersed. The small cell variant was composed of sheets of small to medium sized atypical lymphoid cells which were positive for CD30 and ALK. There was a single case of composite variant which shared components of both Hodgkin-like and lymphohistiocytic variant.

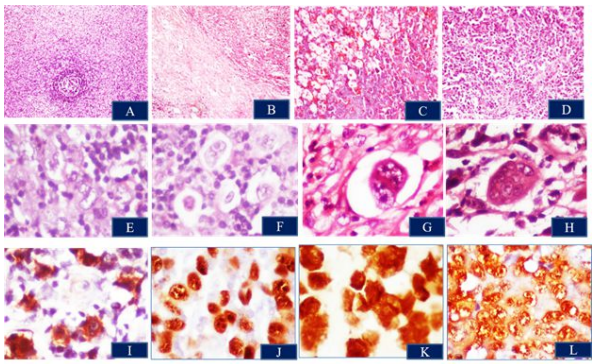



Figure 1: Morphological variants (A) classic variant with involvement by sheets of atypical lymphoid cells (×100) . (B) Hodgkin -like variant with predominant sclerosing component (×100). (C) Lympho-histiocytic variant (×100).(D) Small cell variant (×100) (E) classic embryonated nuclei (×400) .(F) Reniform nuclei and hall mark cells (×400). (G) Binucleated cells with prominent nucleoli resembling RS cells (×400). (H) multinucleated cells resembling tumor giant cells (×400). Immunohistochemistry showing (I) CD30 cytoplasmic positive. (J) ALK stain nucleolar positive, nuclear and cytoplasmic positive (K) and nuclear, cytoplasmic and nucleolar positive (L).

Immunohistochemistry with ALK was done in 41 cases (in 13 cases ALK was not available due to financial constraints faced by the patients). ALK showed strong nuclear and cytoplasmic positivity in majority of the cases (19 cases) followed by only nuclear (5), only cytoplasmic (3), membranous with nuclear and nucleolar (1).

CD30 and EMA were done in all the cases and both were positive in all the cases. The Pan T cell marker (CD3) and B cell marker (CD 20) were done in 39 and 40 cases respectively. CD3 though being a pan T cell marker was expressed in very less few cases (5/20) (25% of ALK+ve cases); (2/9) (22%) of ALK-ve). CD20 was negative in all the cases .CD 15 was done in 13 cases to rule out Hodgkins lymphoma and it was negative in the large cells. The remaining IHC panel included CK, HMB 45, and Vimentin, to differentiate ALCL from metastasis, in view of the sinusoidal pattern.

**Table 1.** Comparison of data of current study with other studies based on the morphologic variants of ALCL.

| | Laxmaiah et al (n=50)2013 | | Savage et al (n=159)2008 | | Current study (n=51)2015 | |
|---|---|---|---|---|---|---|
| **ALK status (% of cases)** | ALK+ve 30(60) | ALK-ve 20(40) | ALK+ve 87(55) | ALK-ve 72(45) | ALK+ve 28(55) | ALK-ve 13(25) |
| **Classic** | 27(90) | 18(90) | 71(82) | 50(70) | 21(75) | 9(70) |
| **Hodgkin like** | - | 1(5) | 6(7) | 10(14) | 2(7) | 4(30) |
| **Lymphohistiocytic** | 1(3) | - | 2(2) | 5(7) | 2(7) | - |
| **Small cell** | 1(3) | - | 5(6) | 3(4) | 2(7) | - |
| **Spindle cell** | 1(3) | - | 2(2%) | 2(3%) | - | - |
| **Giant cell** | - | - | - | 2(3) | - | - |
| **Composite** | - | - | 1(1) | - | | |

**Table 2 :** Comparison of data of current study with other studies on the immunohistochemical stains perfomed.

| IHC stain | | Lakshmaiah et al 7 (n=50)2013 | | Savage et al (n=159)20089 | | Current study (n=51)2015 | |
|---|---|---|---|---|---|---|---|
| **ALK** | ALK+ve 30(60) | ALK-ve 20(40) | ALK+ve 87(55) | ALK-ve 72(45) | ALK+ve 28(55) | ALK-ve 13(25) | ALK u/a 10(20) |
| **CD30** | 30/30 (100) | 20/20 (100) | 87/87 (100) | 72/72 (100) | 28/28 (100) | 13/13 (100) | 10/10 (100) |
| **EMA** | 18/21 (86) | 8/17 (47) | 72/87 (83) | 31/72 (43) | 12/14 (91) | 11/11 (100) | 10/10 (100) |
| **LCA** | 13/14 (93) | 7/8 (88) | - | - | 7/9 (78) | 8/9 (89) | 2/2 (100) |
| **CD3** | 4/21 (19) | 10/17 (59) | 10/87 (12) | 32/72 (45) | 5/20 (25) | 2/9 (22) | 2/10 (20) |
| **CD20** | 0/30 (0) | 0/20 (0) | 0/87 (0) | 0/72 (0) | 0/20 (0) | 0/11 (0) | 0/10 (0) |
| **CD4** | 17/20 (85) | 10/11 (91) | 35/87 (40) | 25/72 (35) | - | - | - |
| **CD8** | 1/21 (5) | 1/11 (9) | 4/87 (5) | 7/72 (10) | - | - | - |

**Bone Marrow Aspiration and Biopsy:**

Bone marrow aaspiration and biopsy was done in 15 patients as part of staging work-up . Of these, only 1 patient showed marrow involvement by ALCL . The marrow showed interstitial pattern of involvement.

**Treatment and follow-up:**

CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), is the standard first-line treatment for ALK+ ALCL. In this study, Out of 51 patients 43 were on treatment with CHOP regimen. Of these 2 patients expired and 3 had relapse. All the relapsed cases were ALK+ve ALCL. 8 patients lost for follow up.

**Discussion:**

During the study period of 14 years, 51 cases of ALCL were diagnosed indicating the rarity of the lymphoma. As the condition has many differential diagnoses, a diagnosis can be made correlating the histomorphology with a large panel of immunohistochemical stains. Of the 51 cases, 28 were ALK positive, 13 were ALK –ve and in 10 cases, ALK results were unavailable. This was due to financial constraints in some cases and non-availability of the paraffin block in some of the older cases. The relative frequency of ALCL among non Hodgkin lymphomas in India in pediatric age group is 11.5% while in adults it is 4.1%. Our hospital does not have a Pediatric department and all the children & adolescent ALCL patients were referred from the Oncology department.

**Age and sex distribution**

In the present study the mean age of the patient was 32.5 yrs with age range from 2-67 years. There was a male predominance in both the subgroups with M: F (ALK+=2.1:1; ALK- =1.6:1). The median age of

presentation in ALK+ cases was 29.5 (age range=2-67 years, with 81% cases <30years of age), whereas in the ALK-ve subgroup, the median age of presentation was 46.5years (age range =11-65 years, only 15.3% cases <30 years of age). These results are in concordance with studies done by Gascoyne et al and Savage et al[8]

**Clinical presentation**

The most common clinical presentation was lymphadenopathy (with cervical lymphadenopathy being most common) followed by B symptoms. Mediastinal lymphadenopathy was extremely rare. The present study also showed primary extranodal involvement to be more common in ALK+group (8/28 cases; 29%) than the ALK –ve (2/13cases; 15%).

**Histomorphology**

Classic type morphology was most common , which was similarly depicted by other studies (table 1).

**Immunohistochemistry**

Cd30, EMA and LCA were positive in almost all the cases ( EMA- 91% in ALK+ve and 100% ALK-ve ; LCA -78% in ALK+ve ; 89% in ALK-ve). This was also represented in studies done by Lakshmaiah et al [7], Gascoyne et al[8] and Savage et al[9] (table 2).

In addition to HL and ALCL, CD30 may also expressed in other lymphomas including angioimmunoblastic T-cell lymphoma (AITL), PTCL or cutaneousT-cell lymphoma (CTCL), diffuse large B-cell lymphoma (DLBCL). Similarly ALK is also expressed in different types of cancers, including glioblastoma, breast cancer, neuroblastoma, Ewing sarcoma, retinoblastoma, DLBCL and melanoma.

CD3 though being a commonly used T cell marker was less expressed in ALCL in our study (25% of ALK+ve cases; 22% of ALK-ve cases) and this was similarly depicted by Lakshmaiah et al [7]. Their study has shown the utility of other T cell markers like CD4 (85% positivity in ALK+ve cases; 91% positivity in ALK-ve cases) to be helpful rather than rely on only one T-cell marker.(Table 2).

**CONCLUSION:**

ALK +ve ALCLs are more common in < 30yrs and ALK –ve ALCLs were more common in >30yrs age group. As the condition has a very varied histology and numerous close differentials include Hodgkins lymphoma, DLBCL, peripheral T cell lymphoma, Burkitts lymphoma, Langerhans cell histiocytosis and metastatic carcinoma , correlation of histomorphology along with an extensive panel of IHC is required to make the diagnosis. Though ALK+ve ALCL is of T-cell origin, CD 3 may be negative in 75 % cases. CHOP regimen is the standard first-line treatment for ALK+ ALCL with good prognosis.

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