HIV ASSOCIATED LYMPHOMA

A FIVE YEAR CLINICOPATHOLOGICAL STUDY

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE

REQUIREMENTS FOR THE M.D. DEGREE BRANCH III (PATHOLOGY)

EXAMINATION OF THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI TO BE HELD IN APRIL 2014

CERTIFICATE

This is to certify that this dissertation titled **"HIV Associated Lymphoma – A Five Year Clinicopathological Study"** is a bonafide work done by Dr. S. Rajalakshmi, in part fulfilment of rules and regulations for the M.D. Branch III (Pathology) Degree examination of The Tamil Nadu Dr. M.G.R. Medical University, to be held in April 2014.

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CERTIFICATE

This is to certify that the thesis entitled **"HIV Associated Lymphoma – A five year Clinicopathological Study"** is the bonafide work done by Dr. S. Rajalakshmi under my guidance, in part fulfilment of the requirement for the M.D. Branch III (Pathology) Degree Examination of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, to be held in April 2014.

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1. INTRODUCTION

Malignancies are more common in immunocompromised individuals as compared to the general population. In the developed countries 34% of AIDS patients suffer from aggressive malignancies which are resistant to treatment and are often fatal (1). On the other hand, in India, the incidence of malignancies in patients with HIV is only 3-4% which is attributed to the under diagnosis and early deaths from opportunistic infections(1).

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CONTENTS

ACRONYMS

AIDS – Acquired Immuno Deficiency Syndrome

ALCL – Anaplastic Large Cell Lymphoma

DLBCL – Diffuse Large B-cell lymphoma

EBV – Epstein Barr Virus

EBER – Epstein Barr Virus Encoded RNA

HHV8/KSHV – Human Herpes Virus 8/Kaposi Sarcoma associated Herpes Virus

HIV – Human Immunodeficiency Virus

LANA – Latent Nuclear Antigen

LMP1 – Latent Membrane Protein 1

LPD – Lymphoproliferative disorder

NHL – Non Hodgkin Lymphoma

NK/T – Natural Killer/T-cell

PD-1 – Programmed death 1

PTCL, NOS – Peripheral T-Cell Lymphoma, Not Otherwise Specified

1. INTRODUCTION

Malignancies are more common in immunocompromised individuals as compared to the general population. In the developed countries 34% of AIDS patients suffer from aggressive malignancies which are resistant to treatment and are often fatal (1). On the other hand, in India, the incidence of malignancies in patients with HIV is only 3-4% which is attributed to the under diagnosis and early deaths from opportunistic infections(1).

The overall risk of all malignancies is increased by 2-3 folds in people with HIV infection and the relative risk as compared to normal population is more (60-200 times) for AIDS defining malignancies such as non Hodgkin lymphoma (NHL), particularly before the introduction of antiretroviral therapy (2,3). The spectrum of malignancies in HIV positive individuals include those that are considered as "AIDS defining illnesses" and "non-AIDS defining illnesses". Among the AIDS defining malignancies in the Western population, the most common is non-Hodgkin lymphoma, others being Kaposi sarcoma and cervical carcinoma. The malignancies considered under non-AIDS defining illnesses are Hodgkin lymphoma, carcinoma lung and carcinoma liver(4). In India as well, the most common AIDS related malignancy is non-Hodgkin lymphoma. Other malignancies include cervical and vaginal malignancies in female, testicular and colonic malignancies in male and anal malignancies in both sexes (5–7).

In HIV infected population, the majority of these malignancies are associated with oncogenic viruses such as EBV, HHV8 and HPV(8). Among these EBV has a strong causal relationship, which most commonly infects B lymphocytes and lead to a wide range of B-

cell non-Hodgkin lymphoma and Hodgkin lymphoma. Sixty six percent of AIDS related lymphomas are EBV associated and in most EBV positive AIDS related lymphomas, all recognizable tumour cells express EBER(9).

Human Herpes Virus 8 (HHV8) is associated with almost all cases of Kaposi sarcoma and 38% of HIV associated immunoblastic/plasmablastic lymphoma. Monoclonal antibody to latent nuclear antigen 1 (HHV8-LANA1) has been considered as a highly sensitive and specific marker for HHV8 in formalin fixed paraffin embedded tissue sections(10,11). Sixty percent of HHV8 positive AIDS related lymphomas are preceded by history of Kaposi sarcoma (80%)(11).

Introduction of antiretroviral therapy has drastically affected the epidemiology of AIDS related malignancies, with the incidence of AIDS defining malignancies such as non-Hodgkin lymphoma decreasing with improved survival and the incidence of non-AIDS defining malignancies such as Hodgkin lymphoma increasing as compared to the pre-HAART era (12–15).

India has a large number of people living with HIV/AIDS, being the second highest in the world. Inspite of this, only sparse data is available on HIV related malignancies especially the lymphoid malignancies, possibly due to under diagnosis and inadequate reporting system. Therefore there is an urgent need to improve the epidemiological data collection system in our country.

2. REVIEW OF LITERATURE

2.1. General features of HIV associated lymphomas:

The occurrence of certain malignancies in the HIV infected individuals indicates the disease progression to Acquired Immuno Deficiency Syndrome (AIDS). The Center for Disease Control (CDC) has determined such malignancies that indicate AIDS and these are called as "AIDS defining malignancies" (16). These include Kaposi Sarcoma, non-Hodgkin lymphoma and cervical carcinoma (16).

Among the AIDS defining malignancies, Kaposi sarcoma was the first to be listed in 1980 (2), followed by the intermediate and high grade B-cell lymphomas in 1985 and finally invasive cervical cancer in 1993 (2). In 1990s, NHL was reported to be the most common AIDS defining cancer followed by Kaposi sarcoma and invasive cervical cancer. The relative risk of NHL among HIV positive individuals is 150-250 times higher than the HIV negative individuals (17,18).

There are other malignancies reported in AIDS patients. However these are not categorized under "AIDS defining malignancies", since they are not included in the diagnostic criteria for AIDS and these are called as "non-AIDS defining malignancies" [Table 1]. The overall incidence of non AIDS defining malignancies has increased from 1 to 4.5/1000 person years over a period of 1985-2011(19). The incidence and the spectrum of non AIDS defining malignancies has increased over time, especially those associated with oncogenic viruses like Human papilloma virus (HPV) and smoking. These include anal, cervical and lung cancers and others (19). Benign neoplasms are more common than

the malignant neoplasms in HIV positive individuals. This frequency of benign and malignant neoplasms remains the same during pre and post HAART era (20). Among the benign neoplasms, hepatic haemangioma is the most common, followed by uterine leiomyoma (20). [Table 2]

Table 1: AIDS and non AIDS defining malignancies: (2,6,19,21)

AIDS defining malignancies	**Non AIDS defining malignancies**
Non Hodgkin lymphoma	Anal malignancy
Kaposi sarcoma	Hodgkin lymphoma
Invasive cervical cancer	Vulval and vaginal cancer
	Lung cancer
	Liver cancer
	Tonsillar cancer
	Melanoma
	Oropharyngeal cancer
	Leukaemia
	Colorectal cancer
	Renal cancer
	Testicular cancer

Table 2: Benign and malignant neoplasia in HIV positive individuals (20)

Benign Neoplasm	**% of total HIV+ individuals**	**Malignant neoplasm**	**% of total HIV+ individuals**
Hepatic haemangioma	4.2	Lymphoid neoplasia	2.7
Uterine leiomyoma	15.7*	Kaposi sarcoma	2.3
Renal adenoma	1.2	Gastric adenocarcinoma	0.8
Renal medullary fibroma	1.2	Gastric malignant GIST	0.4
Follicular thyroid adenoma	0.8	Hepatocellular carcinoma	0.4
Lipoma of large bowel	0.8	Intestinal adenocarcinoma	0.4
Adrenal adenoma	0.4	Malignant testicular teratoma	0.5#
Atrial lipoma	0.4	Papillary thyroid carcinoma	0.4
Benign ovarian teratoma	1.4*	Retroperitoneal embryonal carcinoma	0.4
Gastric benign GIST	0.4		
Meningioma	0.4		
Papilloma of urinary bladder	0.4		
Pituitary adenoma	0.4		

* # percentage among total women and men respectively. GIST – gastrointestinal stromal tumour.

HIV associated lymphomas are more common among males (Male: Female ~ 4.8: 1)(22,23) with median age of 38.5 yrs (Range = 14-67yrs) (22,24,25). Occasional cases of children with AIDS related lymphoma have also been reported. They usually presented with severe immunosuppression and high viral load (26).

Overall, patients presented at advanced stage of disease, with ~86% of the patients having B symptoms with a wide spread disease at the time of diagnosis and a shorter survival (13,27–30). Most cases have an aggressive histological type. This is in contrast to the distribution of lymphoma in normal population, where indolent lymphomas are more common [Fig. 1] (28,31). The main histological subtype of NHL in AIDS patients in the pre-HAART era was DLBCL followed by Burkitt lymphoma and immunoblastic DLBCL (32,33). Burkitt lymphoma had the most aggressive behaviour. The immunoblastic DLBCL occurred in patients with a very low CD4 count (33). Among the AIDS related lymphomas worldwide, high grade B-cell lymphomas constituted the major proportion (92%) (34). The main histological subtype was diffuse large B-cell lymphoma constituting ~50% of the total cases (27). In a study done in Africa on childhood HIV/AIDS population, only two types of malignancies were found; Kaposi sarcoma and non Hodgkin lymphoma. Among these, NHL seems to be less common (9.3%) and rare in childhood population in this region as compared to Kaposi sarcoma (90.7 %) (35)(36).

Immune reconstitution due to treatment with HAART lead to dramatic improvement and prolonged survival of patients with HIV infection which in turn lead to the increasing incidence of AIDS related malignancies including lymphomas in the forthcoming years (37). Non AIDS defining cancers showed a significant increase in incidence in the last

decade(post HAART) and has surpassed the incidence rates of AIDS defining malignancies (21,38). With the advent of HAART, HIV infected individuals are also at increased risk of developing malignancies not related to HIV infection like head and neck cancers (7). The mean time interval from HIV diagnosis to cancer is ~549 days presenting with a lower nadir CD4 count and advanced stage of disease (7).

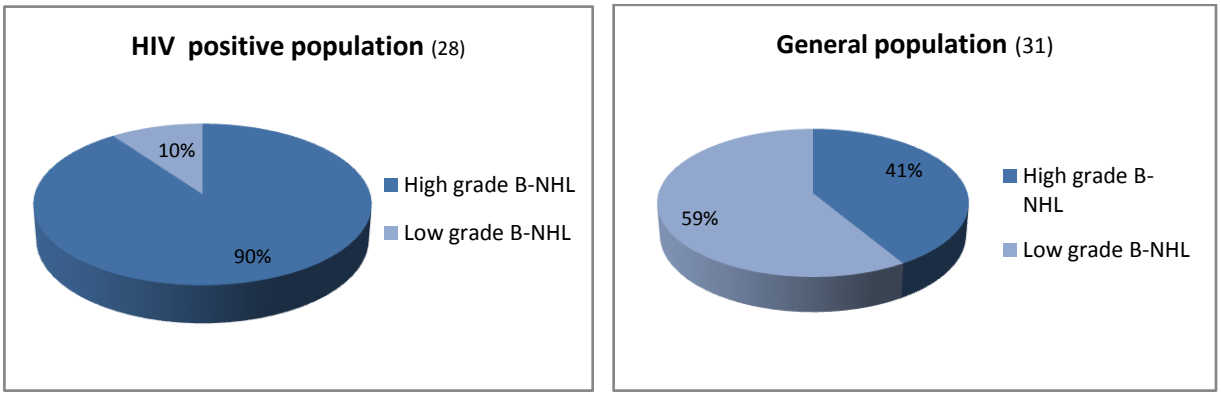


Figure 1: Comparison of aggressive/High grade B-NHL lymphomas in HIV positive and general population

Among the AIDS related lymphomas (including both Hodgkin and non Hodgkin lymphoma), 66% were found to be EBV associated and all these expressed EBER (39). EBV association was found in 20-40% of NHLs in HIV positive patients (17). EBV association varied according to the subtype [Table 3]. It was found to be associated with 100% of primary CNS lymphomas, 50% of DLBCLs, 80-100% of Hodgkin lymphoma and only about 20% of Burkitt lymphoma (28,40). Immunoblast rich subtypes were usually EBV associated (65%) whereas Burkitt type histology show a low association with EBV, the pattern analogous to sporadic Burkitt lymphoma (41).

Table 3: EBV associated lymphomas in immunodeficient hosts (42,43)

Lymphoma	**EBV frequency (%)**	**Latency pattern**
Post transplantation LPD, B-cell	>90	Type 3
Post transplantation LPD NK/T-cell	>70	Type 3
Burkitt lymphoma in HIV	25-35	Type 1
Hodgkin lymphoma in HIV	>80	Type 2
Primary effusion lymphoma	>80	Type 1
Plasmablastic lymphoma	~70	Type 1 or 2
Plasmablastic lymphoma, oral type in HIV	100	Type 1
Primary CNS lymphoma in HIV	100	Type 3
NHL with primary immune disorders	>90	Type 3
Iatrogenic immunodeficiency lymphoma	40-50	Type 3
Diffuse large B cell lymphoma-centroblastic	20-30	Type 3
Diffuse large B cell lymphoma- immunoblastic	80	Type 3

2.2. Subtypes of lymphomas in HIV/AIDS:

These lymphomas can be divided into two groups, those that are also seen in immunocompetent patients and those that are seen specifically in HIV patients (43,44).

1. Lymphoma also occurring in immunocompetent patients:

a. Diffuse large B-cell lymphoma

i. Centroblastic

ii. Immunoblastic

b. Burkitt and Burkitt-like Lymphoma

c. Classical Hodgkin Lymphoma

d. Peripheral T-cell lymphoma

e. Extranodal marginal zone lymphoma of Malt type (rare)

2. Lymphoma occurring more specifically in HIV positive patients

a. Plasmablastic lymphoma of oral cavity

b. Primary effusion Lymphoma

The subtypes of lymphoma and their frequency varied between the HIV positive and the general population (32,45)

2.3. Site of involvement:

These lymphomas most commonly occur in extra nodal sites, among which the gastrointestinal tract is the major site for all subtypes especially DLBCL. This predominantly involves the stomach and duodenum followed by liver and bone marrow(28). Nodal involvement is rare (28). Involvement of unusual sites like oral

cavity, body cavities and jaw can be seen (31). However, since the introduction of HAART therapy the frequency of nodal involvement has increased to 50% while in the pre HAART era nodal involvement occurred only in 14% (32,46).

2.4. Pathogenesis:

AIDS associated B-cell lymphoma has several pathogenetic mechanisms such as chronic antigenic stimulation leading to polyclonal B-cell activation with emergence of monoclonal population, genetic abnormalities, cytokine deregulation and role of oncogenic DNA viruses such as EBV and KSHV. Firstly chronic B-cell hyperactivation leads to genetic alterations which are associated with certain subtypes of AIDS related lymphomas. Secondly, since the oncogenes expressed by EBV are under constant suppression by T-lymphocytes, the loss of immunoregulation in HIV infection, leads to upregulation of these oncogenes leading to transformation of B-cells (18). Finally, the overproduction of B-cell stimulating cytokines such as Interleukin-6 (IL6) and Interleukin-10 (IL10) in AIDS, support the survival and proliferation of nascent lymphoma cell clones (47,48). A viral encoded cytokine like molecule, HHV8vIL6 has also been found to induce B-cell hyperactivation in patients infected with HIV which activates stromal cell derived factor, a chemokine in lymphomagenesis (47). These cytokines influence the tumour biology by either promoting tumour growth or by down regulating the anti-tumour responses (49). Levels of the cytokines, Interferon γ (IFN γ) and Interleukin 12 (IL12) are significantly decreased in AIDS related NHL. IFN γ is a potent antiproliferative factor whose expression is in turn promoted by IL12 (49). However the

levels of IL6 and IL10 are significantly elevated in HIV associated lymphomas as compared to the HIV negative controls (49). These two cytokines are often associated with aggressive B-cell lymphomas and the serum levels are correlated with the outcome (49). Recent studies have also shown the therapeutic role of anti IL6 antibodies and low dose IL2, which promote Th-1 production of IFN γ in HIV-associated NHL.

Mammalian target of rapamycin (mTORC1) signaling pathway is also implicated in lymphomagenesis (50). Activation of this pathway is detected in the neoplastic cells of HIV associated lymphoma and as well as in the transformed cells in reactive conditions such as HIV lymphadenopathy. mTOR is a kinase involved in key cellular functions such as proliferation and protein synthesis. It is associated with several proteins such as raptor and rictor to form mTORC1 and mTORC2, among which mTORC1 pathway is much studied (50). But the exact mechanisms of either of these are not clearly understood until this date. SyK, PI3K (phosphoinositide3 kinase), MEK (Mitogen activating kinase) and ERK (extracellular regulated kinase) are a few kinases that activate mTORC1. These are expressed in transformed B cells and therefore HIV associated lymphoma arising from transformed B-lymphocytes may respond to inhibitors targeting these kinases that ultimately inhibits mTORC1 pathway (50).

2.5. EBV:

Epstein-Barr virus (EBV), a γ - Herpes viruses, infects most of the general population, but causes disease in only a small subset (18). It is involved in human lymphoma genesis, particularly in HIV/ immunocompromised patients (18,44). EBV is capable of infecting a wide range of cell types, preferentially B lymphocytes, others being squamous epithelium

of oropharynx and nasopharynx, stomach, glandular epithelium of thyroid and salivary gland, smooth muscle cells, T lymphocytes, plasma cells, NK cells and follicular dendritic cells(18,51,52). In B lymphocytes, EBV infection results in two outcomes: - 1) production of memory B cells that persists long-term (latent viral persistence) and 2) differentiation towards plasma cells that are destined to die (lytic viral replication)(52). In latent viral persistence, the virus does not replicate to produce virions; instead the EBV genome unites with the host cell nucleus as an episome and is copied by cellular DNA polymerase. There are several viral genes that are expressed during this latency period. Three patterns of latency programs have been described and each of them has different set of viral genes expressed with some degree of overlap. [Table 4] (50, 51).

Table 4: Various EBV gene expressions in different types of latency patterns

Gene Expressed	**EBNA-1**	**EBNA-2**	**EBNA-3A**	**EBNA-3B**	**EBNA-3C**	**EBNA-LP**	**LMP-1**	**LMP-2A**	**LMP-2B**	**EBER**
Product	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	Protein	ncRNA
Latency I	+	-	-	-	-	-	-	-	-	+
Latency II	+	-	-	-	-	-	+	+	+	+
Latency III	+	+	+	+	+	+	+	+	+	+

Some of these genes are under constant suppression by T-cells. Evasion of the immune system as in the case of HIV infection leads to upregulation of these oncogenes leading to B-cell stimulation which along with the acquisition of other mutations leads to lymphoma(18,53). EBV has been implicated in the development of B-cell lymphoproliferative disorders such as Burkitt lymphoma, Hodgkin lymphoma, NK/T-cell lymphoma, non Hodgkin lymphoma, primary central nervous system lymphoma and carcinomas such as nasopharyngeal and gastric carcinoma (18,52). Different types of lymphomas show different latency patterns, depending upon the expression of proteins. [Table 5] (18,43,51,52).

Table 5: Latency patterns in lymphoma:

Latency patterns	Types of lymphoma
Type I	Burkitt lymphoma, Primary effusion lymphoma, DLBCL-centroblastic variant
Type II	Hodgkin lymphoma, peripheral T cell lymphoma, NK/T cell lymphoma, nasal type, AIDS related Burkitt and primary effusion lymphoma
Type III	PTLD, AIDS related immunoblastic or brain lymphoma, DLBCL-immunoblastic variant

Latency type III is usually expressed in HIV associated DLBCL (54). Approximately 60% of the large B-cell lymphomas in HIV positive individuals express EBNA 2 and LMP1&2 when compared to 100% in PTLD. Latency type I is expressed in HIV associated Burkitt

lymphoma in which only 30% are EBV positive and express only EBNA 1 (54). Type I latency can also be found in circulating lymphocytes in healthy viral carriers. Type II latency can also be found in nasopharyngeal carcinoma and type III latency in infectious mononucleosis (52).

Epstein Barr encoded RNA (EBER) is the most abundant viral transcript in latently infected cells (around one million copies per latently infected cell) and therefore considered as the best natural marker of latent EBV infection (51). EBER is expressed in almost all EBV related lesions (both neoplastic and non-neoplastic). The only EBV-related lesion that lacks EBER is oral hairy leukoplakia (lytic type of infection)(52). Detection of EBER expression in lymphoid neoplasm is of tremendous value in proving the association of EBV infection which is considered to be involved in its pathogenesis. According to literature, detection of EBER in biopsy samples by insitu hybridization (ISH) technique is considered to be gold standard (51,52). In most of the AIDS related lymphomas associated with EBV, EBER 1 is expressed in all recognizable viable tumour cells (39). EBER and LMP-1, in combination are considered to be more effective for detecting EBV related Hodgkin lymphoma (55). False positive results are rare and can be obtained if there is confusion with the latently infected background lymphocytes, non specific staining and cross reaction with mucin and yeast(55). False negative results are due to RNA degradation. EBER is almost undetectable (<0.1%) in the background lymphocytes and other cells in the tissue (52). It has been found that EBER can also be done on cytology preparations (52). Sensitivity and specificity of EBER-ISH is very high even when it is done in paraffin embedded tissue sections and is comparable with that of the southern blotting (56). Recent studies have shown the significance of EBV viral load

testing, as a means of early diagnosis and disease monitoring in HIV associated primary CNS lymphoma, Hodgkin lymphoma and nasopharyngeal carcinoma (52). There are two subtypes of EBV, type A and type B. Subtype B is found in about 25% of the HIV associated NHL and has a wider geographical distribution (57). In HIV negative population this subtype is restricted to the nasopharyngeal carcinoma (57).

Viral gene products would be of potential targets for molecule targeted chemotherapy (43). Some studies have shown the prognostic significance of EBV positivity, Hodgkin lymphomas in children which show EBV positivity have a better prognosis whereas in adults the prognosis is better if the tumour is negative for EBV (51).

2.6. KSHV/HHV8:

Kaposi sarcoma associated Herpes virus is most commonly associated to the pathogenesis of primary effusion lymphoma and multicentric Castleman disease. It is also common (38%) in HIV related immunoblastic /plasmablastic lymphomas (11). HHV8 associated lymphomas usually develop in patients with severe immunosuppression in the advanced stage of AIDS and presents most commonly as primary effusion lymphoma (44). HHV8 is associated with 100% of primary effusion lymphomas and virtually all plasmablastic lymphomas occurring in HIV positive individuals (28). HHV8 is also implicated in lymphomas involving solid organs which are classified as extracavitary primary effusion lymphoma (11,58).

The KSHV positive lymphomas are usually preceded by Kaposi's sarcoma in 60% of the HIV associated lymphomas and are predominantly extranodal and involve the gastrointestinal tract in 80% of cases (11). The monoclonal antibody to KSHV/HHV8 latent nuclear antigen 1(LANA-1) is considered as a highly sensitive and specific marker for KSHV/HHV8 infection using immunohistochemical technique on formalin fixed paraffin-embedded tissue section (10).

2.7. Effect of immunosuppression and immune reconstitution:

Mean CD4 count among the patients with HIV associated lymphoma is 83cells/µl with the viral load of 26,000 RNA copies/ml (27). The relative risk for certain AIDS related malignancies such as Kaposi sarcoma, NHL, Hodgkin lymphoma, anal and colorectal malignancies are increasingly associated with decreasing CD4 cell counts (59). Among these, Kaposi sarcoma and NHL also have association with HIV viral load (59). Early treatment with antiretroviral therapy to maintain high CD4 levels might help in reducing the incidence of malignancies in the HIV positive individuals (59). CD4+ T-cell count is higher at the time of diagnosis of lymphoma in the post HAART era i.e., 191 cells/µl vs. 63 cells/µl in the pre HAART era (60). AIDS related Burkitt lymphoma and centroblastic DLBCL arise in the subset of people with maintained CD4 levels (61). Immunoblastic lymphomas occur in patients with low CD4 counts and manifest late in the course of AIDS. HHV8 associated lymphomas and primary CNS lymphoma in HIV positive individuals tend to occur in patients with very low CD4 count (61).

2.8. Lymphoma subtypes:

2.8a. Diffuse Large B-cell lymphoma (DLBCL):

This is the most common (79.8%) lymphoma in HIV positive population (62). Patients usually present at a median age of 40 yrs and are commonly males with a high prevalence of extranodal involvement at the time of diagnosis. They have high or intermediate high IPI score (63) and a median CD4+ T-cell count of 125cells/μl at the time of diagnosis (62).

ETIOPATHOGENESIS: Chronic antigenic B-cell stimulation in a setting of reconstituted immune system in the HAART era is thought to promote the germinal centre pathway in lymphomagenesis (63). The co-expression of germinal centre and activated B-cell phenotype is observed as a unique feature in AIDS related lymphoma (63). Some of the markers that are co-expressed are CD10 and MUM1, and also Bcl-6 and MUM1 which reveal that there is insitu germinal centre differentiation, a unique pathology found in AIDS related lymphomas (63). Association of EBV infection is found in 50% of the DLBCLs (63). Immunoblastic variant of DLBCL is more frequently associated with EBV (65%) and frequently express EBV-LMP1 (44)(41). Immunoblastic variant is also associated with HHV8 in AIDS patients who have a previous history of Kaposi sarcoma with some cases showing co-infection and positivity for both viruses (64). Unusual cases of intravascular large cell lymphomas also have been reported in HIV positive individuals who had co-infection with HHV8 and were negative for EBV infection (65).

MORPHOLOGY: Morphological variants of DLBCL in HIV positive individuals do not differ significantly from the denovo forms. The centroblastic variant can be monomorphic or

polymorphic with a mixture of centroblasts and immunoblasts. The centroblasts are large cells with round to oval vesicular nuclei, 2-3 membrane bound nucleoli and a scant amphophilic cytoplasm. The immunoblasts are large cells with moderate amounts of amphophilic cytoplasm with vesicular nuclei and centrally placed single nucleolus. It is termed as pure centroblastic or immunoblastic, only if it contains >90% of the respective cell type (66). Anaplastic variant, as the name indicates has a variable morphology of large pleomorphic bizarre cells sometimes may even resemble Reed Sternberg cells and cells of anaplastic large cell lymphoma (67). The centroblastic variant of DLBCL is more common and accounted for 25-30% of the HIV associated lymphomas and was EBV positive in 30% of the cases. Immunoblastic variant with plasmacytoid features accounted for 10% and was positive for EBV in 90% of the cases (68).

IMMUNOPHENOTYPE: DLBCL are always positive for CD20 (63). These are mostly germinal centre phenotype (60%), which is peculiar to AIDS related lymphomas in contrast to only 38% of DLBCL being germinal centre phenotype in HIV negative patients. Atypical phenotype such as co-expression of germinal centre and activated B-cell phenotype is also unique in HIV positive individuals as described earlier (63,69).

PROGNOSIS: Median survival time is ~ 10 months in a follow-up period of 4 yrs (62). Survival is affected by two major factors; 1) Development of AIDS before the diagnosis of lymphoma and 2) IPI score (62). In a multivariate analysis it was found that an increased IPI score and failure to achieve complete remission following chemotherapy was independently associated with shorter survival both in the pre-HAART and post HAART era (70) and that decreased CD4 count (<100cells/μl) was associated with decreased

survival only in pre-HAART era (70). Outcome of DLBCL in HIV positive individuals is comparable to that of the HIV negative individuals in the post HAART era due to immune reconstitution (70). Median survival increased from 8.3 months to 43.2 months in the post HAART era (70). EBV positive subset of DLBCLs generally shows poor response with conventional chemotherapy (18).

2.8b. Burkitt lymphoma:

In countries which are holoendemic for malaria, this is the most common malignancy among children (71). In non-endemic areas, it tends to be more common among the immunosuppressed individuals, especially the HIV positive patients (71). It occurs more commonly in children and young adults (median age 30 yrs) (72,73). In immunosuppressed individuals, nodal involvement is more frequent as compared to extranodal sites in the immunocompetent individuals (31). In HIV infected patients this occurs in those patients with maintained levels of CD4+ T-cell count (71).

ETIOPATHOGENESIS: This is the first tumour discovered to have chromosomal translocation involved in the pathogenesis, causing activation of an oncogene, c-myc (71). Burkitt lymphoma in AIDS shows certain molecular lesions, such as activation of c-myc, inactivation of p53 and infection with EBV (44). Plasmodium falciparum and chronic HIV infection leads to chronic antigenic stimulation of B-lymphocytes (71). Markers of B-lymphocyte activation such as CD23 and CD30 are found at high levels in the serum and predict development of lymphoma in HIV positive individuals (71). Apart from chronic antigenic stimulation, chromosomal rearrangement can be seen in B-lymphocytes which

is induced by over expression of the enzyme actin induced cytidine deaminase (AID), which is detected in HIV positive patients with lymphoma (71). Blood levels of EBV DNA also have an impact in the development of lymphoma in HIV positive individuals (74). HIV positive individuals with high levels of EBV DNA in the peripheral blood mononuclear cells tend to develop systemic B-cell lymphomas in contrast to those with undetectable levels of EBV DNA who did not develop lymphoma over a period of 3yr follow up (74). EBV is associated with 45% of all Burkitt lymphoma in general and the association is stronger in the paediatric age group (75), but it is seen only in about 25-35% of HIV associated Burkitt lymphoma (28). Most of these cases found to have EBV subtype A. HHV8 is not associated with Burkitt lymphoma including those in the immunocompromised individuals (75).

MORPHOLOGY: Burkitt lymphoma is characterized by a monomorphic population of medium sized lymphoid cells with scant cytoplasm exhibiting "squared off" borders, round to oval nuclei with clumped chromatin and multiple paracentrally located basophilic nucleoli. The cytoplasm appears deeply basophilic with lipid vacuoles which are better appreciated in imprints (31). "Starry-sky pattern" is quite characteristic of this lymphoma indicating that the rapid proliferation rate. As this is the feature of rapid proliferation, this pattern can also be found in other high grade lymphomas (31).

IMMUNOPHENOTYPE: This lymphoma is positive for pan B-cell antigen (CD20) and for germinal centre cell markers, CD10 and Bcl-6. Expression of clonal surface immunoglobulins is also observed in Burkitt lymphoma (63,76). Ki-67 proliferation index is highest(>95%) in Burkitt lymphoma among all aggressive lymphomas (77). AIDS

related Burkitt lymphoma typically fail to express LMP1 and EBNA2 (44). Atypical immunophenotype is not uncommon in Burkitt lymphoma (76). It may show lack of CD10, negativity for surface immunoglobulins and B cell antigens (76). In this situation diagnosis is made on the basis of morphology and confirmed by cytogenetic analysis (76). Therefore immunophenotype should not be interpreted alone. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma are classified under "grey zone lymphomas". In a majority of these lymphomas the morphology is intermediate between DLBCL and Burkitt with some cells smaller than DLBCL resembling Burkitt lymphoma but with more variation in size and contour of the nuclei and some cells larger than Burkitt resembling DLBCL with a high proliferation index and an immunophenotype consistent with Burkitt lymphoma. In some cases, the morphology may be typical of Burkitt lymphoma, but with an atypical immunophenotype or genetic features. These lymphomas are usually positive for B cell antigens and surface immunoglobulins (sIg).

PROGNOSIS: Outcome is better in children and poorer in adults (71). Rituximab, a monoclonal antibody against CD20 has less toxic effects with improved outcome (71).

2.8c. Plasmablastic lymphoma:

The unique feature of this lymphoma is that it occurs almost exclusively in the immunocompromised individuals. Studies show that ~89% of patients are immunosuppressed, among which 79% are HIV positive individuals (78,79). In HIV

positive individuals, it occurs in association with a very low CD4+ T-cell count (<100cells/μl) (18,28). Patients usually presents at a median age of 38 yrs with a male predominance (7:1) (80,81). The most common primary site is the oral cavity followed by other mucosal sites such as the gastrointestinal tract, sinonasal region and lymph nodes (80). In the oral cavity the most common location is the gingivobuccal complex (84%) (82). In a few multicentric retrospective studies it was found that around 90% had extra nodal manifestation (81,83) with oral cavity as the primary site is 27% of the cases. Sixty nine percent presented with advanced stage of disease (83) and 17% had bone marrow involvement (81).

ETIOPATHOGENESIS: EBV association as shown by EBER-ISH is positive in 60-75% of plasmablastic lymphomas and 100% in plasmablastic lymphomas of oral cavity (18,28,84). Myc rearrangement is found in up to 50% of the patients and is more common among those that are EBV positive suggesting its possible role in lymphomagenesis (18,84). This implies the important role of EBV in the pathogenesis of plasmablastic lymphomas (78). Myc/IgH gene rearrangement in plasmablastic lymphomas predicts an aggressive clinical course and all these cases are EBV positive and HHV8 negative (85). In contrast one study, found that, lymphomas occurring in oral cavity in HIV positive population were strongly associated with EBV and HHV8 (86).

MORPHOLOGY: The classical oral type of plasmablastic lymphomas usually show a predominant population of plasmablasts which are monomorphic large cells with moderate amounts of amphophilic cytoplasm with round nucleus, high nucleo-cytoplasmic ratio, and centrally placed prominent nucleoli and high apoptotic activity and

absent mature plasma cells (82,87). The categorization of these tumours can be made with immunohistochemistry and virus association (88). If both EBV and HHV8 are positive in HIV associated lymphomas with plasmablastic morphology it is categorized as PEL; if only HHV8 is positive, it is large B-cell lymphoma arising in HHV8 associated multicentric Castleman disease and negativity for both the viruses, can be classified as plasmablastic lymphoma (88). Plasmablastic lymphomas occurring in oral cavity in HIV positive individuals usually does not show features of plasmacytic differentiation and have a relatively uniform population of plasmablasts, whereas those that are occurring in the extra-oral sites in HIV negative individuals tend to show plasmacytic differentiation with a variable mixture of large plasmablasts and small cells with plasmacytic differentiation (87).

IMMUNOPHENOTYPE: Characteristic immunophenotype of these lymphomas which is considered as the essential diagnostic criteria is CD20 negativity, CD138 positivity and a high MIB1 index of >60% (82). The other antigens expressed are EMA (Epithelial Membrane Antigen) in ~74% of the cases, VS38c positivity correlating with pronounced plasma cell differentiation and CD79a positivity in about 50% of those with plasmacytic differentiation. CD79a is negative in pure oral plasmablastic lymphoma (44,80,82,87).

PROGNOSIS AND TREATMENT: Poor prognostic factors include ECOG status ≥ 2, advanced stage and myc rearrangement (83). Median survival time after diagnosis is 6 months to 11 months(84,89). The general outcome of plasmablastic lymphoma in HIV positive individuals remains poor even in the era of HAART (83). Nevertheless, there has also been a case report of prolonged survival (61 months) in a HIV positive female with

oral Plasmablastic lymphoma that has been treated with HAART, 10 weeks before the start of chemotherapy and attained complete remission after 2 cycles of CHOP regimen (90). Its consistent association with immunosuppression indicates that all patients with a diagnosis of plasmablastic lymphoma should be investigated for the presence of HIV infection (78,79) . Since this subtype of lymphomas occurs in severely immunocompromised individuals, HAART should be initiated at the time of diagnosis (18).

2.8d. Hodgkin lymphoma:

There has been an increasing incidence of Hodgkin lymphoma in HIV patients and is ~7 times more common in them than in the HIV negative population (40,91). Hodgkin lymphoma tends to occur in young men, ~28 yrs of age and have a high incidence (86%) among the IV drug abusers (40). Presentation in HIV positive population is unique in that it usually presents at advanced stage III-IV (90%) with a high incidence of B symptoms (81%), aggressive histology with bone marrow involvement (50%), low CD4+ T-cell counts (<100cells/μl), association with opportunistic infections and cytopenias before the initiation of treatment (18,40,91–93).

ETIOPATHOGENESIS: EBV association is detected in ~80-100% of the of the HIV positive patients with Hodgkin lymphoma as compared to 30-60% in immunocompetent individuals with Hodgkin lymphoma (40,94). LMP1 oncogene is almost always expressed (18). Because of its strong association with EBV, it is considered as the main etiology in

the pathogenesis of classical Hodgkin lymphoma and HIV infection may in turn predispose to this condition (31,40).

MORPHOLOGY: Mixed cellularity and lymphocyte depleted subtypes are more common in HIV positive individuals, whereas nodular sclerosis is less common (18). This is because there is decrease in cell mediated and humoral immunity, which results in low incidence of inflammatory immune response (91). Histological features in HIV positive individuals with Hodgkin lymphoma are highlighted in the publication by Thompson et al., (40) This includes, syncitial formation by tumour cells, nodularity, thick fibrous capsule, sclerotic banding, granuloma formation, stromal fibrosis, necrosis, lymphocyte depletion in the background, sarcomatoid changes and extra capsular extension of the tumour cells. Among these the thick fibrous capsule is more common in nodular sclerosis and granuloma formation is more commonly found in mixed cellularity type (40). Isolated cases of bone marrow involvement are not uncommon (95). In bone marrow, a nodular and diffuse pattern of involvement is seen. Reed Sternberg cells were identified in these cases both by histology and by immunohistochemistry (95). Characteristic stromal changes were also observed, like fibrosis, necrosis and sinusoidal dilatation, among which pronounced fibrosis of the uninvolved areas was found consistently in all cases. EBER-ISH was positive in all cases (95). Reed Sternberg cells are more concentrated in HIV positive Hodgkin lymphoma than in HIV negative patients (18).

The characteristic cell of classical Hodgkin lymphoma is the Reed Sternberg cell, a large cell with abundant amphophilic cytoplasm, which are bilobated or binulceated with

prominent nuclear membrane, pale to vesicular chromatin with a single large centrally placed eosinophilic inclusion like nucleolus, which is usually scattered in a polymorphous inflammatory background (31). Other variants of Hodgkin cells such as mononuclear, lacunar and LP cells/popcorn cells can also be found (31).

IMMUNOPHENOTYPE: Is similar to Hodgkin lymphoma in immunocompetent patients. The Reed Sternberg cells in classical Hodgkin lymphoma are typically positive for CD15 and CD30 which show a characteristic membrane and Golgi staining pattern and are usually negative for CD20 (31). A weak staining is observed with PAX5. EBV LMP1 is positive in almost all cases in HIV positive individuals as compared to 30-60% in general population (18,31,94). Lymphocyte predominant type have LP cells that are CD20 positive and EBV LMP1 negative and are characterized by negativity for CD15 and CD30 with a co expression of Oct2 and BOB1 and EMA positivity (31).

PROGNOSIS AND TREATMENT: Factors associated with poor outcome are high stage at presentation, lymphocyte depleted background and sarcomatoid features on histology (40). Lymphocyte predominant type has a clinical course of frequent relapses like a low grade NHL but a few early deaths (91). Almost all of the patients with isolated bone marrow involvement are male with a median age of 35 yrs with an average CD4 cell count of 70 cells/μl at the time of diagnosis, had a shorter survival of 4 months (95). Response to chemotherapy is poor with a poor bone marrow tolerance and significantly low survival time (91,92). HAART can be initiated at the time of diagnosis of lymphoma or after the completion of chemotherapy (18).

2.8e. Primary effusion lymphoma (PEL):

This is the first human neoplasm in which dual herpes viral infection with HHV8 and EBV was found (96). PEL is a large B cell lymphoma presenting as serous effusions without detectable tumour masses (31). History of Kaposi sarcoma is present in 50% of the patients with PEL (28). Primary effusion lymphoma usually presents in HIV infected patients with advanced immunosuppression and is almost always associated with HHV8 (44). EBER1 is positive in all patients with primary effusion lymphoma, EBNA1 is expressed in latency type I and II cases whereas LMP1&2 are expressed at a low levels. Thus PEL shows a restricted pattern of latency type I with EBNA1 positivity in all cases (96).

Extracavitary primary effusion lymphoma:

This lymphoma is characterized by extracavitary location, nodal and/or extranodal. This is an unusual type that more specifically occurs in HIV positive males with PEL like morphology and a plasma cell related phenotype on immunohistochemistry (44,58). This lymphoma is more common among the HIV positive patients than the primary effusion lymphoma and they can have secondary effusions (11). They present usually at advanced stage of disease with involvement of multiple extranodal sites and generalized lymphadenopathy (44).

MORPHOLOGY: Usually immunoblasts like cells, but in a few cases variable morphology including pleomorphic, anaplastic or plasmablastic features can be found (44,58).

KSHV/HHV8 positive immunoblastic/plasmablastic lymphomas occurring in solid organs are categorized as extracavitary PEL (11).

IMMUNOPHENOTYPE: The neoplastic lymphoid cells are usually positive for CD20, CD79a and CD138 and show variable expressivity for CD45 (-/+), EMA (-/+) and CD30 (-/+) (58). All cases express syndecan-1 (CD138), which acts as the receptor for KSHV (11). These tumours also express EBER by ISH (11,58). Though there are similarities between extracavitary and the classical form, now the consensus diagnostic terminology recommended for this entity is "KSHV-associated Large B-cell Lymphoma (KSHV-LBL)" (58).

PROGNOSIS AND TREATMENT: This lymphoma has an aggressive behavior with rapidly fatal outcome in almost all patients (44) and a poor overall survival, ~43% died within 2 months of diagnosis (58).

2.8f. T-cell NHL:

T cell lymphomas comprise only 3% of the AIDS related lymphomas, which is much lower than that seen in the normal population[12%] (97,98). T cell lymphomas differ from B cell HIV related lymphomas in terms of more frequent extra nodal sites of the disease but carry same poor prognosis as their B cell counterparts (97). Incidence of T-cell NHL has increased with a relative risk of 15 (54). Median age at presentation is 38yrs with a male predominance (5:1). These patients usually have low CD4+ T-cell counts (173 cells/mm^3)

and a high viral load (334,787 copies/ml). Most of them present with an advanced stage (75%) of disease and with B symptoms (66%) (99).

MORPHOLOGY: The most common subtype is peripheral T-cell lymphoma, not otherwise specified (PTCL NOS) ~61%, and followed by anaplastic large cell lymphoma (ALCL) ~ 22%. Other subtypes are rare (99). NK/T-cell lymphomas are rare subtypes with increased prevalence among HIV and post transplant patients (100). In a study done in our institution, a similar pattern was seen in the general population, where it was found that PTCL NOS is the most common type (26%), followed by ALCL (23%) and cutaneous lymphomas (101).

IMMUNOPHENOTYPE: Most of the patients with PTCL NOS have a CD3+ phenotype with CD4>CD8. ALCL cases show positivity for CD30 (membrane and Golgi pattern of staining) which can be ALK positive or negative (31). In a case series of 3 cases of HIV positive ALCL, all of them were found to be ALK negative which indicates a poor prognosis (102).

PROGNOSIS AND TREATMENT: The overall median survival is 12 months. Use of HAART therapy and IPI score are independently associated with the overall survival (99). Long term remission rates are observed with HAART therapy alone without additional chemotherapy. This antitumour effect of HAART is due to strong immune reconstitution achieved by this therapy (103).

2.8g. Primary CNS lymphoma (PCNSL):

Among all NHLs in HIV positive individuals, the incidence of primary CNS lymphoma has decreased drastically over recent years, which is attributed to the use of HAART (60,104–106). In Indian population this subtype is rare(2.8%) as compared to the Western population(17%) in the era of HAART (32,60).

ETIOPATHOGENESIS: Development of PCNSL could be possibly due to the reactivation of latent infection of EBV in an immunocompromised individual (106). Though immune reconstitution is achieved by HAART therapy which is considered to be the cause for decline in the incidence of PCNSL, there have been a few studies stating that HIV positive individuals develop PCNSL irrespective of the CD4+ T-cell count. The possible reason of which could be the lack of EBV specific CD4+ T-cells, though the absolute CD4+ T-cell count is maintained (107).

MORPHOLOGY: Primary CNS lymphomas usually display centroblastic/immunoblastic morphology and are of B-cell origin (44). The tumour characteristically shows a perivascular arrangement (31).

IMMUNOPHENOTYPE: Primary CNS lymphomas are of B-cell origin and express pan B-cell markers, CD20, strong positivity for IRF4/MUM1, Bcl6 and sometimes CD10 and Bcl2. EBV-LMP1 is frequently expressed indicating its etiological significance (44).

PROGNOSIS AND TREATMENT: Treatment for HIV associated PCNSL is a multidisciplinary approach, i.e., whole brain radiation therapy, anti EBV therapy and HAART. The combination of radiotherapy and chemotherapy result in increased response rate and

survival (106). The beneficial effect of HAART, which caused a drastic decline in the incidence, was observed to be strong up to 10yrs post treatment (104).

2.9. Molecular genetics:

The specific feature of HIV associated immunosuppression is polyclonal B-cell activation. Heterogeneity of molecular lesions that occurs in HIV associated lymphomas accounts for the heterogeneity of AIDS associated NHL (44). Approximately 40% of HIV associated NHLs have p53 mutation; c-myc rearrangement is seen in 100% of Burkitt lymphoma, 30-40% of HIV associated DLBCL have Bcl-6 gene rearrangement whereas it is only 20% when all NHL is considered together (44)(54). Molecular lesions in AIDS related Burkitt lymphoma are activation of c-myc, infection with EBV and inactivation of p53 (44) . Burkitt lymphoma in AIDS resembles the sporadic form of Burkitt lymphoma in the pattern of c-myc translocation breakpoint causing crippling mutation that helps in its survival from apoptosis (54). c-Myc gene rearrangement can also be seen in plasmablastic lymphoma (108). In general c-myc rearrangement is a genetic mechanism which is considered to impart a plasmablastic morphology and aggressive clinical course in B-cell lymphomas (85,108), suggesting that cytogenetic studies to be done routinely in all plasmablastic lymphomas (85). Bcl-1 and Bcl-2 gene abnormalities have not been found in AIDS related NHL (54). In Hodgkin disease, NF-κB pathway is involved in cell survival from apoptosis (54).

Histogenesis of AIDS related lymphomas can be ascertained by the expression of CD138/syndecan-1 and Bcl-6 (Fig. 2). B-cells within the germinal centre express Bcl-6 but

not syndecan-1 (Burkitt and centroblastic DLBCL) whereas the B-cells that has exited the germinal centre show maturation towards plasma cells and exhibit Bcl-6-/syn-1+ phenotype (immunoblastic DLBCL and HHV8 associated lymphomas). Different pathways highlighted by Carbone et al., are that involving AIDS related Burkitt lymphoma which are characterized by mild immunodeficiency but involving multiple genetic alterations like c-Myc activation, disruption of p53 and less frequently EBV infection. Typically these lymphomas fail to express EBVLMP1 and EBNA2. The other pathway involves the AIDS related DLBCL, majority of which are associated with EBV infection but only immunoblastic variant expresses EBV LMP1. Thus the expression of LMP1 and Bcl6 segregates the pathway for centroblastic and immunoblastic variant. The final pathway involves the lymphomas associated with HHV8 which show positivity for HHV8 and are usually co-infected with EBV (44).

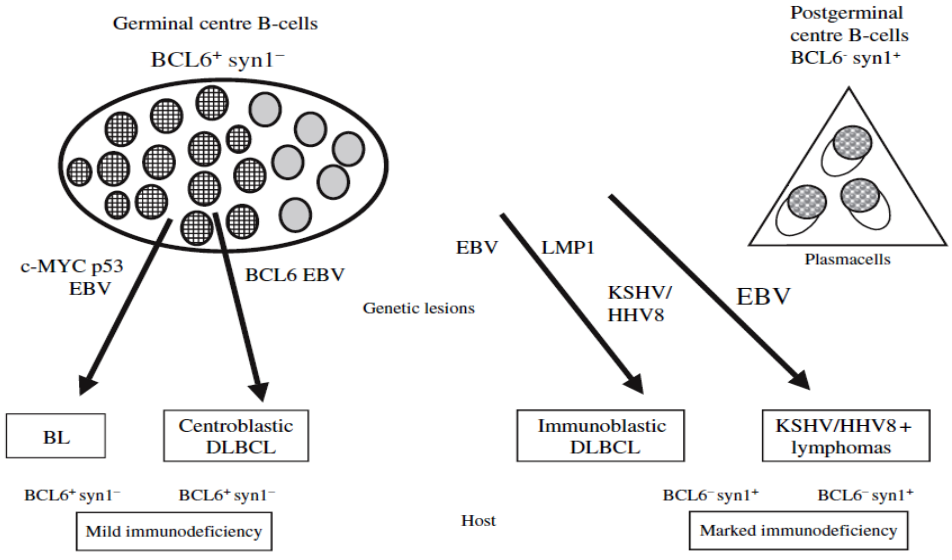


Figure 2: Histogenesis of AIDS related non Hodgkin lymphoma with linkage to molecular pathways {Courtesy: Antonio Carbone et al. (44)}

2.10. Geographic Distribution of AIDS and non AIDS Defining Malignancies:

The incidence of lymphomas has drastically decreased after the introduction of antiretroviral therapy in the resource rich regions in contrast to the resource limited regions like Africa, where lymphoma is still found to be an increasingly common complication (109).

In a study done in Western Europe over the time period of 1992-2010, 68% of the HIV positive patients had AIDS defining cancers, among which NHL is the most common type and 32% had non AIDS defining cancers, among which lung malignancy was most common (110). In Switzerland, the incidence of NHL declined from 13.6/1000 person years to 1.8/1000 person years which is attributed to the introduction of HAART in 1996. This decline was drastic for primary CNS lymphoma than other types of NHL. This beneficial effect of HAART was strong up to 10yrs post treatment (104). In UK, there is an increased preponderance of HIV associated lymphomas in male homosexuals and those who are infected predominantly through IV drug abuse (111).

In USA, the cancer risk for the two major AIDS defining malignancies, Kaposi sarcoma and NHL has decreased, especially Kaposi sarcoma and the risk for developing Hodgkin lymphoma as well as other non AIDS defining malignancies have increased over the time period of 1990-2002, which is attributed to the introduction of HAART in 1996 (15,17). In an another study done in USA during the same time period, it was found that the incidence of non AIDS defining cancers significantly increased as compared to the general population (112). Non AIDS defining cancers constituted 31.4% of cancers in HIV infected

individuals before the introduction of HAART in 1996 as compared to 58% in the post HAART era, with marked decline in the incidence of AIDS defining cancers such as Kaposi sarcoma and NHL, reflecting the HAART related immune reconstitution (4,113).

In Africa, prevalence of AIDS related lymphoma was surprisingly lower (2.3%) as compared to the general population (4.4%). In a total of 9 cases of AIDS associated lymphoid neoplasms (1993-2008), no single case of Burkitt lymphoma was present despite the fact that this part of the world is endemic for Burkitt lymphoma(29).

In the developing countries, the risk of developing lymphoma in HIV infected individuals is 10 times less than that in the developed countries (RR of 400 for high grade NHL) probably because of under diagnosis and early death from opportunistic infections (114,115).

In China, NHL (28.6%) is the most common malignancy among the HIV infected individuals, followed by cervical cancer (22.2%) and liver malignancies (17.5%) in a publication during the time period of 2004-2008 (30).

In India, the first reported study (35 cases over a period of 8 yrs) of HIV associated lymphoid neoplasms, NHL was found to be the most common, followed by Hodgkin lymphoma and plasmacytoma (32). Among the various malignancies reported in HIV infected patients in India, the presence of Kaposi sarcoma is very rare and NHL was more common (1,17). Another study during the period of 2001-2005 also observed an increased proportion of NHL with the absence of Kaposi sarcoma (6,116). Studies done during 2003-2007 had not reported single case of primary CNS lymphoma (24). The spectrum of malignancies observed in this part of the world differed from other countries,

with rarity of primary CNS lymphoma and Kaposi sarcoma (6,116). Certain subtypes of AIDS related lymphoma like plasmablastic lymphoma which is known to occur in this group of population is more common in India than in world literature (17). However, the most common subtype of AIDS related lymphomas is DLBCL followed by Burkitt lymphoma and plasmablastic lymphoma (17,32). In a study done in Indian population, the EBV association in AIDS related lymphomas was as follows; 100% in Hodgkin lymphoma and 23% in DLBCL and 67% in Burkitt lymphoma. In this study they also found that the lymph nodes were the most common site of involvement as mentioned in the other studies in the era of HAART. The primary site was lymph node in 67% of NHLs and 100% of Hodgkin lymphoma (32). With the advent of HAART, HIV infected individuals are at increased risk of developing non AIDS defining malignancies(6,7,116). There is a sparse data available on HIV associated lymphomas from India, possibly due to under diagnosis and inadequate reporting system. Therefore, there is a need to improve epidemiological data collection system in our country.

2.11. Prognosis:

Median survival time is low, ~ 5.8 months (27). The median survival (14 months vs. 4.8months) and the remission rate for chemotherapy (58% vs. 14%) was better among the individuals with HIV-NHL who received HAART when compared to the similar population who did not receive HAART (113). Patients with CNS lymphomas had a shorter survival (49 days) as compared to those with extra cerebral lymphomas (149 days) with an overall median survival of 103 days (117). Patients with non-CNS lymphoma had a survival for more than 2 yrs after diagnosis but most of these had a CD4+ T-cell count of

>200 cells/µl and a viral load (HIV RNA) of <400copies/ml (117). HIV associated NHLs which are disseminated at presentation, having a typical B-cell phenotype and harboring EBV in the tumour genome, carry a poor prognosis (49). The overall median survival time of Hodgkin lymphoma in the cohort of those who acquired HIV infection through IV drug abuse and those who acquired through homosexuality appeared to be similar (111). As with general population, DLBCL with c-myc translocation carry a poor prognosis and constitute 10-15% of HIV associated lymphomas (28). The only significant risk factors associated independently with poor overall survival are age ≥40yrs, diagnosis of AIDS before lymphoma, elevated levels of LDH, CD4+ T-cell count <100x10^6/L and impaired performance status. The histological subtypes or the chemotherapy regimens used to treat different subtypes were not associated with prognosis and do not predict the overall survival (22,33). The overall survival in patients with AIDS related lymphoma has significantly improved in the post HAART era which is now comparable to that of the general population with aggressive lymphomas (37,44,60,105). The median survival of HIV infected patients in a large Indian study was 92 months. This prolonged survival leads to increased risk of development of non AIDS defining malignancies more than the AIDS defining malignancies (112) (5) .

2.12. Treatment:

HIV associated lymphomas especially the aggressive forms require intensive chemotherapy. Infusion regimen [EPOCH-R – etoposide, prednisolone, vincristine (oncovin), doxorubicin hydrochloride and rituximab] is considered as highly effective as first line chemotherapy. For relapse, high dose chemotherapy with stem cell support is the

accepted mode of treatment (44)(38). Other most common regimen like CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine and prednisolone) can also be used (118). HAART treatment is associated with immune reconstitution i.e., increase in CD4+ T-cell count (44). Therefore, institution of HAART therapy early in HIV infection before the development of lymphomas has a significant impact on survival (105). In a multicentric study done on HIV infected children with lymphoma, it was observed that around 83% achieved complete remission with intensive first line chemotherapy and none of these developed relapses over a 72 month period of follow up. Those who did not respond to first line chemotherapy had a poor prognosis and died due to the progression of disease (26).

Drug interactions between antiretroviral agents particularly protease inhibitors and chemotherapeutic agents are a major problem and make the communication between HIV treatment provider and haemato-oncologist very important (44). A combination of HAART, use of Rituximab and supportive care has shown improved rates of complete remission and survival in some studies (38).

3. AIMS AND OBJECTIVES

I. To ascertain the frequency and subtypes of HIV associated lymphomas diagnosed in the pathology department of Christian Medical College and Hospital during a five year period, January 2007 till December 2011.

II. To sub classify the types of HIV associated lymphomas using the WHO 2008 classification and to compare this with the lymphomas occurring in the general population during the same period and with the world literature.

III. To find out the association of Epstein Barr virus and Human Herpes Virus 8/Kaposi Sarcoma associated Herpes Virus with these lymphomas by insitu hybridization and immunohistochemical techniques respectively.

IV. To evaluate the clinical profile of these patients including staging, CD4 count, IPI score, prognosis and follow-up.

4. MATERIALS AND METHODS

The study was performed in the Norman Institute of Pathology, Christian Medical College and Hospital, Vellore, India. The study period was 5 years from January 2007 to December 2011.This is a partly retrospective and partly prospective study. All lymphoma cases diagnosed in our department over this five years period were identified and among these only the patients who were positive for HIV by serology (ELISA) were included in this study. The tissue blocks were obtained from the archives of our department and the patients' clinical details were obtained from the clinical workstation and from patients' charts obtained through medical records department or from the biopsy request forms for outside referral biopsies.

4.1. INCLUSION CRITERIA: All patients diagnosed to have lymphoma and who were positive for HIV serology (ELISA) over the period January 2007 to December 2011 were included in this study.

4.2. EXCLUSION CRITERIA:

Patients who were seronegative for HIV, those for whom HIV serology was not done and the outside referral cases for which HIV status were not known, were excluded.

4.3. PARAMETERS EVAULATED:

1. Histological subtype: Based on WHO 2008 classification (31).
2. Immunohistochemistry: This was used to detect EBV LMP1 and HHV8/KSHV by LANA antibody. On review of slides additional CD markers were done whenever necessary to help in classification {Appendix 1}.
3. In situ hybridization: To detect the expression of EBV encoded RNA (EBER) in paraffin sections. {Appendix 2}.
4. Clinical profile: Age, sex, site of involvement and B symptoms.
5. Haematological and lab parameters: Haemoglobin, total and differential leukocyte count, platelet count, LDH level, CSF analysis (cytospin study) and CD4+ T cell count.
6. Tumour staging: Ann Arbor staging {Appendix 3} was done based on the number of nodal and extranodal sites involved, hepatomegaly, splenomegaly and bone marrow infiltration.
7. International Prognostic Index {IPI} for aggressive non Hodgkin lymphomas {Appendix 4} and International prognostic score {IPS} for Hodgkin lymphomas {Appendix 5} were calculated.

All tissue samples were fixed in 10% neutral buffered formalin and processed in an automatic processor for13 hours and embedded in paraffin wax. 4μ tissue sections were taken using a microtome and the slides were incubated and stained with Haematoxylin and eosin (H&E) and mounted with DPX. Immunohistochemistry was done for all cases using Ventana automated immunostainer {Appendix 6}. A primary panel for lymphoma

(CD3, CD20 and MIB1) was done for all cases. For the CD20+ cases (B cell lymphomas) further sub typing were done using additional markers such as CD10, Bcl6, Bcl2, MUM1 and CD79a. For cases that were negative for CD20 with a plasmablastic morphology, CD138 was done. For the cases suspicious for Hodgkin lymphoma, primary panel included, CD3, CD20, CD15 and CD30. Pax5, Oct2 and BOB1 were also done in a few Hodgkin lymphoma cases when a differential diagnosis of anaplastic large cell lymphoma or T-cell/histiocyte rich large B-cell lymphoma needed to be excluded. For cases that were positive for CD3 (T cell lymphomas), CD4 and CD8 subset markers were done. Additional markers done included PD1 and CD21 to rule out angioimmunoblastic T cell lymphoma, CD56 (NK cell antigen) and cytotoxic markers such as Granzyme B for NK/T cell lymphoma. CD30 and Alk were done for anaplastic large cell lymphoma. Tdt was done for cases with blastic morphology. The details of antibodies used including their source, dilution and pretreatment are shown in table 6.

Table 6: Antibodies used in immunohistochemistry:

Antigen Designation	Normal Cellular Distribution	Source *	Dilution **	Pre-treatment
Alk 1	Anaplastic lymphoma kinase -1; T cells	D	1:25	EDTA
Bcl2	Anti-apoptotic factor	D	1:25	Citrate
Bcl6	Germinal centre B cells	L	1:50	EDTA
BOB1	B cell specific Octamer Binding protein-1, Germinal centre B cells (B cell transcription factor)	C M	P	Citrate
CD3	Mature T cell (Pan T cell marker)	D	1:50	EDTA
CD4	Helper T cells	C M	P	EDTA
CD5	T cell and a small subset of B cells	L	1:50	EDTA
CD7	Mature T cells	D	1:25	EDTA
CD8	Cytotoxic T cells and some NK cells	D	1:25	EDTA
CD10	Pre-B cells and germinal centre B cells	D	1:25	EDTA
CD15	Granulocytes, Reed Sternberg cells & variants	D	1:50	EDTA
CD20	Mature B cells (pan B cell marker)	D	1:500	Citrate
CD21	Follicular dendritic cells	D	1:25	Trypsin
CD30	Reed Sternberg cells & variants; activated B and T cells	D	1:50	EDTA
CD43	Pan T-cell marker	D	1:50	Citrate
CD56	Natural Killer cells and a subset of T cells	L	1:50	Citrate
CD79a	Mature B cells	D	1:50	Citrate
CD138	Syndecan1, Plasma cells	D	1:250	Citrate
Granzyme B	Enzyme granules in cytotoxic T cells	D	1:50	EDTA
LCA	All leukocytes (Leukocyte Common Antigen)	D	1:50	Citrate
MIB1	Marker for Ki 67- cell proliferation index	D	1:100	EDTA
MUM1/IRF4	Multiple Myeloma1/Interferon Regulatory Factor4; post germinal centre B cells	D	1:75	EDTA
Oct2	B cell octamer transcription factor	L	1:50	EDTA
PD1	Programmed death-1; germinal centre associated T helper cells	C M	P	EDTA
TdT	Terminal deoxy-nucleotidyl-transferase – pre-B and pre-T lymphoblasts	L	P	EDTA
VS38	Plasma cells	D	1:50	Citrate

* D – Dako, L – Leica and CM – Cell Marq. ** P – Pre-diluted

4.4b. IMMUNOHISTOCHEMICAL TECHNIQUE FOR EBV LMP1 AND HHV8 {Appendix 1}**:** Association of EBV and HHV8/KSHV was studied using immunohistochemical technique to detect the EBV LMP1 and LANA protein respectively. Immunohistochemistry was done in 62 cases for which the tissue were adequate. Hodgkin lymphoma was used as the positive control for EBV LMP1. Kaposi sarcoma in an HIV positive individual was used as the positive control for HHV8-LANA. This procedure was done manually using the Envision technique/two step polymer method. The primary antibodies used for LMP1 for EBV and LANA for HHV8 are shown in table 7. Cytoplasmic granular staining was considered as positive for EBV LMP1 and nuclear staining was considered as positive for LANA.

Table 7: Primary antibodies

Protein	**Antibody**	**Source**	**Dilution**	**Pretreatment**
EBV – Latent Membrane Protein 1 (LMP 1)	Mouse monoclonal	DAKO	1:200	EDTA (1mmol/L at pH 9.0)
KSHV/HHV8 – Latent Nuclear Antigen (LANA)	Mouse monoclonal	NOVOCASTRA	1:25	CITRATE (0.01M at pH 6.0)

4.4c. INSITU HYBRIDIZATION TECHNIQUE {Appendix 2}**:** This procedure was done for detecting EBER using Benchmark XT autostainer, Ventana Medical Systems. The probe used was INFORM EBER Probe 800-2842 from Ventana Medical Systems. It is a fluorescein-labelled oligonucleotide which has specific affinity to the target sequence. This hybridized site was detected using VENTANA ISH *i*VIEWBlue Detection Kit, which

shows a nuclear staining (blue precipitate) that was readily visualised under light microscopy.

4.5. STATISTICAL METHODS:

1. Data entry was done using epi data software.
2. Data analysis was done using SPSS soft ware.
3. To compare the subtypes of lymphoma with HIV negative population two proportion test was used.
4. Pearson correlation was used to find the correlation of CD4 with the patients' status.
5. Log rank statistics was used for assessing the outcome predictors such as IPI score and staging.
6. Kaplan Meir survival curve was plotted for survival analysis.

5. RESULTS

A total of 3346 lymphomas were diagnosed during a 5 year period 01/01/2007 to 31/12/2011 in Normal Institute of Pathology, Christian Medical College and Hospital, Vellore. Of these 73 patients (2%) were found to be positive for HIV infection by serology (ELISA). The rest of them were seronegative or biopsies from outside hospitals with HIV status unknown.

5.1. Age and Sex ratio: The median age of presentation was 40 years {range 8-66 years}. There was a definite overall male preponderance, males – 74% (n=54); females – 26% (n=19) with male to female ratio of 2.8:1 [Fig. 3].

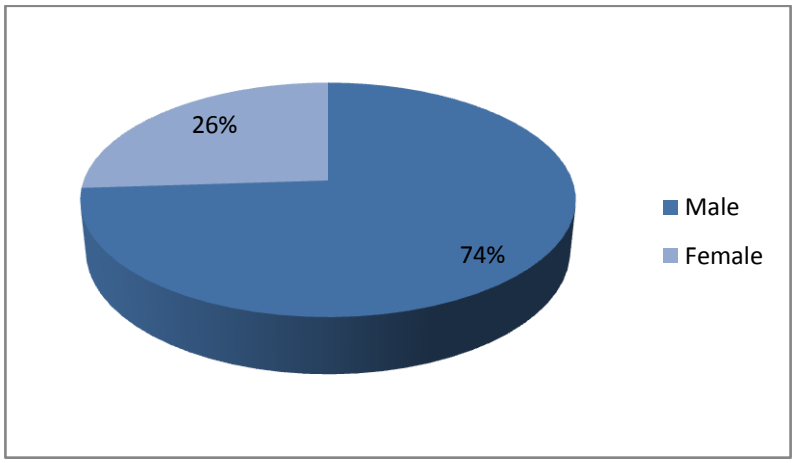


Figure 3: Proportion of males and females in all HIV associated lymphomas

5.2. Sites of involvement: These lymphomas most commonly involved lymph node {56% (n=41)} as the primary site of presentation and a high frequency of extranodal sites as well {44% (n=32)} [Fig. 4]. Among the nodal sites, axillary {32.5% (n=13)} was the most common site, followed by cervical {30% (n=12)}, abdominal {15% (n=6)}, inguinal {5% (n=2)}, supraclavicular {2.5% (n=1)} and tonsillar lymph node {2.5% (n=1)}. Lymph node site was not known in 5 cases {12.5% (n=5)} [Table 8]. Among the extranodal sites, the most common primary sites were the gastrointestinal tract and liver 19% each (liver, n=6; stomach, n=3; small intestine, n=2; large intestine, n=1), followed by soft tissue 16% (chest wall, n=2; trunk, n=1; thigh, n=1, retroperitoneum, n=1) upper aerodigestive tract 13% (n=4), oral cavity 9% (n=3) and other less common sites include ischiorectal fossa, orbit, pleura, submandibular region, perianal region and omentum, constituting 3% each (n=1 each) [Table 9 & Fig. 5].

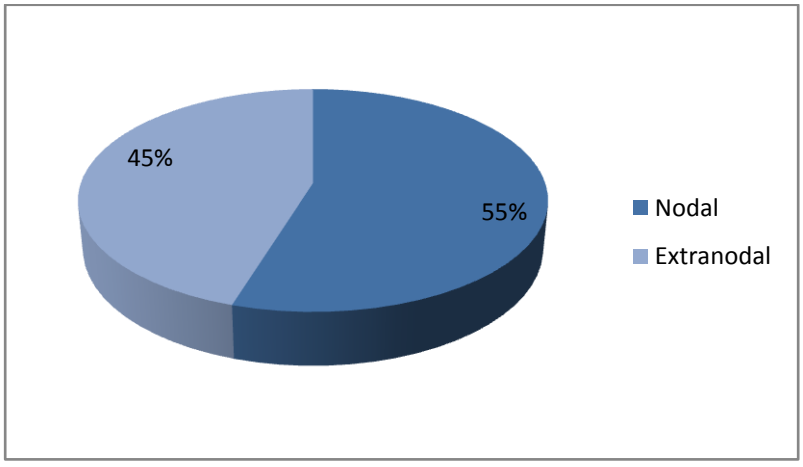


Figure 4. Sites of involvement

Table 8: Nodal - Sites of involvement

Nodal site	Number of cases	Percentage (%)
Axillary	13	32.5
Cervical	12	30
Abdominal	6	15
Inguinal	3	5
Supraclavicular	1	2.5
Tonsillar	1	2.5
Unknown	5	12.5
Total	**41**	**100**

Table 9: Extranodal - Sites of involvement

Extranodal site	Number of cases	Percentage (%)
Liver	6	19
Gastrointestinal tract	6	19
Soft tissue	5	16
Upper aerodigestive tract	4	13
Oral cavity	3	9
Ischiorectal fossa	2	6
Omentum	1	3
Orbit	1	3
Pleura	1	3
Perianal region	1	3
Submandibular region	1	3
Unknown	1	3
Total	**32**	**100**

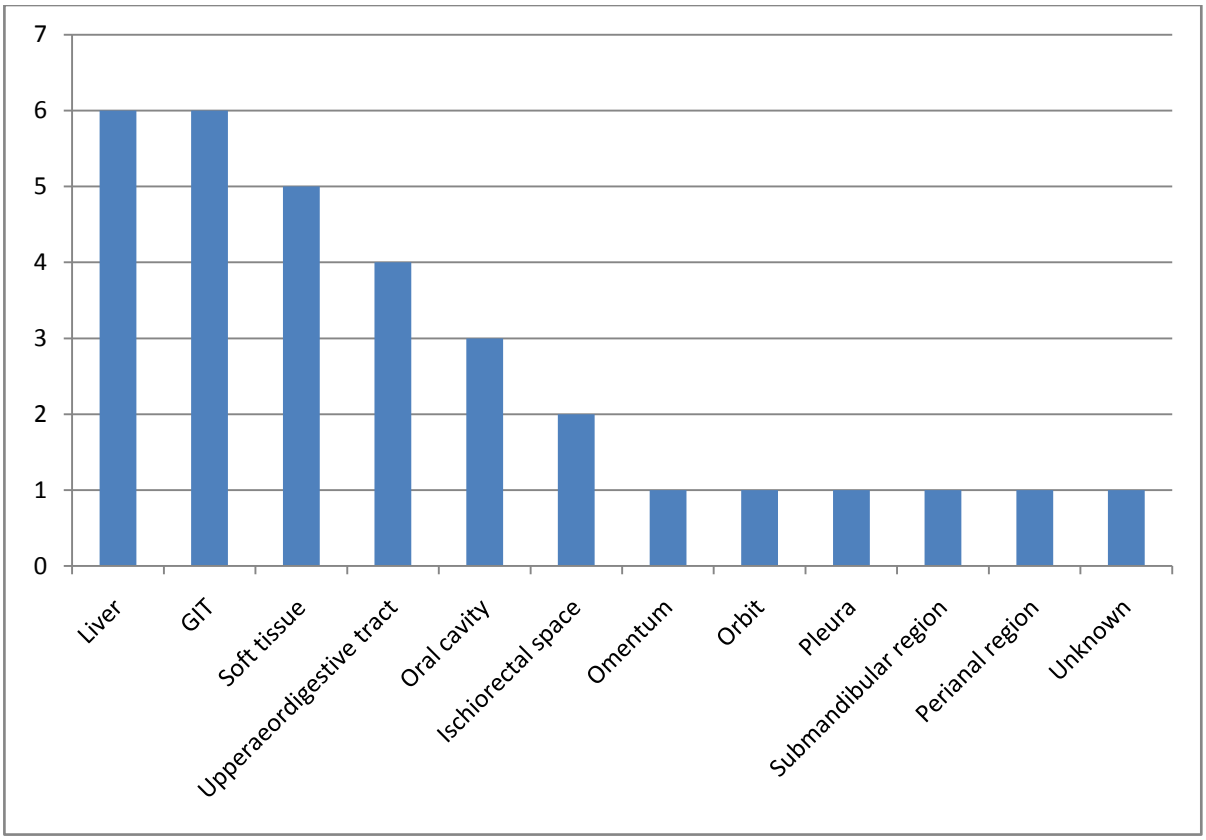


Figure 5. Extranodal sites of involvement

5.3. Clinical presentation and laboratory findings: B symptoms were present in 78% (n=57) of the patients. Hepatomegaly was seen in 50% (n=34) and splenomegaly in 47% (n=32) of the cases. Marrow involvement at presentation was seen in 37% (n=18) of all lymphomas; 87% (7 of 8 cases) in Hodgkin lymphoma and 27% (11 of 41 cases) in non Hodgkin lymphoma. Serum LDH level was elevated in 89.5% of the cases with a mean value of 2356 IU/L (n=51). The mean CD4+ T-lymphocyte count was 195 cells/µL {Range = 14 – 872}. The mean CD4 count was lowest in plasmablastic lymphoma and highest in DLBCL. [Fig. 6 & Table 10]. Clinical features of individual lymphomas are shown in table 11.

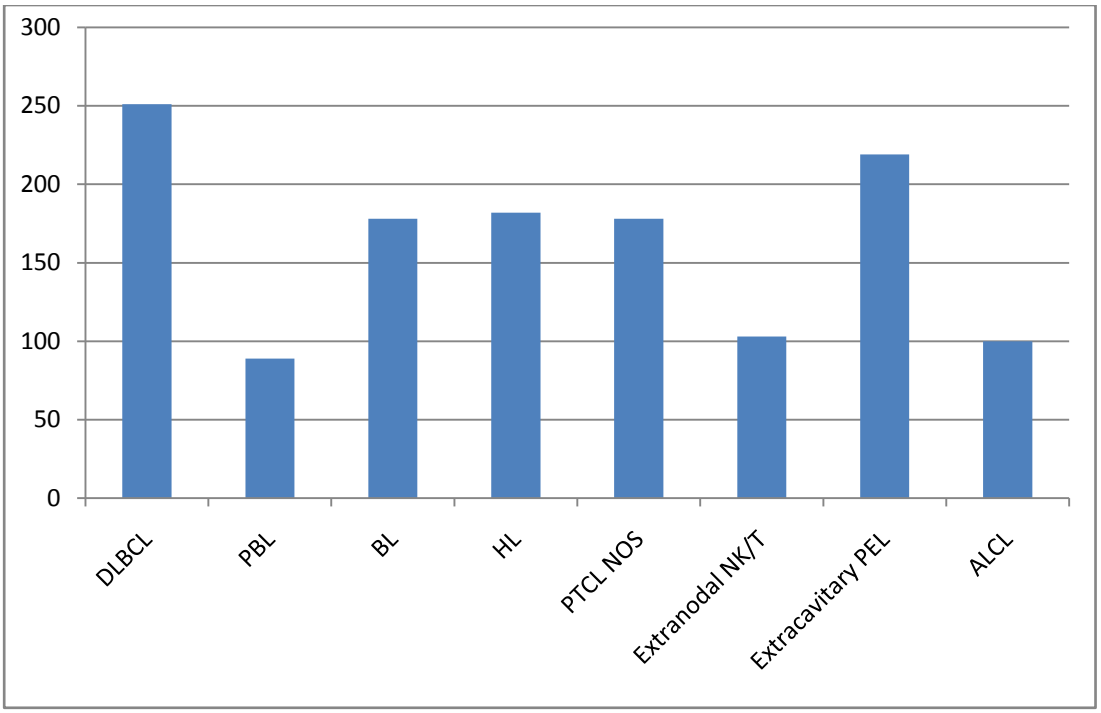


Figure 6. CD4+ T-lymphocyte count in different subtypes of lymphomas

Table 10. CD4+ T-lymphocyte count in different subtypes of lymphomas

Subtypes of lymphoma	**Mean CD4 count (cells/µL)**
DLBCL	251
PBL	89
BL	178
HL	182
PTCL NOS	178
Extranodal NK/T	103
Extracavitary PEL	219
ALCL	100

Table 11. Clinical presentation and laboratory parameters of HIV associated lymphoma

Clinical Details	**DLBCL**	**PBL**	**BL**	**HL**	**PTCL NOS**	**Extranodal NK/T**	**ALCL**	**Extracavitary PEL**	**HGNHL**
Median age in years	41	42	41	34	41	41	37	66	17
Range in years	(8-54)	(30-61)	(26-59)	(21-60)	(35-45)				
Sex {M:F}	1.4:1	11:1	10:1	3.5:1	3 (male)	Male	Male	Male	Female
Hepatomegaly	47%	44%	33%	78%	33%	--	Present	Present	Present
Splenomegaly	50%	33%	22%	78%	33%	--	--	Present	--
Marrow involvement	21%	50%	29%	78%	33%	--	--	--	--
Elevated LDH	87%	100%	100%	86%	50%	690	698	247	1059
Median (IU/L)	(831)	(1916)	(2397)	(686)	(449)				
Median CD4 T-cell count(cells/µL)	149	52	189	77	109	103	100	219	375
Stage III/IV	74%	50%	55%	89%	100%	II	IV	II	III
B symptoms	82%	50%	73%	89%	100%	Present	Present	Present	Present

5.4. Subtypes of lymphomas: Non Hodgkin lymphoma {87.6%; n=73} was more common than the Hodgkin lymphoma {12.4%; n=9} [Fig. 7]. Of the 73 HIV positive patients, diffuse large B cell lymphoma was the most common subtype {46.6%; n=34 cases}, plasmablastic lymphoma {16.4%, n=12 cases}, Burkitt lymphoma {15%, n=11 cases}, peripheral T cell lymphoma NOS {4%, n=3 cases}, high grade non Hodgkin lymphoma, unclassified {1.4%, n=1 case}, extracavitary PEL {1.4%, n=1 case}, Extranodal NK/T-cell lymphoma {1.4%, n=1 case} and anaplastic large cell lymphoma {1.4%, n=1 case}. Hodgkin lymphoma constituted 12.4% (n=9) of cases. Of these the commonest subtype was mixed cellularity (MC), 89% (n=8 cases) followed by nodular sclerosis (NS), 11% (n=1 case) [Fig. 8]. There were no cases of lymphocyte depleted, lymphocyte rich or nodular lymphocyte predominant type Hodgkin lymphoma. In all subtypes, male predominance was seen [Fig. 9].

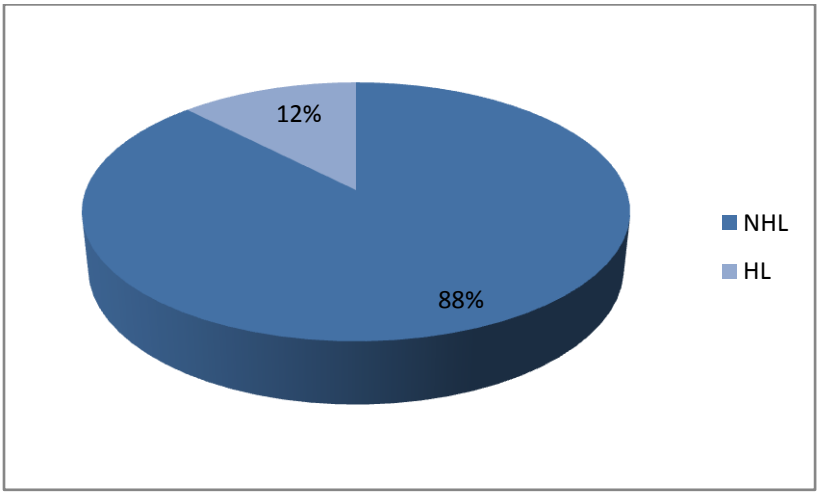


Figure 7: Relative frequencies of major subtypes of lymphomas

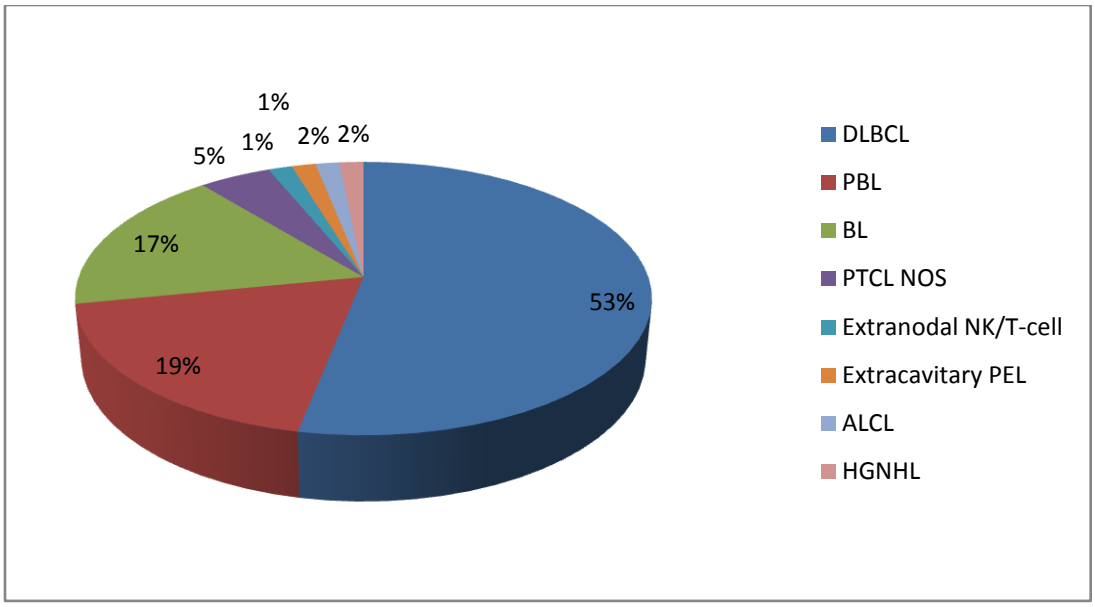


Figure. 8a: Relative frequencies of NHL - subtypes

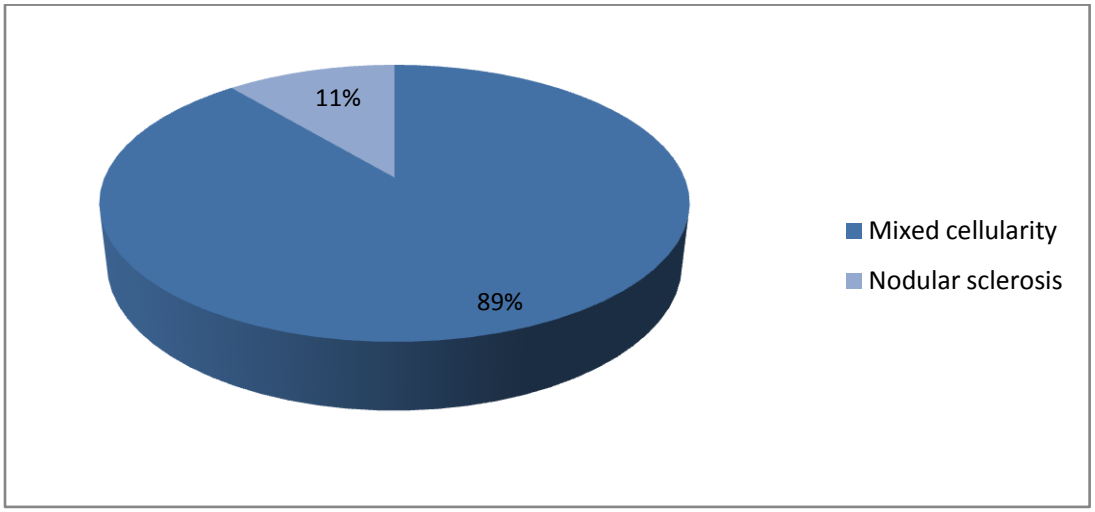


Figure. 8b: Relative frequencies of Hodgkin lymphoma - subtypes

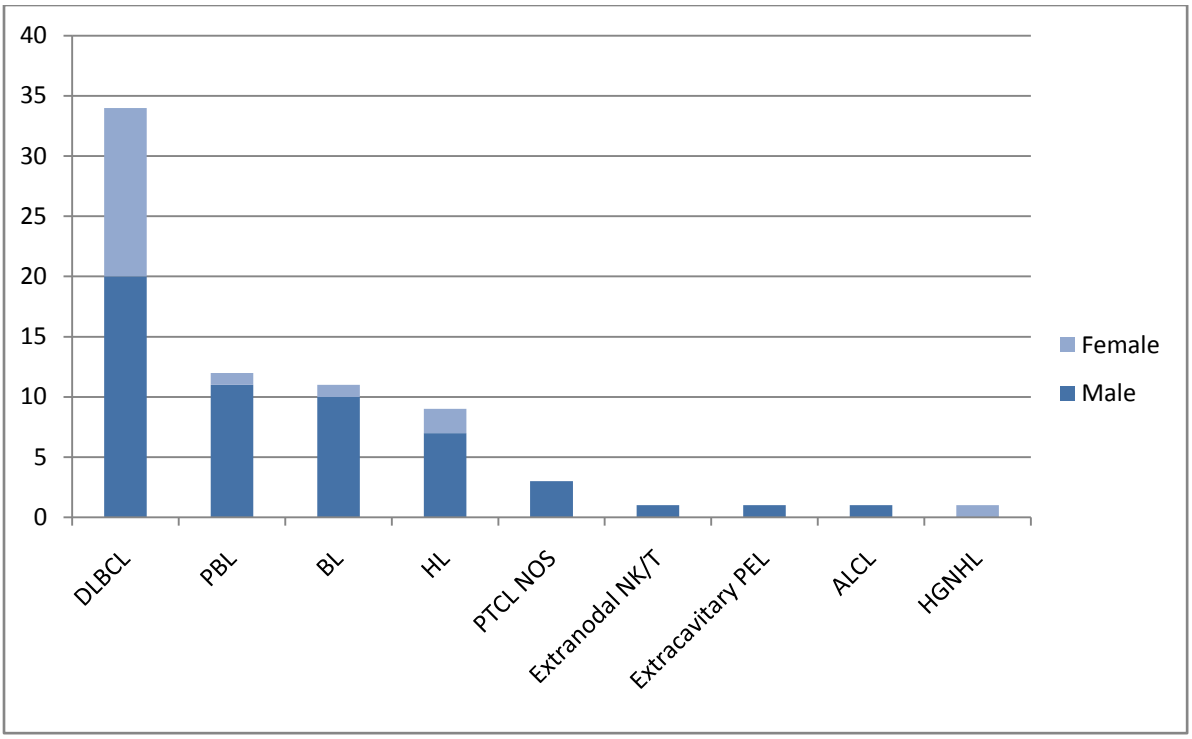


Figure 9: Frequency of lymphoma subtypes with gender distribution

5.5. EBV and KSHV association: EBER in situ hybridization was done in 60 cases. Among these 47% {n=28} were positive and 53% {n=32} were negative. EBV LMP1 immunohistochemistry was done in 62 cases, among which 23% {n=14} were positive and 77% {n=48} were negative. EBV LMP1 was positive in only 13 of 28 cases that were positive for EBER-ISH and the tissue was inadequate for EBER-ISH in the 14th case that showed positivity for EBV LMP1. The overall positivity for EBV by EBV LMP1 and EBER-ISH was 47% {29 of 62 cases}. Of these 20 cases were NHLs {38% positivity; 20 of 53 cases of NHL} and 9 cases were Hodgkin lymphoma {100% positivity; all 9 cases of HL} [Fig.10]. Among the different subtypes of NHLs, the proportion of cases that were positive for EBV was as follows; 8 of 27 (29.6%) DLBCLs, 1 of 10 (10%) Burkitt lymphoma, 9 of 10 (90%) plasmablastic lymphoma, 1 of 2 (50%) PTCL NOS, one extranodal NK/T-cell lymphoma and

one extracavitary PEL. All 8 cases (100%) of Hodgkin lymphoma tested for EBV were positive.

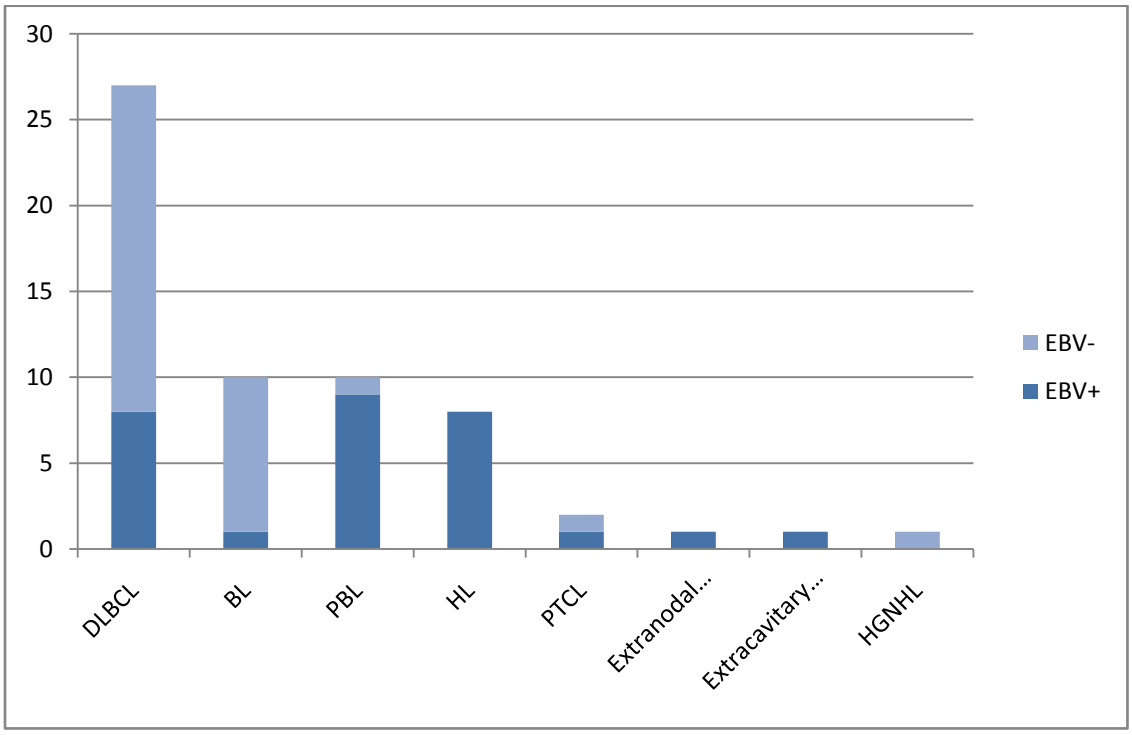
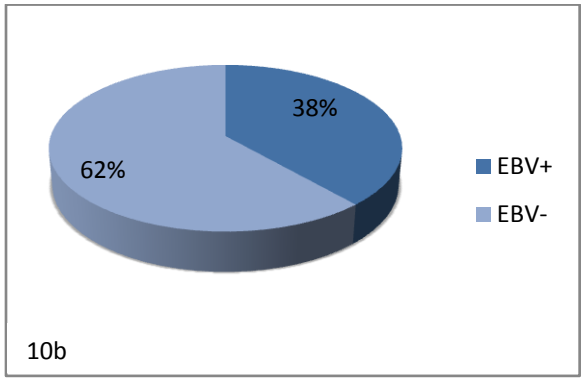


Figure 10a: Proportion of EBV positive cases in different subtypes of lymphomas



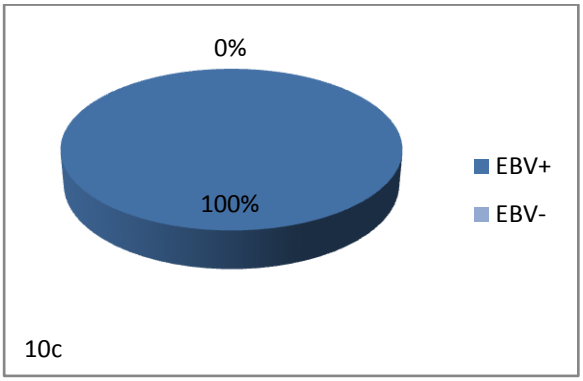


Figure 10b & 10c: Proportion of EBV positive cases in Non Hodgkin and Hodgkin lymphomas

KSHV-LANA immunohistochemistry was done in 62 cases, out of which only one {1.6%} was positive and the rest were negative. This single positive case was extracavitary PEL involving the stomach and small intestine.

5.6. Staging & prognostic scoring: Staging was done for 72 patients. Among this 69% (n=50) of the patients presented with wide spread disease (stage III/IV) and 31% (n=22) presented at an early stage (stage I/II). Of the 50 patients with advanced stage, 33 were stage IV disease and 17were of stage III. Of the 22 patients, 8 were stage II and 14 were stage I. In one case, staging was not done. Among the patients with Hodgkin lymphoma, 89% (n=8; stage IV-7; stage III-1) of the cases presented with advanced stage. Among the patients with non Hodgkin lymphoma, 67% (n=42; stage IV-26; stage III-16) of the cases presented with high stage. [Fig. 11].

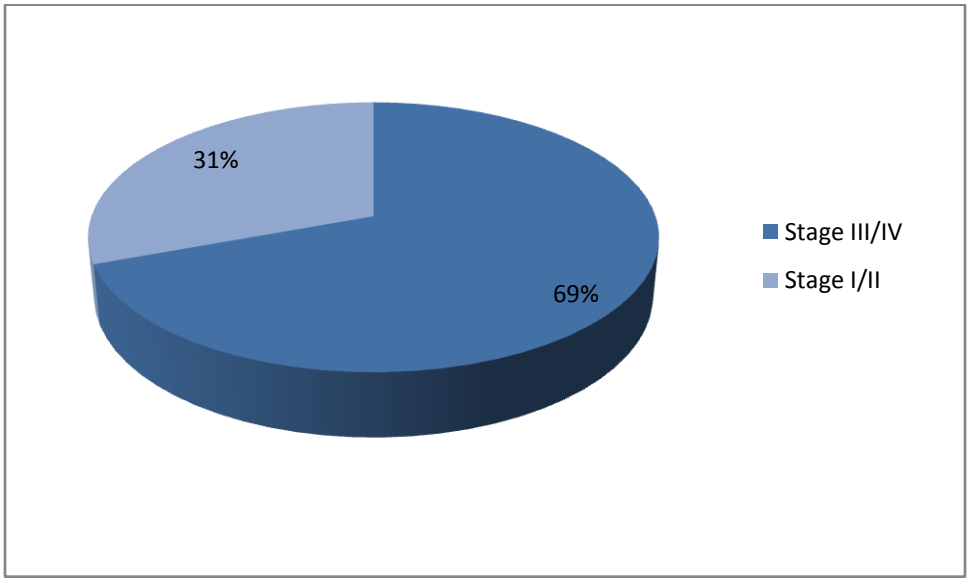
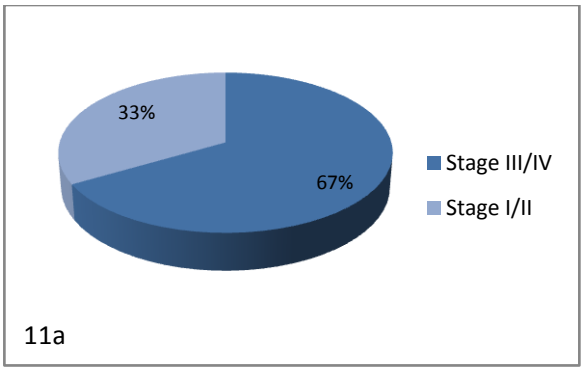


Figure 11: Stage of disease at presentation [All HIV positive lymphomas]



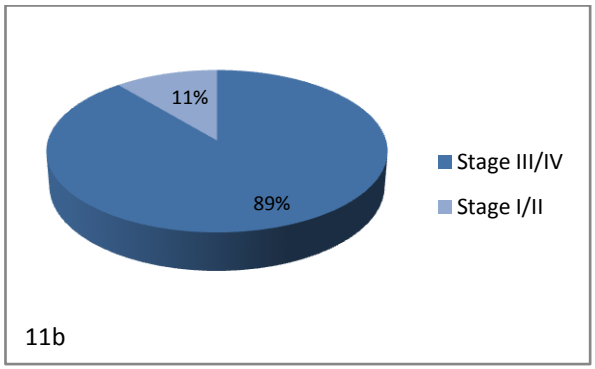


Figure 11a. Stage of disease at presentation-NHL

Figure 11b. Stage of disease presentation - Hodgkin lymphoma

IPI scoring was done only in 54 of 64 NHLs. Of these 54% (29 of 54 cases) had high/high intermediate score and 46% (25 of 54 cases) had low/low intermediate score. IPSS score for Hodgkin lymphoma were done in all 9 cases. Of these 55.6% of patients had a poor risk (n=5), 44.4% had a fair risk (n=4) and none of them had good risk.

5.7. Prognosis: Follow up was available in 36 of 73 patients. The clinical course was aggressive in most patients. Thirteen patients (36%) died during the disease course, among which 12 died within first three months and 1 died in 6 months. Ten patients are in remission until the recent follow-up with a maximum survival period of 5 years after diagnosis. One patient developed CNS relapse at 18 months. Five patients had progressive disease and lost to follow up. Rest of the 7 patients were also lost to follow up (at 3, 6 and 12 months) but were in remission until the last day of visit.

5.8. DIFFUSE LARGE B-CELL LYMPHOMA

5.8A. Frequency: DLBCL was the most common subtype of all HIV associated lymphomas and constituted 45.4% {34 of 73 cases}.

5.8B. Age and Sex ratio: The median age at presentation was 41 years {range 8-54 years}. There was a slight male preponderance with male to female ratio 1.4:1 {20 males and 14 females}. This included two children; 8yr and 14yr old female and male respectively.

5.8C. Sites of involvement: Lymph node was the primary site in 62% {21 cases}, among which cervical and axillary nodes were more common {32% each; cervical – 6 cases, axillary – 6 cases), followed by abdominal {2 cases}, inguinal {2 cases} and tonsillar {1 case}. The primary site was extranodal in 39% {13 cases}, among which the most common site was liver 31% {4 cases} followed by upper aerodigestive tract 23% {3 cases}, GIT 15% {2 cases; stomach and small intestine}, submandibular gland {1 case}, iliac fossa {1 case}, ischiorectal space {1 case} and retroperitoneum {1 case}.[Fig. 12]. Multiple lymph node involvement was seen in approximately 50% {17 cases}. Marrow involvement was seen in 21% {5 of 24 cases}. For the rest of the cases marrow examination was not done. CSF examination was done in 15 cases and secondary involvement of CNS was present in 20% {3 of 15 cases}.

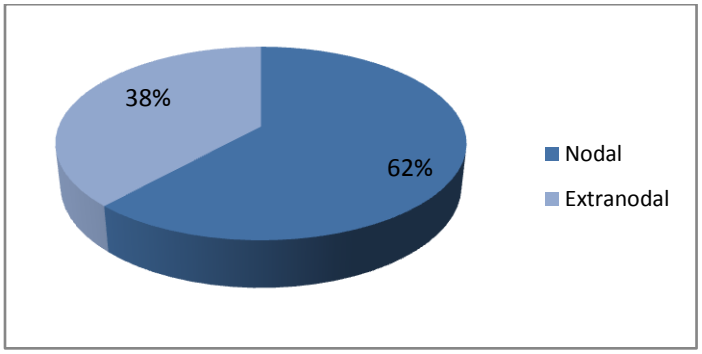


Figure 12: DLBCL – Sites of involvement

5.8D. Clinical presentation and laboratory findings: B symptoms were seen in 82% {28 of 34 cases}. Hepatomegaly was seen in 47% {16 of 34 cases} and splenomegaly in 50% {17 of 34 cases}. Elevated LDH was seen in 87% {26 of 30cases} with a range of 153 to 16,100 IU/L and the median CD4+ T cell count was 149 cells/μL {range = 16-872}.

5.8E. Ann Arbor Staging and International prognostic index {IPI}: 74% of patients {25 of 34 cases; Stage IV – 12 cases; Stage III – 13 cases} presented with stage III/IV disease. Nine {26%} patients presented with early stage of disease {Stage I – 6 cases; Stage II – 3 cases}. IPI scoring was done in only 30 patients. Of these 14 patients (47%) were in low/low intermediate risk and 16 patients (53%) were in high/high intermediate risk.

5.8F. Morphology: Lymph nodes showed effacement of architecture in all cases due to a diffuse infiltrate of large sized lymphoid cells with moderate amounts of amphophilic cytoplasm, round to oval vesicular nuclei with 2-3 membrane bound nucleoli resembling centroblasts in 26 cases [Fig. 13]. One case showed abundant pale to clear cytoplasm [Fig. 14]. There were 6 cases of immunoblastic type [Fig. 15] with the large cells having vesicular nuclei with single centrally placed prominent nucleolus, out of which one showed plasmacytoid features [Fig. 16]. Anaplastic morphology with pleomorphic large cells, some resembling Reed-Sternberg like cells were seen in 2 cases [Fig. 17]. The frequencies of various histological subtypes are shown in figure 18.

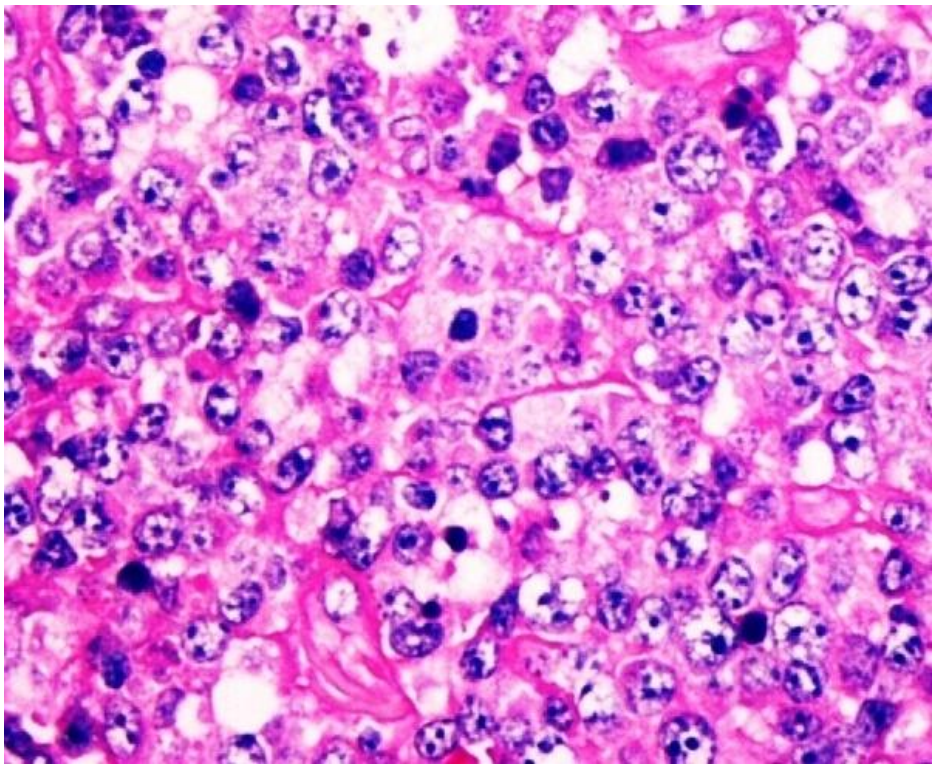


Figure 13: Diffuse large B cell lymphoma – Centroblastic type – Centroblasts with moderate amounts of amphophilic cytoplasm, vesicular nuclei and 2-3 membrane bound nucleoli *H&E 400x*

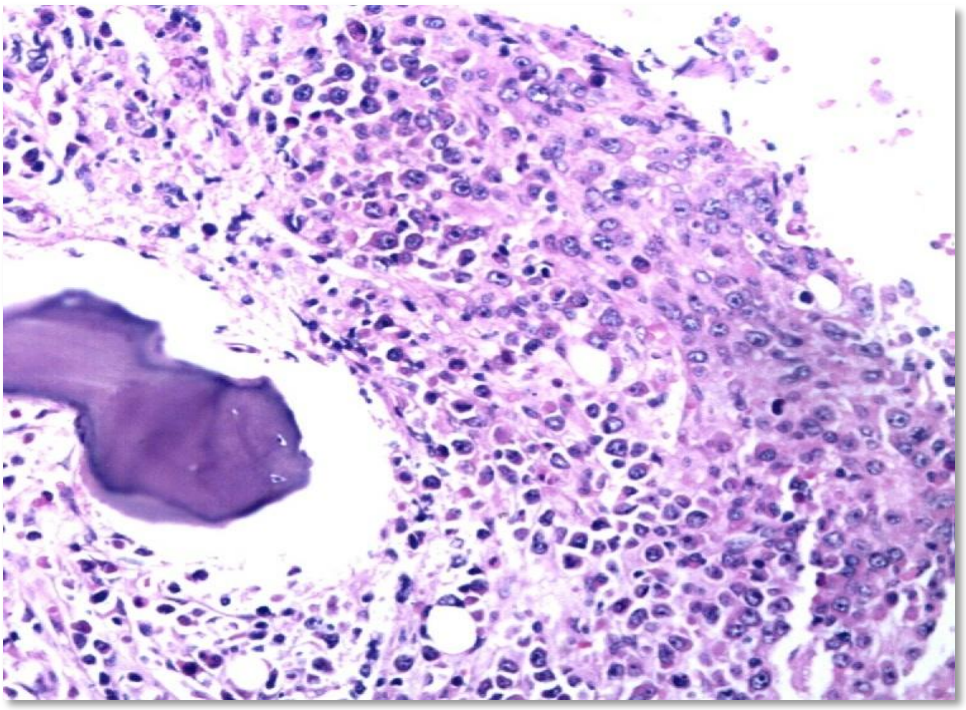


Figure 13a: Diffuse large B-cell lymphoma – Centroblasts infiltrating the bone marrow *H&E 200x*

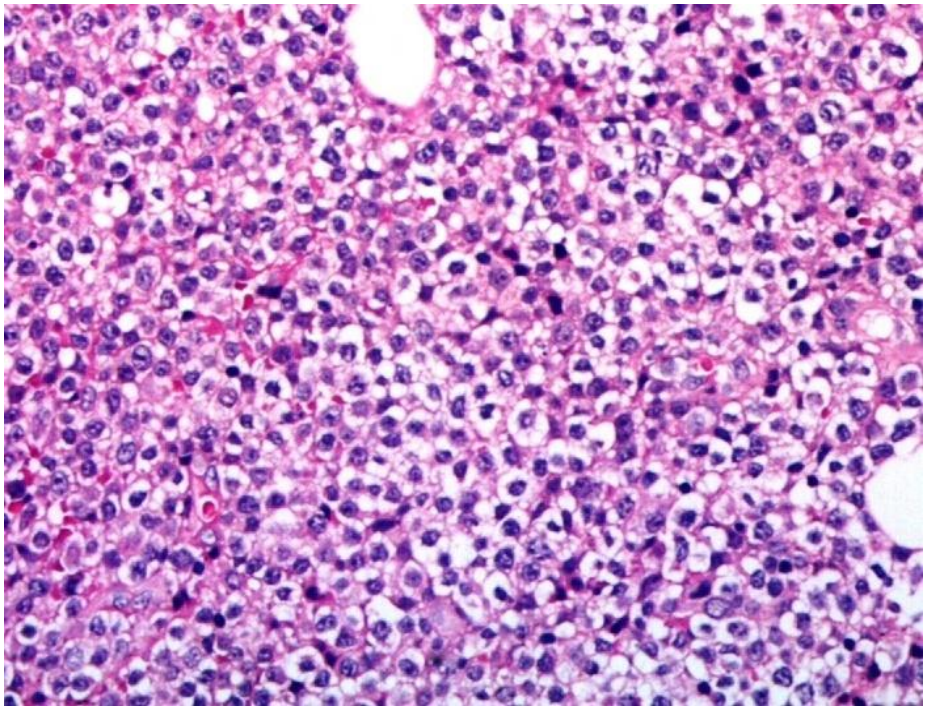


Figure 14: Diffuse large B-cell lymphoma – clear cell type with abundant pale to clear cytoplasm *H&E 200x*

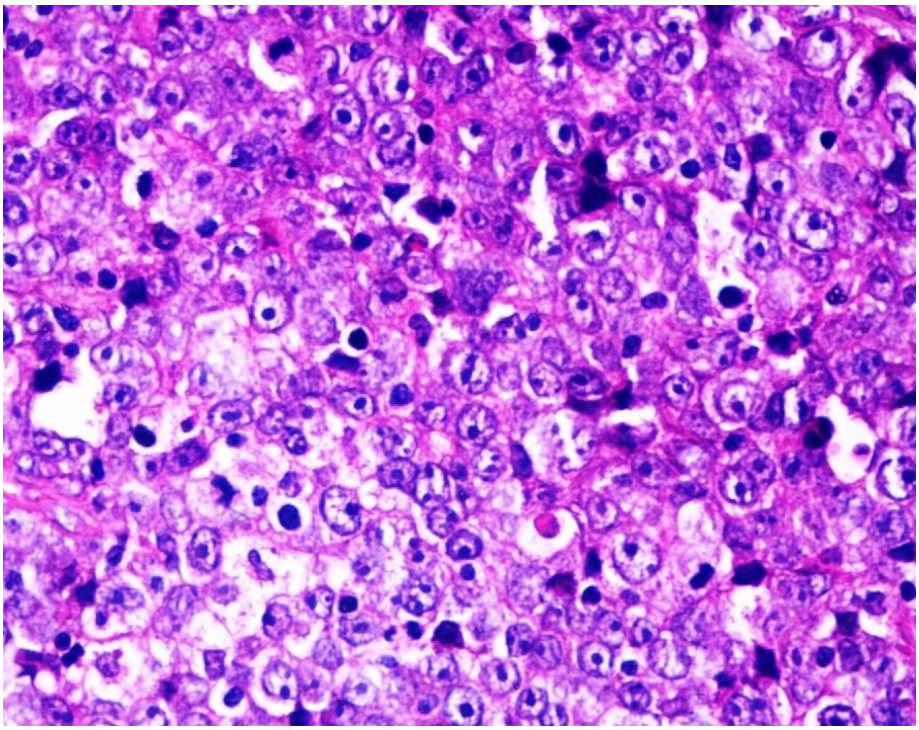


Figure 15: Diffuse large B-cell lymphoma – Immunoblastic type; Immunoblasts with moderate amounts of amphophilic cytoplasm, vesicular nuclei and single centrally placed prominent nucleoli *H&E 400x*

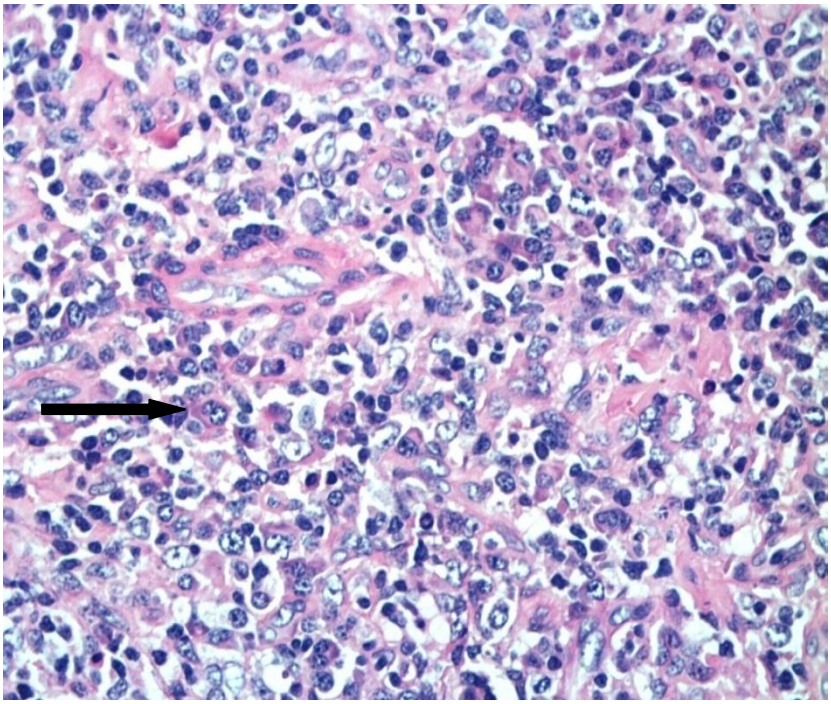


Figure 16a: Immunoblasts and plasmacytoid cells (arrow) *H&E 400x*

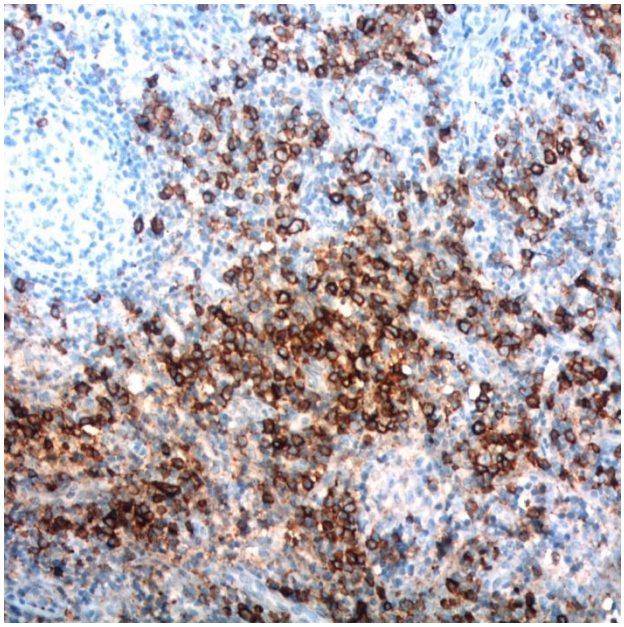


Figure 16b: CD138 positivity in plasmacytoid cells *200x*

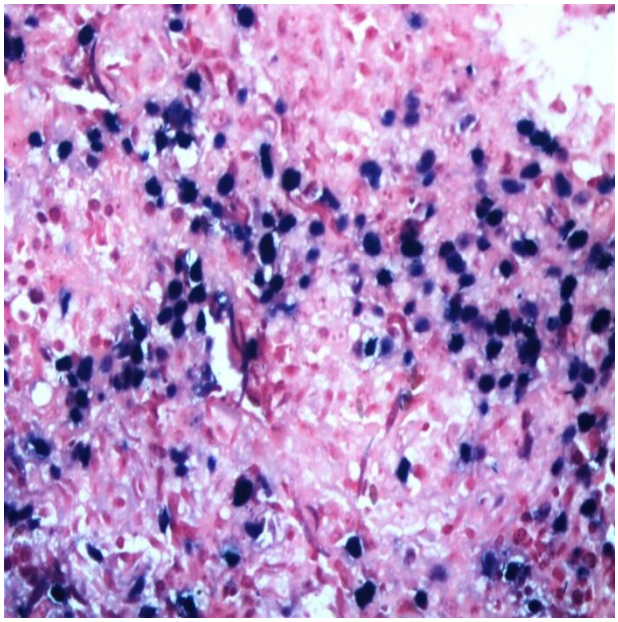


Figure 16c: EBER-ISH – Nuclear positivity in neoplastic cells *400x*

Figure 16: Diffuse large B cell lymphoma – Immunoblastic type with plasmacytoid differentiation

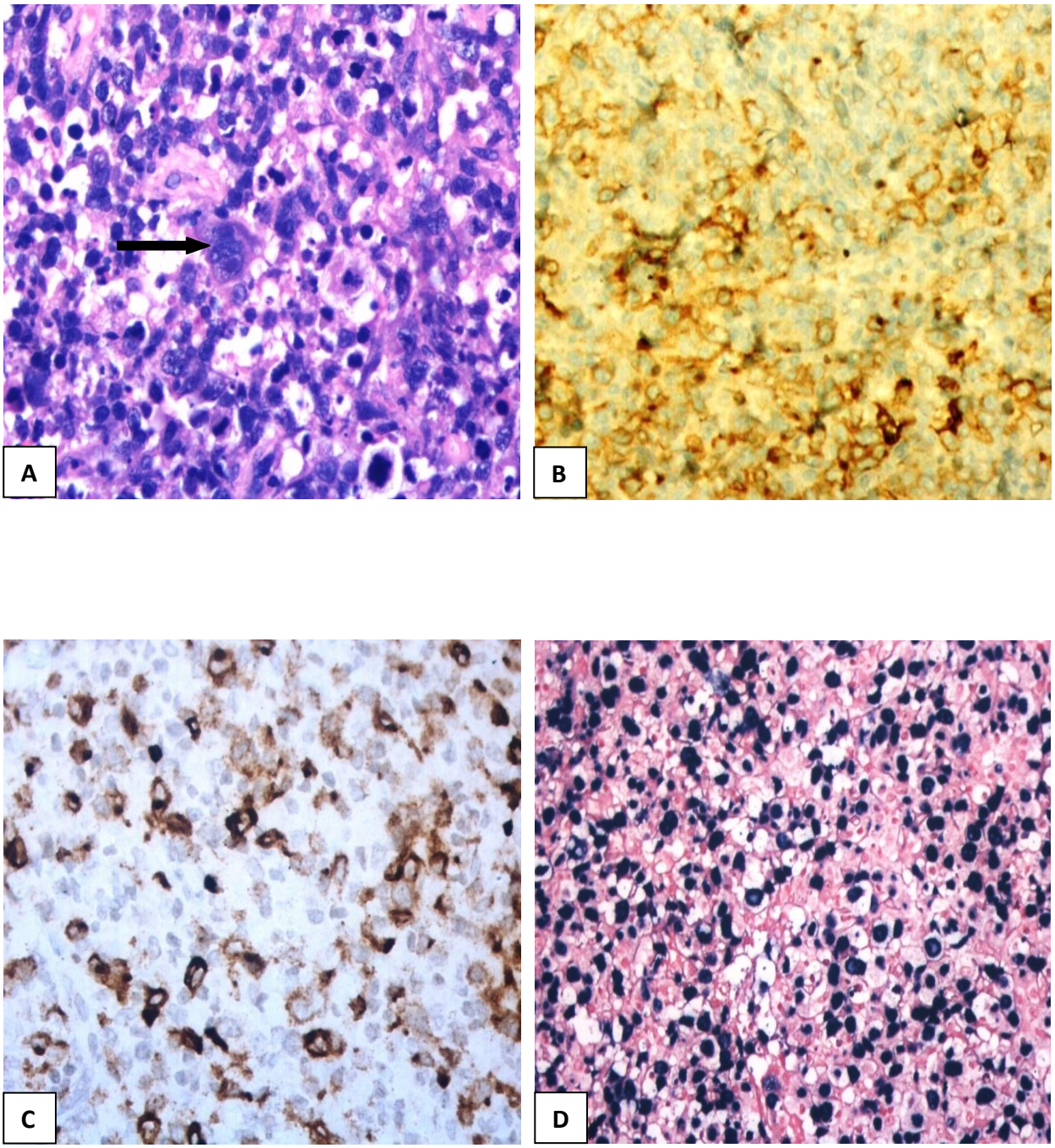


Figure 17: Diffuse large B cell lymphoma – Anaplastic type – Morphology and immunophenotype; A) Multinucleate bizarre cell with prominent nucleoli (arrow) H&E *400x;* B) Membrane staining for CD30 *200x;* C) Cytoplasmic granular staining for EBV LMP1 *200x;* D) Nuclear positivity for EBER-ISH *200x*

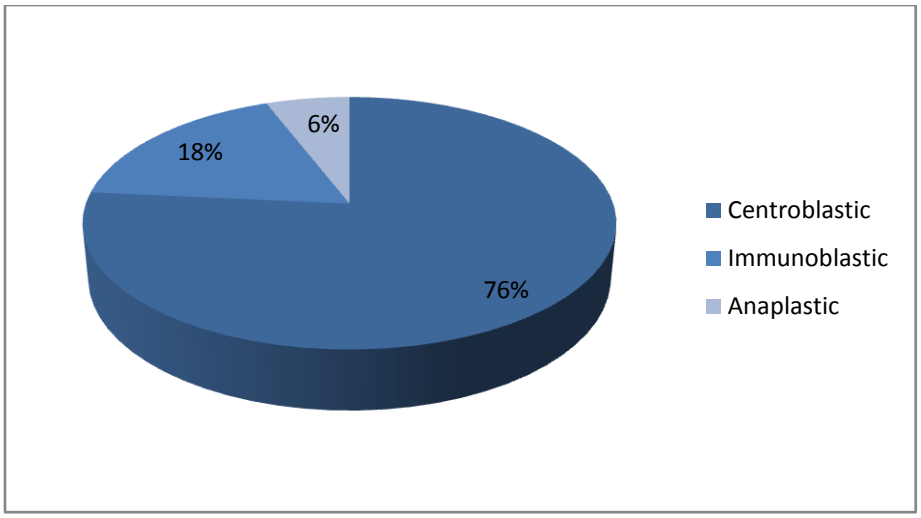


Figure 18: DLBCL – Morphological subtypes

5.8G. Immunohistochemistry: All the cases were CD20+ [Fig. 19a] with MIB1 [Fig. 19b] labeling index of 80-100% (median – 95%). The immunoblastic type with plasmacytoid differentiation showed positivity for CD138 [Fig. 16b]. The anaplastic type showed positivity for CD30 [Fig. 17b].

5.8H. EBV&KSHV: EBV association was found in 30% {8 of 27 cases} by EBER-ISH technique. Of these 3 cases were of immunoblastic variant (3 of 6 cases; including the case with plasmacytoid features [Fig. 16c]), 3 cases were centroblastic [Fig. 19c] (3 of 26 cases) and 2 cases were anaplastic (2 of 2 cases). EBV LMP1 was negative in 5 of these 8 cases. Three cases which were positive for EBV LMP1 were, 2 cases of anaplastic variant [Fig. 17c] and one case of immunoblastic variant. Frequency of association of EBV in different variants is shown in Fig. 20. KSHV LANA was negative in all the cases.

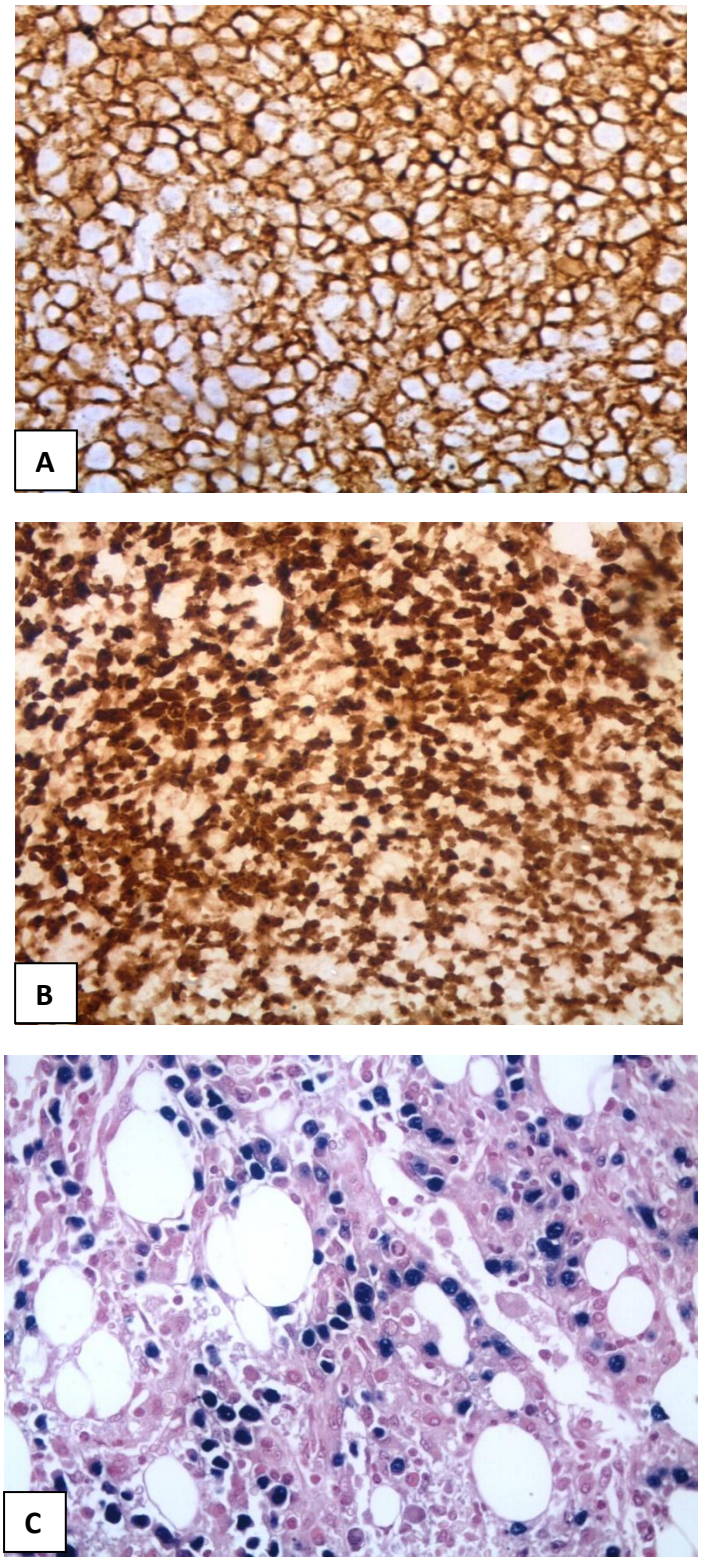


Figure 19: Diffuse Large B-cell lymphoma – Immunohistochemistry; A) Membrane staining – CD20 *400x;* B) Nuclear staining – MIB1 *400x;* C) Bone marrow - EBER-ISH - nuclear staining *400x*

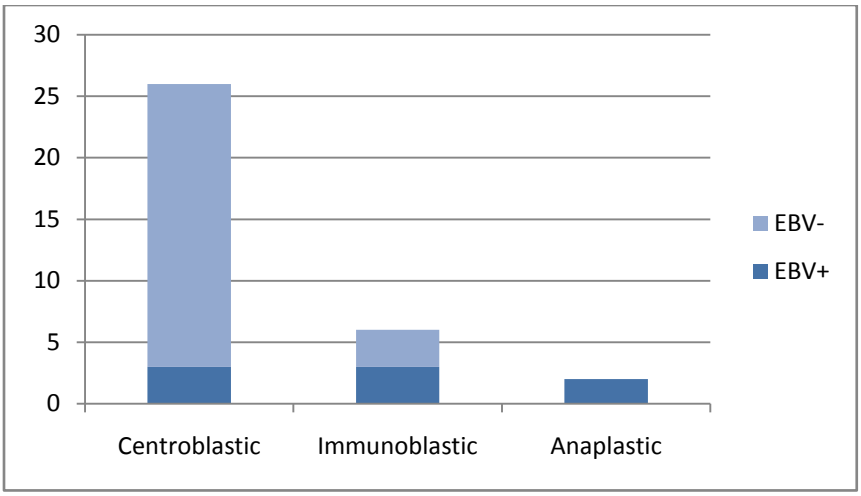


Figure 20: Proportion of EBV positivity in different subtypes of DLBCL

5.8I: Prognosis: Follow up was available in only 17 patients, out of which 4 patients [3 are stage IV] died within 3 months. One had CNS relapse after 18 months. Among the rest 5 had progressive disease and lost follow up later. Three patients are in remission until recent follow up date with a maximum survival period of 4 years.

Childhood DLBCL: Two children (8yr old female and 14 yr old male) had centroblastic type of DLBCL. The 8yr old child presented at stage IVB disease with generalized lymphadenopathy, hepatosplenomegaly, B symptoms and bone marrow involvement. This child had a very high LDH value of 5959 IU/L and the trephine biopsy showed positivity for EBER-ISH. This child died within 3 months of diagnosis inspite of taking chemotherapy. The other 14yr old male child presented at stage II with B symptoms and negative for EBER-ISH. Follow up was not available for this patient.

Other associated neoplasms: Two of the female patients with DLBCL also had cervical intraepithelial neoplasia.

5.9. PLASMABLASTIC LYMPHOMA

5.9A. Frequency: Plasmablastic lymphoma constituted a significant proportion of HIV associated lymphomas, 16% {12 of 73 cases} forming the second commonest type.

5.9B. Age and Sex ratio: The median age at presentation was 42 years {range 30-61 years}. There was a definite male preponderance with male to female ratio 11:1 {11 males and 1 female}.

5.9C. Sites of involvement: The most common site of involvement was extranodal, constituting 92% {11 of 12 cases}. [Fig. 21]. Among these the most common site was oral cavity, 27% {3 of 11 cases}, followed by chest wall {2 cases}, large intestine, trunk, pleura, ischiorectal fossa and perianal region {1 case each}. [Table 12]. In one case only bone marrow biopsy was available and the primary site was not known. Lymph node {abdominal} was the primary site in one case {8%}. Multiple lymph node involvement was seen in approximately 25% {3 cases}. Marrow involvement was seen in 50% {3 of 6 cases}.

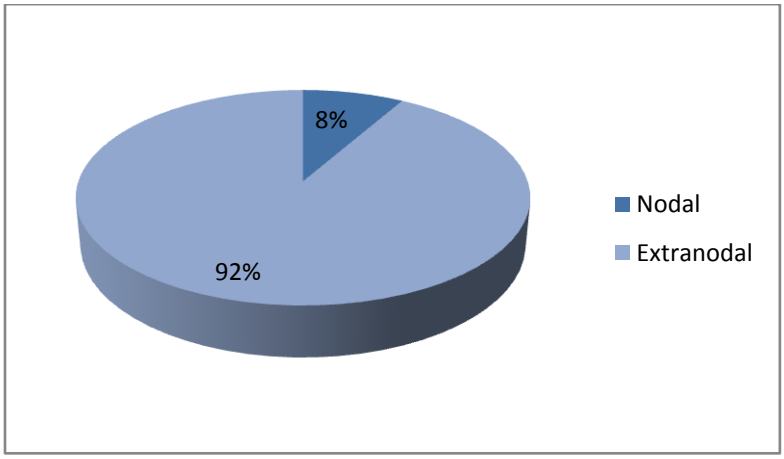


Figure 21: Plasmablastic lymphoma – Sites of involvement

Table12: Plasmablastic lymphoma – extranodal sites

Extranodal sites	Number of cases	Percentage (%)
Oral cavity	3	28
Chest wall	2	18
Large intestine	1	9
Trunk	1	9
Pleura	1	9
Ischiorectal fossa	1	9
Perianal region	1	9
Unknown	1	9

5.9D. Clinical presentation and laboratory findings: B symptoms were seen in 42% {5 of 12 cases}. Hepatomegaly was seen in 44% {4 of 9 cases} and splenomegaly in 33% {3 of 9 cases}. Elevated LDH was seen in all cases {100%} with a mean value of 1916 IU/L {range = 587 to 8889}. The median CD4+ T cell count was 52 cells/μL {range = 18-285}.

5.9E. Ann Arbor Staging and International prognostic index {IPI}: 55% {6 of 11 cases} presented with stage IV disease. Five {45%} patients presented at an early stage (stage II-2; stage I-3). IPI scoring was done in only 9 patients. Of these 5 patients were in low/low intermediate risk and 4 patients (44%) were in high/high intermediate risk.

5.9F. Morphology: Oral plasmablastic lymphomas showed diffuse subepithelial infiltrate of plasmablasts. A diffuse infiltrate of monomorphic population of large lymphoid cells with moderate amounts of amphophilic cytoplasm, round nuclei, high nucleo-cytoplasmic ratio

and centrally placed prominent nucleolus resembling plasmablasts. A high apoptotic and mitotic activity with a "Starry-sky" pattern were seen in 3 cases. [Fig. 22].

5.9G. Immunohistochemistry: CD138 was positive in all cases [Fig. 23a]. All the cases except one were CD20 negative. One case showed focal CD20 positivity. MIB1 labeling index was high with a median of 98% {range = 95-100%}.

5.9H. EBV &KSHV: EBV association by EBER-ISH technique was found in 90% {9 of 10 cases} [Fig. 23b]. EBV LMP1 was positive in 2 of these cases. The case which was negative for EBER-ISH was from pleura. KSHV LANA was negative in all the cases.

5.9I. Prognosis: Six out of 12 patients were available for follow up. Four patients (all were stage IV) died within 3 months. The other two were in remission but lost to follow up after 3 and 12 months respectively.

5.10. BURKITT LYMPHOMA

5.10A. Frequency: Burkitt lymphoma constituted 15% (n=11) of HIV associated lymphomas.

5.10B. Age and Sex ratio: The median age at presentation was 41 years {range 26-59 years}. There was a definite male preponderance with male to female ratio 10:1 {10 males and 1 female}. There were no childhood Burkitt lymphomas in this study.

5.10C. Sites of involvement: Primary site of presentation was extranodal, constituting 54% {6 of 11 cases}. [Fig. 24]. Among the extranodal sites, GIT was the most common site {2 cases; 1-stomach, 1-small intestine}, followed by liver {1 case}, thigh {1 case}, orbit {1 case} and omentum {1 case}. Lymph node was the primary site in 45% {5 of 11 cases},

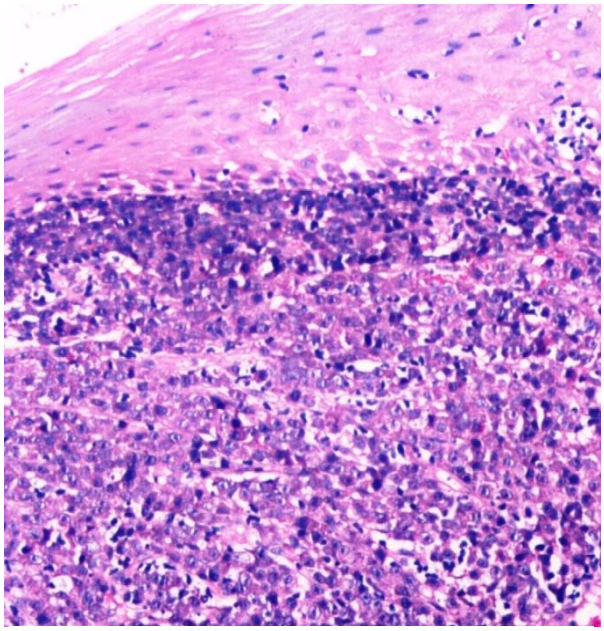


Figure 22a: Oral plasmablastic lymphoma – with overlying stratified squamous epithelium of buccal mucosa *H&E 200x*

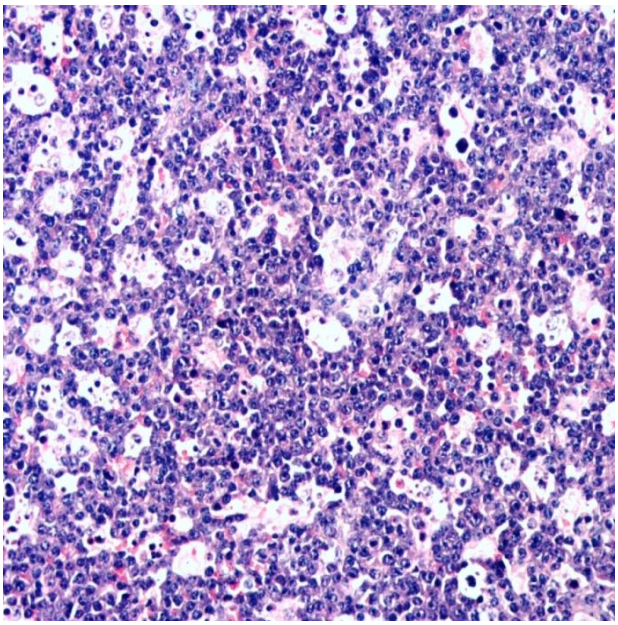


Figure 22b: Oral plasmablastic lymphoma - "Starry sky pattern" – multiple tingible body macrophages scattered among the plasmablasts

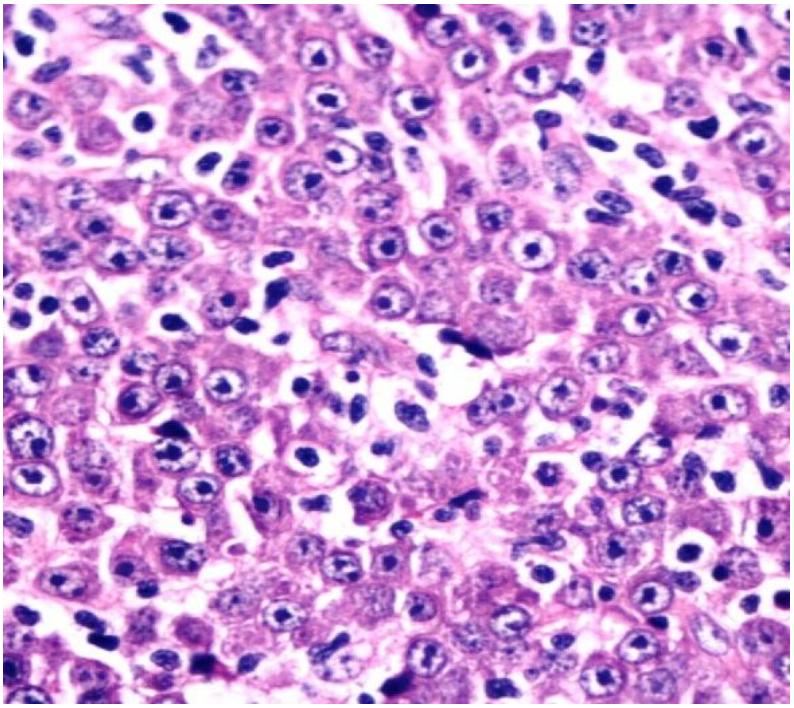


Figure 22c: Plasmablastic lymphoma - large cells with abundant amounts of amphophilic cytoplasm, eccentric vesicular nuclei with single centrally placed prominent nucleolus resembling plasmablasts H&E *400x*

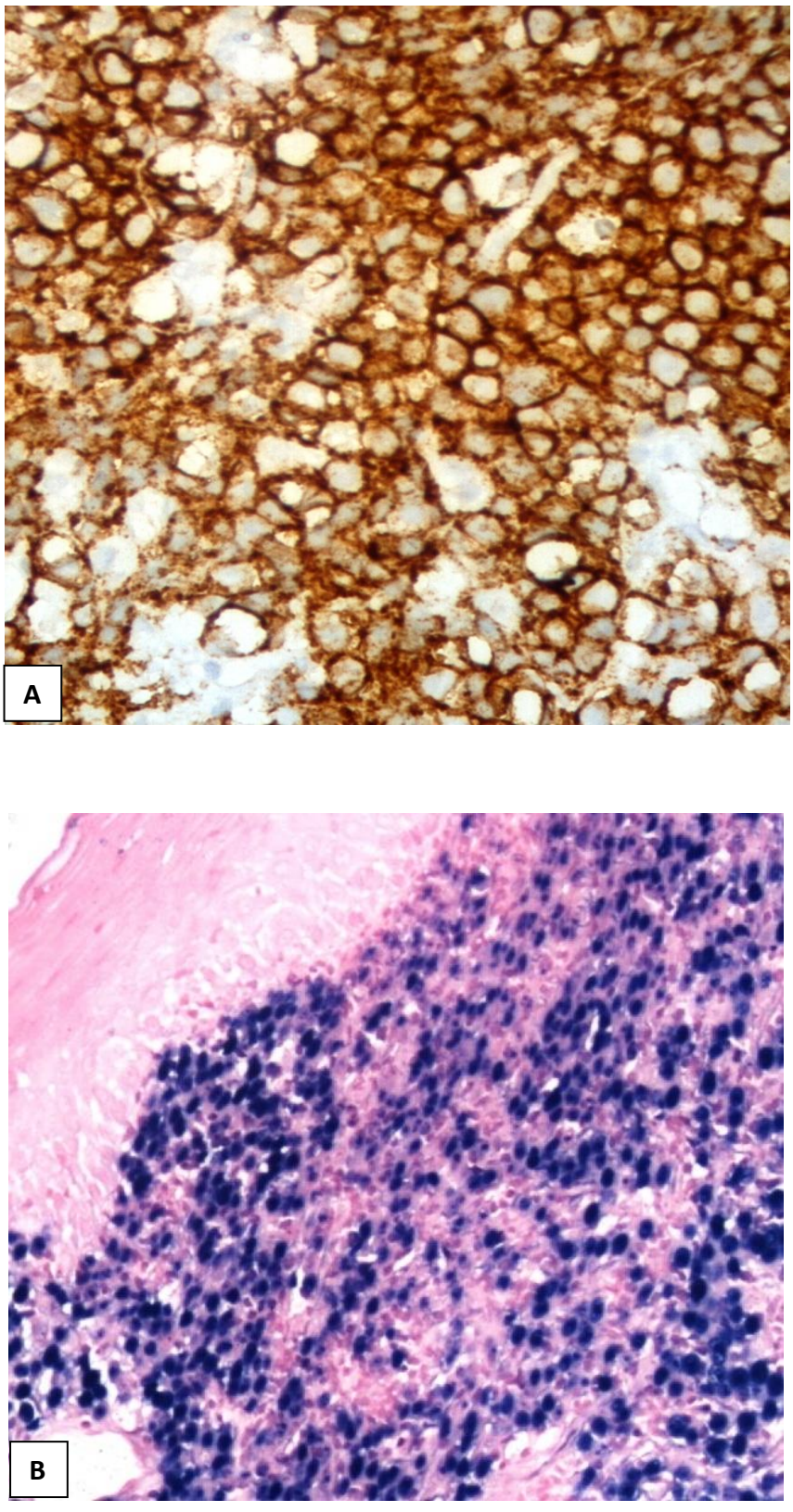


Figure 23: Plasmablastic lymphoma – Immunophenotype; A) Plasmablasts showing membrane positivity for CD138 *400x;* B) nuclear positivity for EBER-ISH *200x*

among which cervical and axillary nodes were more common {cervical – 2 cases, axillary – 2 cases), followed by abdominal lymph node. Multiple lymph node involvement was seen in approximately 36% {4 cases}. Marrow involvement was seen in 29% {2/5} of the cases in which bone marrow was examined.

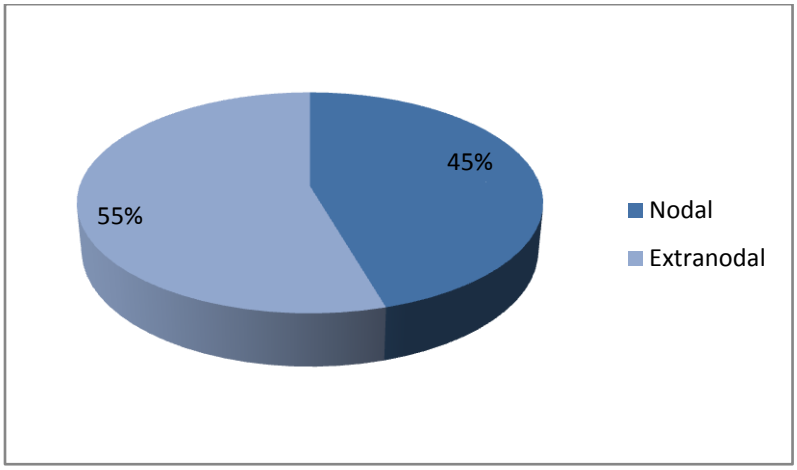


Figure 24: Burkitt lymphoma – Sites of involvement

5.10D. Clinical presentation and laboratory findings: B symptoms were seen in 64% {7 of 11 cases}. Hepatomegaly was seen in 33% {3 of 9 cases} and splenomegaly in 22% {2 of 9 cases}. Elevated LDH was seen in all cases {100%} with a mean value of 2397 IU/L {range = 584 – 9479} and the median CD4+ T cell count was 189 cells/µL {range = 16-382}.

5.10E. Ann Arbor Staging and International prognostic index {IPI}: 55% {6 of 11 cases} presented with stage IV disease. Five {45%} patients presented at an early stage of disease {Stage I – 1 case; Stage II – 4 cases}. IPI scoring was done in only 9 patients. Of these 2

patients were in low/low intermediate risk and 7 patients (78%) in high/high intermediate risk.

5.10F. Morphology: Lymph nodes showed diffuse effacement of architecture due to a diffuse infiltrate of medium sized lymphoid cells with scant cytoplasm, round to oval nuclei with clumped chromatin and multiple small nucleoli [Fig. 25]. In all cases, a "Starry-sky" pattern was observed [Fig. 26]. One of the cases showed involvement of duodenum with infiltration of neoplastic lymphoid cells in the lamina propria [Fig. 27a].

5.10G. Immunohistochemistry: All the cases were CD20+ [Fig. 27b] with a very high MIB1 labeling index of 100% {range = 95-100%}.

5.10H. EBV &KSHV: EBV association was found in 10% {1 of 10 cases} by EBER-ISH technique [Fig. 27c]. EBV LMP1 was negative in all cases. The frequency of EBV association is shown in figure 28. KSHV LANA was negative in all cases.

5.10I: Prognosis: Follow up was available for 5 patients. Two patients [Both are stage IV] died within 3 months. One had progressive disease and lost follow up later and 2 are in remission until the recent follow up date with a maximum survival period of 5 years.

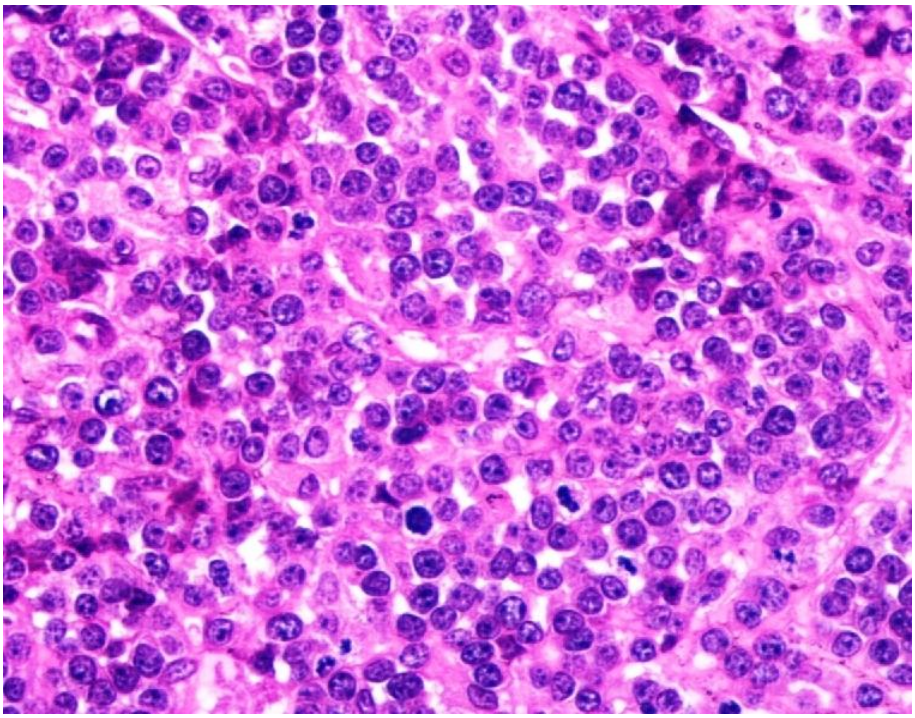


Figure 25: Burkitt lymphoma – Monotonous population of medium sized lymphoid cells with scant cytoplasm, round to oval nuclei, clumped chromatin and multiple small nucleoli *H&E 400x*

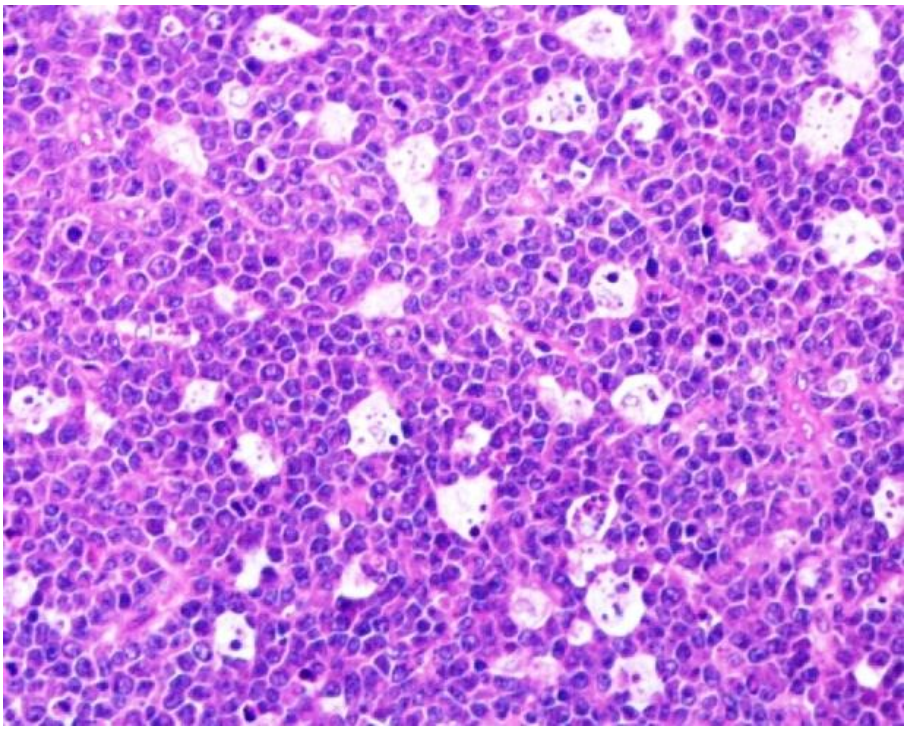


Figure 26: Burkitt lymphoma – "Starry-sky pattern" *H&E 200x*

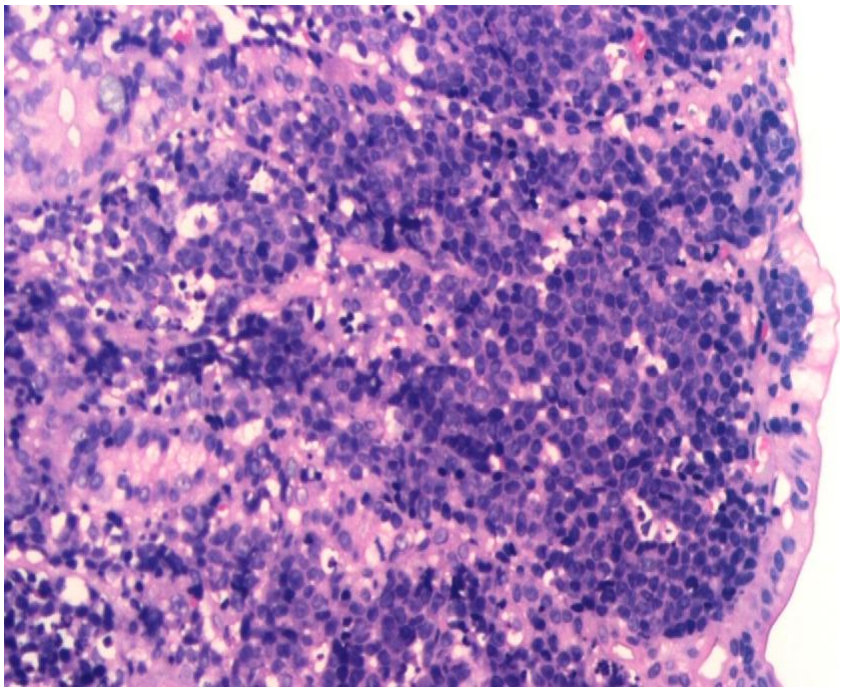


Figure 27a: Monotonous population of medium sized lymphoid cells infiltrating the lamina propria of duodenum *H&E 200x*

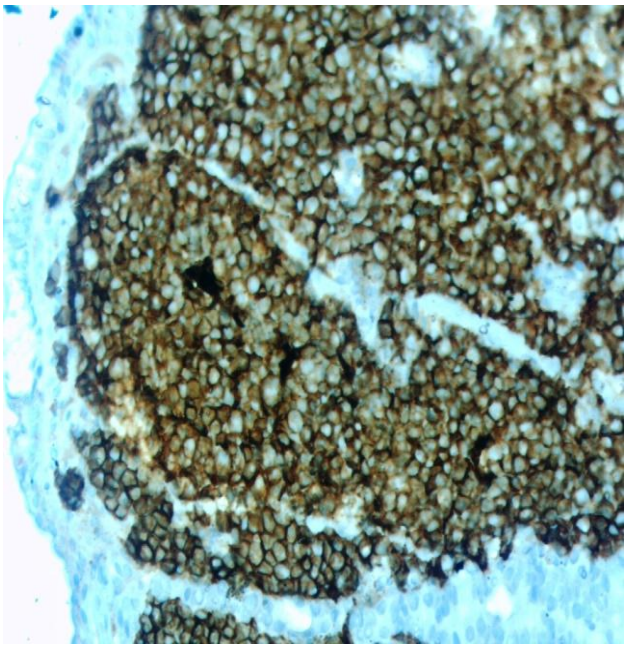


Figure 27b: Membrane positivity for CD20 200x

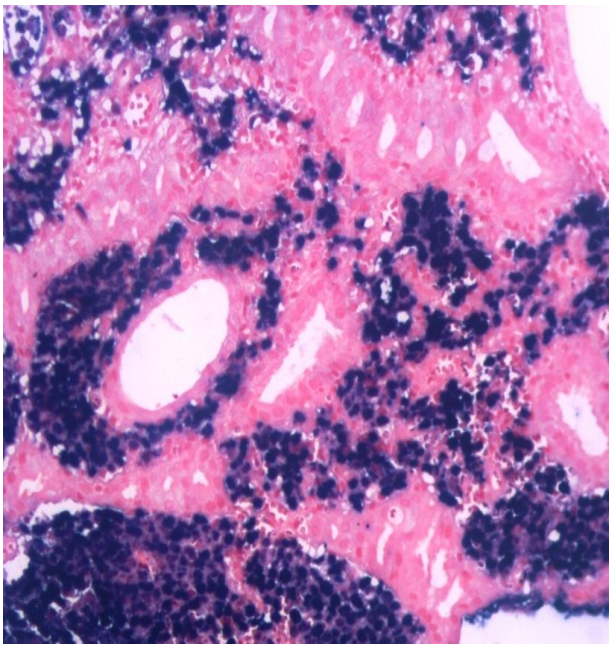


Figure 27c: Nuclear positivity for EBER-ISH 200x

Figure 27: Burkitt lymphoma – Duodenum

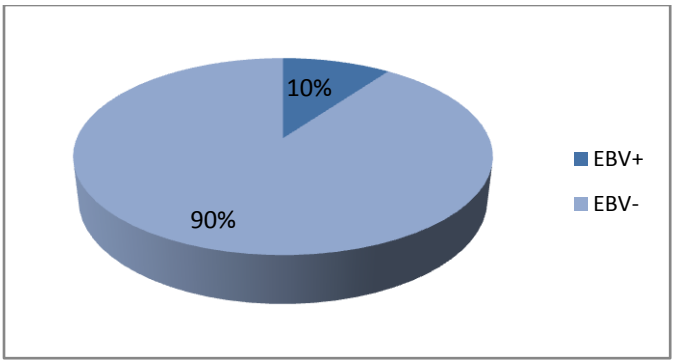


Figure 28: Burkitt lymphoma – EBV status

5.11. HODGKIN LYMPHOMA

5.11A. Frequency: Hodgkin lymphoma also constituted 12% {9 of 73 cases} of HIV associated lymphomas.

5.11B. Age and Sex ratio: The median age at presentation was 34 years {range 21-60 years}. There was a definite male preponderance with male to female ratio of 3.5:1 {7 males and 2 females}.

5.11C. Sites of involvement: Lymph node was the primary site in all patients. Among these the most common site involved was axillary {3 cases}, followed by cervical {2 cases}, abdominal {2 cases} and supraclavicular lymph node. Multiple lymph node involvement was seen in approximately 25% {3 cases}. Marrow involvement was seen in 87.5% {7 of 8 cases}.

5.11D. Clinical presentation and laboratory findings: B symptoms were seen in 89% {8 of 9 cases}. Hepatomegaly was seen in 78% {7 of 9 cases} and splenomegaly in 78% {7 of 9 cases}. Elevated LDH was seen in 86% {6 of 7 cases} with a mean value of 1466 IU/L {range = 407 to 6169 IU/L} and the median CD4+ T cell count was 77 cells/µL {range = 14-745}.

5.11E. Ann Arbor Staging and International prognostic index {IPI}: 89% {8 of 9 cases} presented with advanced stage {stage IV – 7 cases; stage III – 1 case}. One {11%} patient presented with stage I disease. IPSS scoring was done in all patients. Of these 5 patients {55.6%} were found to have a poor risk, 4 patients {44%} had fair risk and none of the patients had good risk.

5.11F. Morphology: All were classical Hodgkin lymphoma. Of these, 8 cases were mixed cellularity and 1 was of nodular sclerosis type. There were no cases of lymphocyte rich, lymphocyte depleted or nodular lymphocyte predominant type. All cases showed a polymorphous infiltrate of small lymphocytes, plasma cells, histiocytes and eosinophils, scattered among which are large cells with abundant amphophilic cytoplasm, which are bilobated or binulceated or multinucleated with prominent nuclear membrane, vesicular chromatin and a single large centrally placed eosinophilic inclusion like nucleolus resembling classical Reed-Sternberg cells [Figure 29&30]. Other variants of RS cells such as mononuclear and lacunar cells were also seen. One case was of nodular sclerosis type with nodules separated by fibrocollagenous septae with lacunar variant of Hodgkin cells.

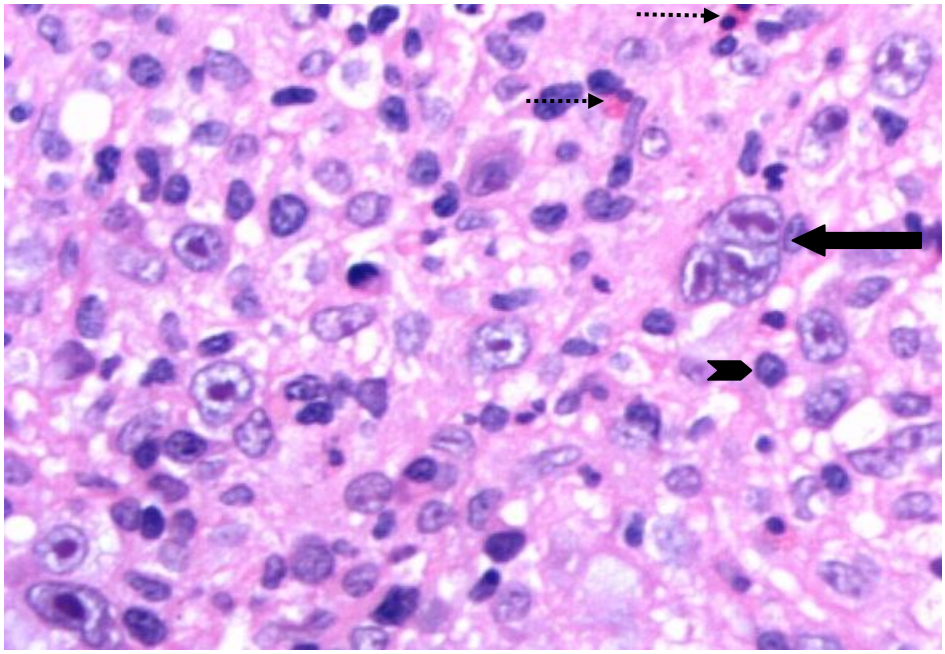


Figure 29: Classical Hodgkin lymphoma – Multinucleate Reed-Sternberg cell (block arrow) with moderate amounts of amphophilic cytoplasm, vesicular nuclei with prominent eosinophilic inclusion like nucleolus. Background shows plasma cells, small lymphocytes (arrow head) and eosinophils (dotted arrows) *H&E 400x*

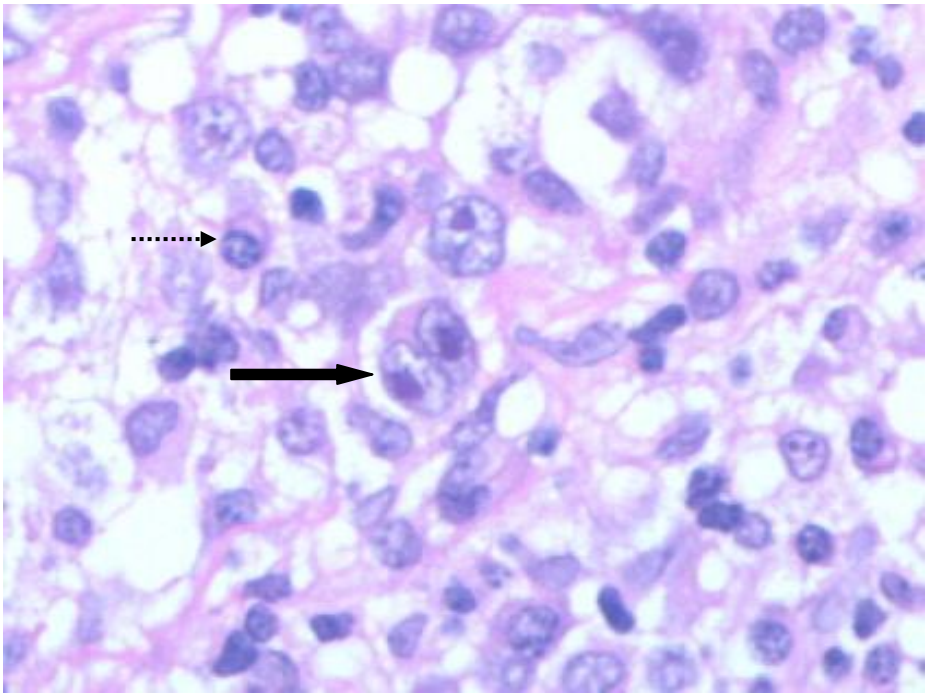


Figure 30: Classical Hodgkin lymphoma – Binulceate Reed Sternberg cell with moderate amounts of amphophilic cytoplasm, vesicular nuclei and prominent eosinophilic inclusion like nucleolus. Background shows plasma cells (dotted arrows), histiocytes and small lymphocytes *H&E 400x*

5.11G. Immunohistochemistry: CD30 was positive in all cases (100%) and CD15 was positive in 67% {6 of 9 cases} [Fig. 31a&b]. All the cases except one were CD20 negative.

5.11H. EBV&KSHV: EBV association was found in all cases {100%} by EBER-ISH technique [Fig. 31c]. EBV LMP1 was positive in 78% {7 of 9 cases} [Fig. 31d]. KSHV LANA was negative in all the cases.

5.11I. Prognosis: Four patients were available for follow up. Two patients (Both were stage IV) died within 3 and 6 months. The other two were in remission until recent date of follow up with a maximum survival period of 44 months.

5.12. PERIPHERAL T-CELL LYMPHOMA, NOS

5.12A. Frequency: Peripheral T-cell lymphoma constituted only 4% {3 of 73 cases} of the entire study population.

5.12B. Age and Sex ratio: The median age at presentation was 41 years {range 35-45 years}. All were male patients.

5.12C. Sites of involvement: Lymph node was the primary site in all patients. Among these the most common was involved was axillary {2 cases}. Multiple lymph node involvement was seen in all 3 cases. Marrow involvement was seen in 33% {1 of 3 cases}.

5.12D. Clinical presentation and laboratory findings: B symptoms were seen in all patients {100%}. Hepatomegaly was seen in 33% {1 of 3 cases} and splenomegaly in 33% {1 of 3 cases}. Elevated LDH was seen in 50% {1 of 2 cases} with a range of 378 - 520 IU/L and the median CD4+ T cell count was 109 cells/μL {range = 66-152}.

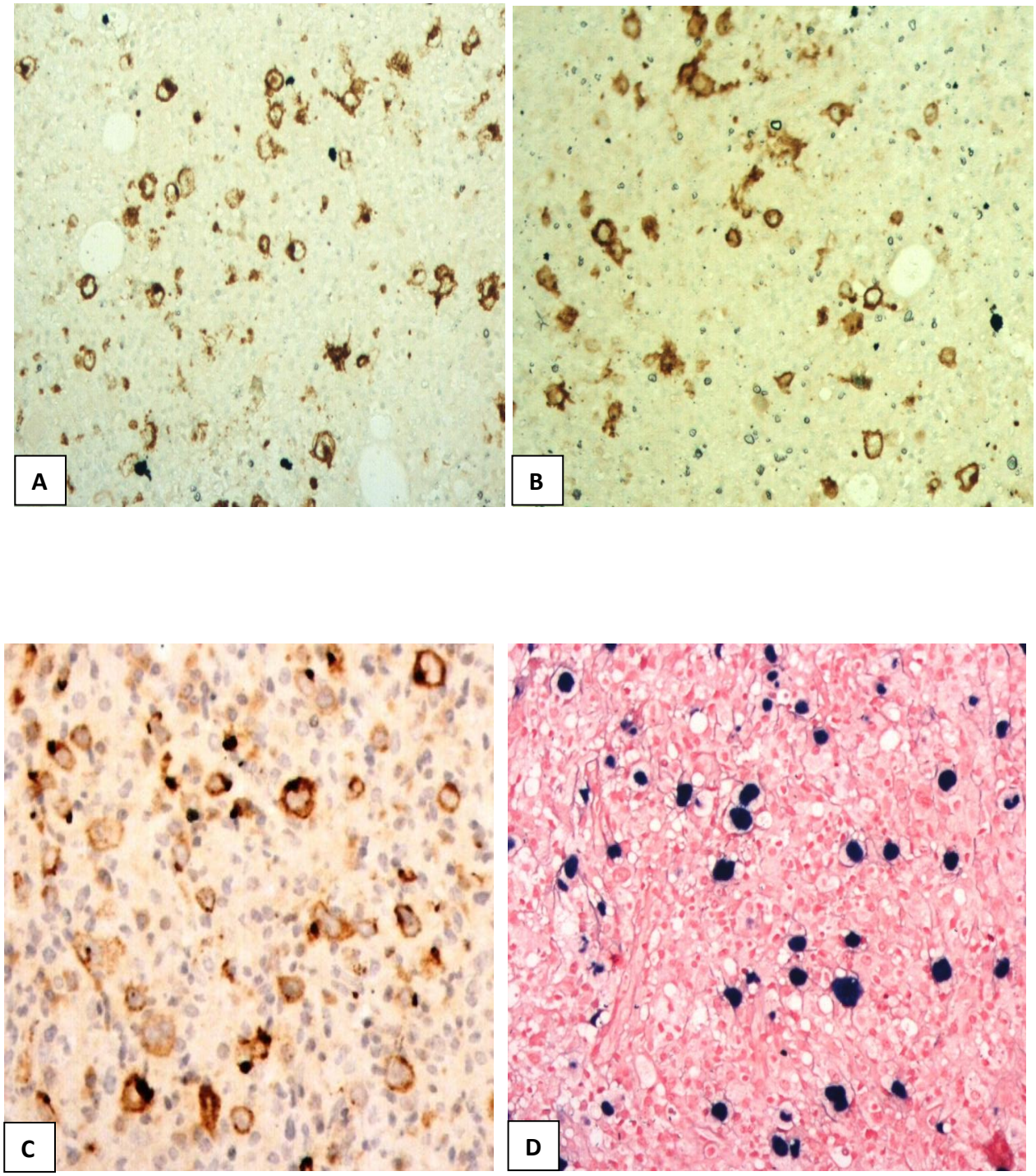


Figure 31: Classical Hodgkin lymphoma – Immunophenotype; Reed Sternberg cells showing membrane and golgi body staining for A) CD15 *200x;* B) CD30 *200X*; C) cytoplasmic granular staining for EBV LMP1 *200x* and D) Nuclear positivity for EBER-ISH *200x*

5.12E. Ann Arbor Staging and International prognostic index {IPI}: All cases {100%; Stage IV-1 case; Stage III-2 cases} presented with advanced stage. IPI scoring was done in 2 patients and both of them were in low intermediate risk (score 2).

5.12F. Morphology: Lymph nodes showed partial to diffuse effacement of architecture due to a polymorphous infiltrate of small to large sized lymphoid cells with irregular round to oval indented vesicular nuclei, some with prominent nucleoli and scant to moderate pale eosinophilic cytoplasm. Admixed among these were small lymphocytes, plasma cells and a few eosinophils. One case showed scattered large cells (immunoblasts). In one case there were arborizing prominent high endothelial venules.

5.12G. Immunohistochemistry: All the cases were CD3+ and CD20-. One showed predominantly CD4+ T-cells and the other two showed predominantly CD8+ T-cells. CD56 and PD1 were negative in the case in which these were done. PD1 was negative in that case with prominent high endothelial venules. The MIB-1 labeling index was 50% {range = 50-80}.

5.12H. EBV&KSHV: EBV association was found in 50% {1 of 2 cases} by EBER-ISH technique and EBV LMP1. KSHV LANA was negative in all the cases.

5.12I. Prognosis: Follow up was available for 2 patients, both of which are in remission until the recent follow up date with a maximum survival period of 5 years.

5.13. EXTRANODAL NK/T-CELL LYMPHOMA

5.13A. Frequency: There was only a single case of this subtype, constituting only 1.3% of all HIV associated lymphomas.

5.13B. Age and Sex: This was a 41 year old male.

5.13C. Sites of involvement: Upper aerodigestive tract was the primary site. Generalized lymphadenopathy was present. Bone marrow was not involved.

5.13D. Clinical presentation and laboratory findings: B symptoms were present. Organomegaly was not present. Serum LDH was 690 IU/L and the CD4+ T cell count was 103 cells/μL.

5.13E. Ann Arbor Staging and International prognostic index {IPI}: The patient presented with stage II disease with an IPI score of 1 (low risk).

5.13F. Morphology: The lining stratified squamous epithelium showed ulceration covered by necrotic debris and acute inflammatory exudate. There was a subepithelial diffuse infiltrate of small to medium sized lymphoid cells with moderate amounts of pale eosinophilic cytoplasm and irregular nuclei with finely granular to vesicular chromatin and prominent nucleoli exhibiting mitotic activity. Extensive necrosis was present.

5.13G. Immunohistochemistry: The lymphoid cells were CD3+, Granzyme B+ and negative for CD20 and CD56. The MIB-1 labeling index was 60%.

5.13H. EBV &KSHV: Both EBV LMP1 and EBER-ISH were positive. KSHV LANA was negative.

Followup was not available for this patient.

5.14. HIGH GRADE NON HODGKIN LYMPHOMA - UNCLASSIFIED

5.14A. Frequency: There was only a single case of this subtype, constituting only 1.3% of all HIV associated lymphomas.

5.14B. Age and Sex: This was a 17 year old female patient.

5.14C. Sites of involvement: Lymph node was the primary site. Generalized lymphadenopathy was present. Bone marrow was not involved.

5.14D. Clinical presentation and laboratory findings: B symptoms were present. Hepatomegaly was present but no splenomegaly. Serum LDH was elevated up to 1059 IU/L and the CD4+ T cell count was 375 cells/μL.

5.14E. Ann Arbor Staging and International prognostic index {IPI}: The patient presented with stage III disease with an IPI score of 2 (low intermediate risk).

5.14F. Morphology: There was a complete effacement of lymph node architecture due to diffuse infiltration by monomorphic population of small to medium sized lymphoid cells with scant cytoplasm, round to oval nuclei with finely dispersed chromatin and inconspicuous nucleoli. Extensive areas of necrosis were observed in this case.

5.14G. Immunohistochemistry: The lymphoid cells were CD43+ and CD7 focal positive. Tdt and MPO were negative. CD3 and CD20 stained only reactive lymphocytes. The MIB-1 labeling index was 95%.

5.14H. EBV &KSHV: Both EBER-ISH and EBV LMP1 were negative. KSHV LANA was also negative.

5.14I. Prognosis: This patient died within first 3 months in spite of chemotherapy.

5.15. ANAPLASTIC LARGE CELL LYMPHOMA

5.15A. Frequency: There was only a single case, constituting only 1.3% of all HIV associated lymphomas.

5.15B. Age and Sex: This was a 37 year old male patient.

1.15C. Sites of involvement: Liver was the primary site. Lymph nodes or bone marrow were not involved.

5.15D. Clinical presentation and laboratory findings: B symptoms were present. Hepatomegaly was present but no splenomegaly. Serum LDH was elevated up to 698 IU/L and the CD4+ T cell count was 100 cells/μL.

5.15E. Ann Arbor Staging and International prognostic index {IPI}: The patient presented with stage IV disease, with an IPI score of 3 (high intermediate risk).

5.15F. Morphology: Sheets of large cells with pleomorphic hyperchromatic nuclei, some of which are eccentrically placed and horseshoe shaped with an eosinophilic region near the nucleus resembling hall mark cells were seen. The background and intervening fibrocollagenous septae showed a few inflammatory cells.

5.15G. Immunohistochemistry: The lymphoid cells were CD3+, CD30+ and are negative for CD20 and Alk. The MIB-1 labeling index was 95%.

5.15H. EBV&KSHV: EBV LMP1, EBER-ISH and KSHV LANA were not done due to unavailability of tissue block.

5.15I. Prognosis: The patient took treatment for both lymphoma and AIDS and was in remission but lost follow-up after 12 months.

5.16. EXTRACAVITARY PEL

5.16A. Frequency: There was only a single case, constituting only 1.3% of all HIV associated lymphomas.

5.16B. Age and Sex: This was a male patient with 66 years of age at presentation.

5.16C. Sites of involvement: Gastrointestinal tract was the primary site. Abdominal lymphadenopathy was present. Bone marrow was not involved.

5.16D. Clinical presentation and laboratory findings: B symptoms were present. Hepatosplenomegaly was not present. Serum LDH was within normal limits {247 IU/L} and the CD4+ T cell count was 219 cells/μL.

5.16E. Ann Arbor Staging and International prognostic index {IPI}: The patient presented with stage II disease with an IPI score of 3 (high intermediate risk).

5.16F. Morphology: The bowel wall was infiltrated by a diffuse infiltrate of large lymphoid cells with moderate amounts of amphophilic cytoplasm, round vesicular nuclei, some of which are eccentrically placed with single centrally placed prominent nucleolus resembling plasmablasts [Fig. 32a]. There was an increase in the mitotic and apoptotic activity. The overlying mucosa showed ulceration in foci.

5.16G. Immunohistochemistry: The lymphoid cells were CD20+ and CD30+. The MIB-1 labeling index was 95%.

5.16H. EBV &KSHV: EBER-ISH and KSHV LANA were positive [Fig. 32b&c]. EBV LMP1 was negative.

This patient was lost to follow up.

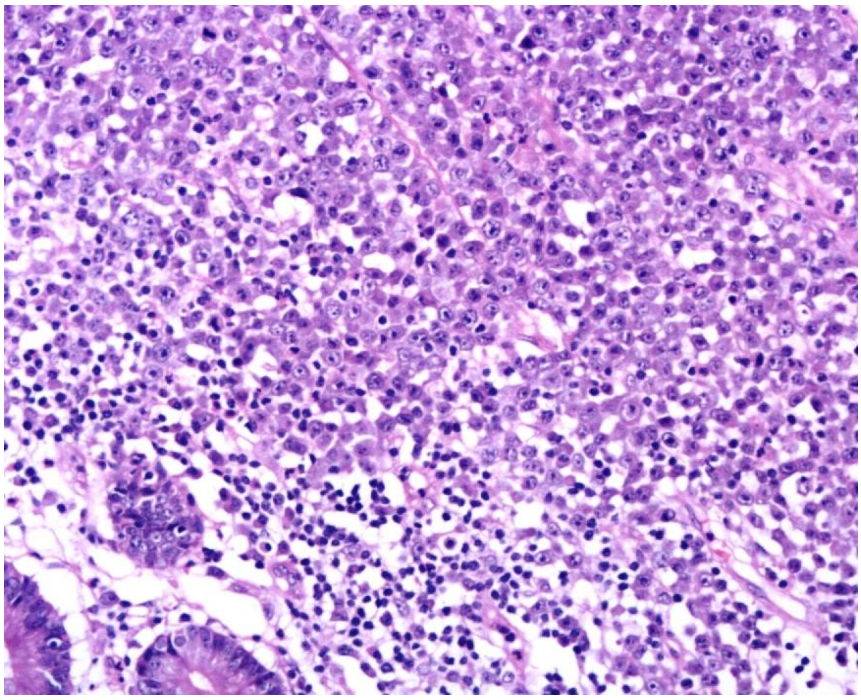


Figure 32a: Extracavitary PEL – Plasmablast like cells infiltrating the lamina propria of the small intestine H&E *200x*

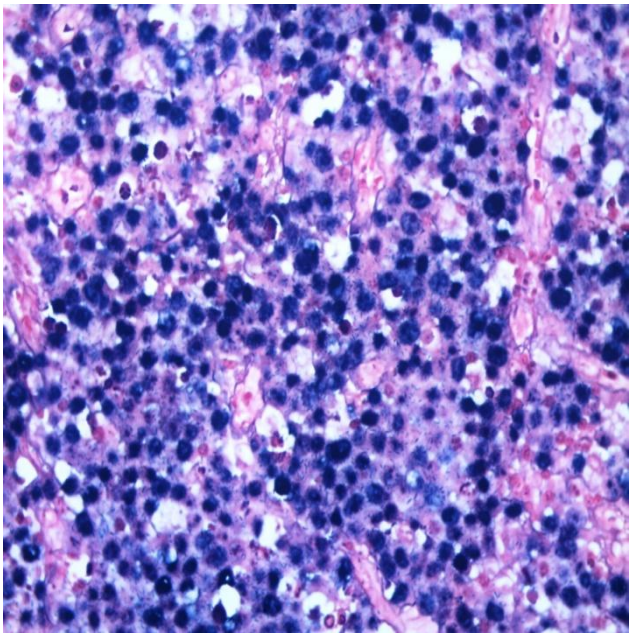


Figure 32b: Extracavitary PEL – small intestine-lymphoid cells positive for EBER-ISH *400x*

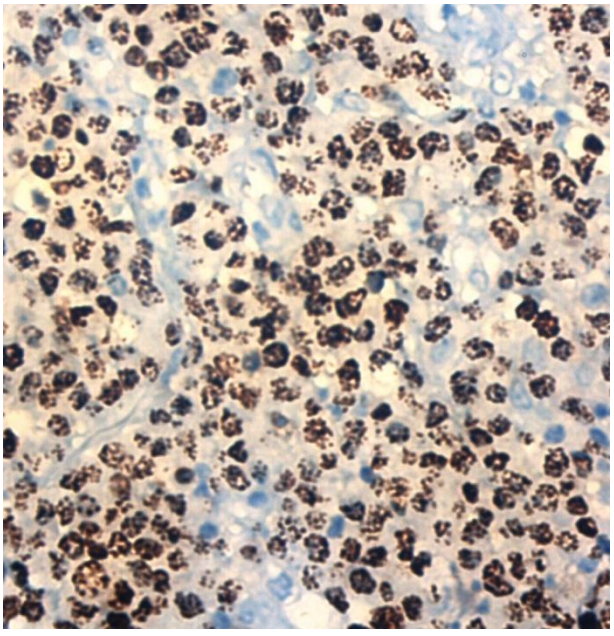


Figure 32c: Extracavitary PEL – small intestine - lymphoid cells showing granular nuclear positivity for HHV8-LANA

Figure 32: Extracavitary primary effusion lymphoma

6. DISCUSSION

A total of 3346 lymphomas were diagnosed during the 5 year period (January 2007 to December 2011) in our Norman Institute of Pathology, Christian Medical College and Hospital, Vellore. Of these 73 (2%) patients were found to be positive for HIV serology (ELISA). The rest of them were seronegative (2446 cases), patients with HIV status unknown (260 cases) and biopsies from outside hospitals with HIV status unknown (567 cases).

This is the first large detailed Indian study on HIV associated lymphomas in a five year period including a wide spectrum of histological subtypes, EBV and HHV8 status, follow-up and survival analysis. One previous large Indian study presented 77 cases but with no detailed histological sub typing (81). In eastern countries such as Korea and China the frequencies of HIV associated lymphomas reported were 23 and 18 cases in the 8 year and 5 year study period respectively (30,119). In reports from America, the frequencies were as follows; 65 cases in a 10 year study period (North America) and 55 cases in a 16 year study period (South America) (23,117). In UK, as well, the frequency was lower when compared to our study, 51 cases in a 15 year study period (111). Even in developing countries such as Africa, only 9 cases have been reported in a study period of 15 years (29). The comparison of frequencies of HIV associated lymphomas between our study and studies across the world is shown in Fig. 33.

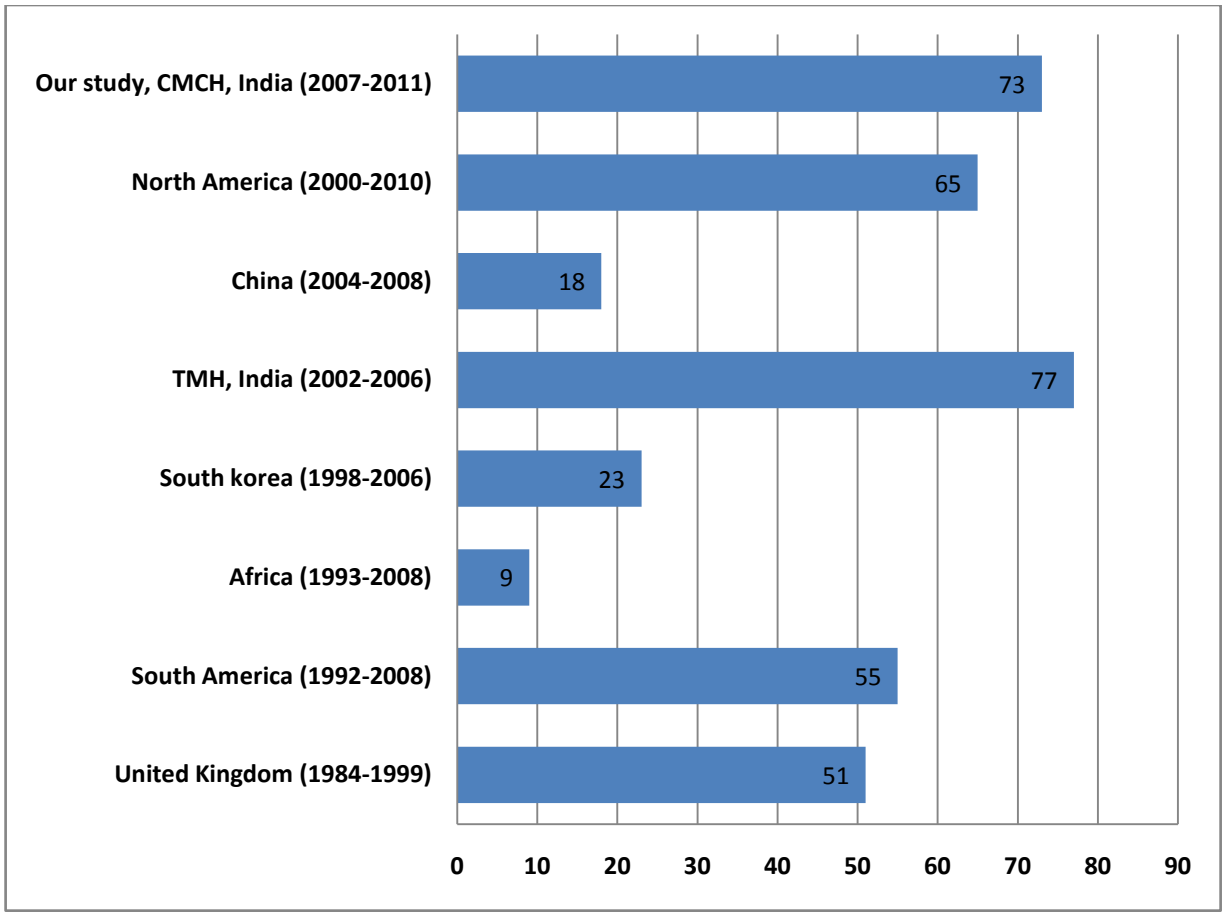


Figure 33: Frequencies of HIV associated lymphomas in our study as compared to the other countries (23,29,30,81,111,117,119)

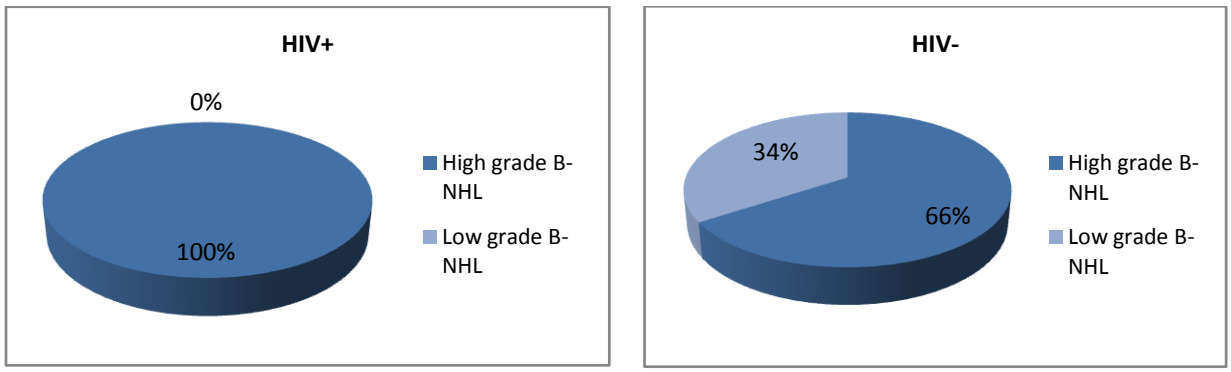


Figure 34: Proportion of aggressive lymphomas in CMCH (2007-2011)

6.1. HIV associated lymphoma – Subtypes and its comparison with the HIV negative population:

High grade/aggressive B-cell non Hodgkin lymphomas were much more prevalent in HIV patients (58 of 58 cases – 100%) as compared to HIV negative population (844 of 1282 cases – 66%) in our institution during the same study period [Fig. 34]. This difference was statistically significant with a p value of 0 **{p<0.05}**.

Among the HIV associated lymphomas, DLBCL was the most common subtype accounting about half of the cases (n=34; 46.6%). DLBCL is also the most common subtype of NHLs in non HIV individuals. However this constituted only 29% (less than 1/3rd) of all lymphomas in this patient group. This difference was found to be statistically significant **{p value - 0.0009}**.

The next most common type in our study was plasmablastic lymphoma {n=12; 16.4%}, which is known to occur specifically in the immunocompromised individuals especially due to HIV infection. The frequency of this lymphoma in the HIV negative population was very low {n=5; 0.2%} with a statistically significant difference **{p < 0.0001}.**

The third common subtype was Burkitt lymphoma (n=11; 15%). This was also found to be a significantly higher frequency when compared to the HIV negative individuals (1.7%) with a statistically significant difference **{p < 0.0001}.**

The frequency of Hodgkin lymphoma was much lower than non Hodgkin lymphoma. There were only 9 cases of Hodgkin lymphoma (12.3%). This was much less than that in the negative population (n=626; 25.6%). This difference was statistically significant {p value

0.0111}. The frequencies of various subtypes in HIV positive and negative population are shown in Fig. 35 and their statistical significance is given in Table 13.

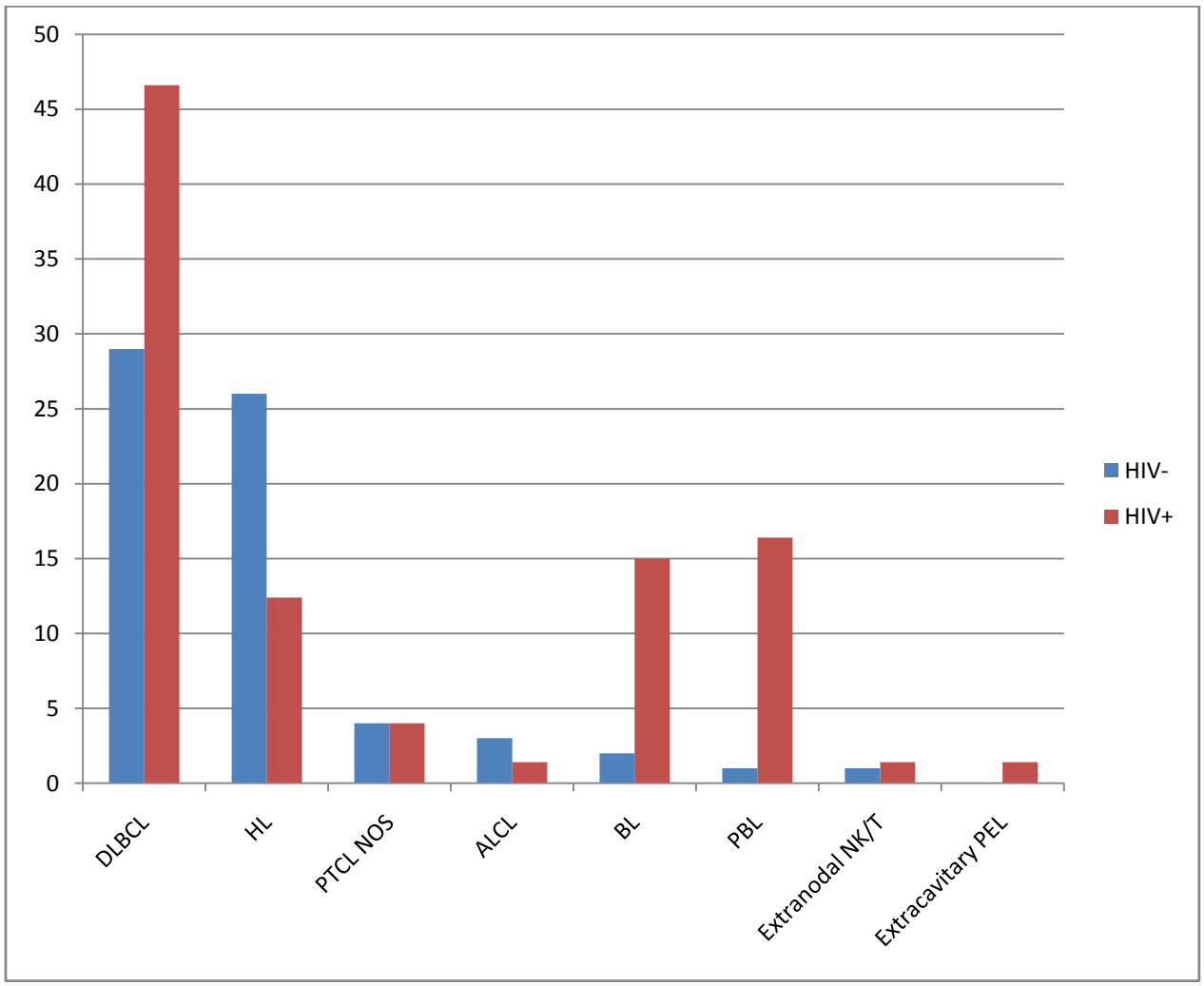


Figure 35: Comparison of frequency distribution of different lymphoma subtypes among HIV positive and HIV negative individuals over a 5 year period (2007-2011) in CMC Vellore

Table 13: Lymphoma subtypes – Frequency distribution in HIV+ and HIV- population:

Subtypes	**Frequency in HIV+ (%)**	**Frequency in HIV- (%)**	**p value**
DLBCL	34 (46.6)	709 (29.0)	**Significant (0.0009)**
Plasmablastic	12 (16.4)	5 (0.3)	**Significant(<0.0001)**
Burkitt	11 (15.0)	42 (1.7)	**Significant (<0.0001)**
Classical Hodgkin	9 (12.3)	626 (25.6)	**Significant (0.0111)**
PTCL NOS	3 (4.1)	100 (4.1)	Not significant (>0.9999)
ALCL	1 (1.3)	78 (3.2)	Not significant (0.3594)
Extracavitary PEL	1 (1.3)	……	…….

In the previous studies on HIV associated lymphomas, both in Indian and Western literature, very few cases of peripheral T cell lymphomas have been reported. In this study we had 5 cases of peripheral T cell lymphoma including PTCL NOS (n=3 cases; 4.1%), ALCL (n=1;1.3%) and extranodal NK/T cell lymphoma (n=1; 1.3%), which are considered as rare among the HIV positive individuals (31).

Not a single case of primary effusion lymphoma or primary CNS lymphoma was found in our study, whereas these were common among HIV positive individuals in the Western and the Eastern population constituting 33% to 17.4% of their NHLs (111,119,120). All

primary CNS lymphoma cases in our institution were in immunocompetent individuals. A single rare case of extracavitary PEL was seen in the stomach and small intestine of an HIV positive individual in our study.

6.2. Site of involvement:

Nodal involvement (n=41 cases; 56%) was slightly higher than extranodal involvement (n=32 cases; 44%), but the proportion of extranodal cases in HIV positive individuals was significantly higher as compared to the HIV negative population (30%). This difference was statistically significant **{p - 0.0102}**. Though nodal sites had become the predominant primary site of involvement in the HAART era (32,46), extranodal site is more commonly involved than in the HIV negative population. As mentioned in the literature gastrointestinal tract remains the most common extranodal primary site of involvement followed by soft tissue. Gastrointestinal tract is also the commonest extranodal site of NHLs in the general population.

6.3. Age and sex:

These lymphomas involved a wide range from childhood to elderly age group (8-66yrs). The median age was 40 years which is similar to the previous studies (22,24,25). There were two cases in childhood age group, 8 years and 14 years old respectively.

6.4. EBV and KSHV:

The association of EBV was found to be 47%, when all HIV associated lymphomas were included. EBV association with NHLs was 38% (20 of 53 cases) similar to that mentioned in the literature (17) and with Hodgkin lymphoma, it was 100% (all 9 cases). Varied association of EBV with Hodgkin lymphoma ranging from 80-100% has been quoted in the literature (40,120). The frequency of EBV association varied widely among the different subtypes. The frequency of EBV association and comparison with the literature are shown in Table 14. The frequency of EBV association in DLBCLs was low in our study group (30%) when compared to the literature (50%). This could be due to the absence of primary CNS lymphoma in our study group, which is known to have 100% association with EBV (18).

Table 14: Frequency of EBV association in different subtypes of lymphomas:

Subtype	**Our study (%)**	**Literature (%)**
DLBCL – centroblastic	12	20-30
DLBCL – immunoblastic	63	80
PBL - extra oral	90	~70
PBL – oral cavity	100	100
BL	10	25
CHL	100	80-100

The frequency of EBV association with plasmablastic lymphomas was found to be higher in our study (90%) as compared to the literature, which varies from 60-75% (28). The EBV association among Burkitt lymphomas was unusually low (10%) in our study. The frequency of EBV association with Hodgkin lymphomas (100%) was significantly high as compared to the HIV negative (30-60%) reported in the literature (40,94). EBV LMP1 was positive in only 13 of 28 cases that were positive for EBER-ISH. This proves that the EBER-ISH technique is highly sensitive and superior to immunohistochemical techniques. Also EBERs are expressed in all three latency patterns of EBV infection (51,52,56).

KSHV association was found only in a single case of extracavitary PEL. In literature KSHV has been reported in 10% of HIV associated lymphomas, but 60% of these had previous history of Kaposi sarcoma (11). None of our cases were associated with Kaposi sarcoma. This difference is probably due to geographic location. There is no other study in India with KSHV status for comparison.

6.5. Staging and IPI score:

Most of our patients (n=50 cases; 69%) presented with advanced stage (III/IV) disease and with B symptoms (78%) as mentioned in the literature (89%) (13,27,29,30,120). Only about half (55%) of the DLBCLs in the HIV negative population presented at stage III/IV, in our institution.

6.6. CD4 count:

The mean CD4 count was 194 cells/μL. The CD4 count was very low in plasmablastic lymphoma (89 cells/μL). As mentioned in the literature, this subtype occurs in the severely

immunosuppressed individuals (18,28). As most of the patients received HAART (n=43) therapy for AIDS, CD4 count was maintained above 100 in most of them.

6.7. Specific features of individual subtypes:

6.7a. Diffuse large B cell lymphoma:

This was the most common subtype (n=34; 46.6%) and included a wide range of age group including two children (8yr and 14 yr). DLBCL in HIV infected children have been rarely reported in the literature so far. DLBCLs so far reported in children were mainly of primary CNS type (121,122). Both the children in our study had lymph node as the primary site of involvement. EBV association was found in one of these. In a study done in Western country it has been reported that 100% of the lymphomas in HIV infected children were associated with EBV (122). The 8 year old child presented with an advanced disease (stage IVB) and died on the 5th admission day and the 14 yr old child presented with stage IIB disease and was lost to follow up. In the literature, it has been quoted that prognosis is better in the childhood age group with complete remission (121,123).

6.7b: Plasmablastic lymphoma:

This was the second most common subtype in our study population accounting for 16.4% (n=12) of all cases. This is very high when compared to the Western population (one study reported only 6 cases in a 6 year study period) (108). However a previous large Indian study has also reported a much higher frequency (44% - 34 of 77 cases) (81). This shows that there is geographic variation in the frequency of plasmablastic lymphoma with large numbers of this type occurring in HIV population in India (17). Male sex predilection was

more readily observed in our study group (11:1) as compared to the previous Indian study with large number of plasmablastic lymphomas (2.4:1) (81). The median CD4+ T-cell count was 52 cells/μL as mentioned in the literature, that this subtype occurs in the severely immunocompromised individuals with a CD4 count of <100 cells/μL (18,28).

6.7c. Burkitt lymphoma:

This was the third most common subtype (n=11; 15%) in our study group. The age group of patients with this subtype of lymphoma ranged from 26-59yrs. There were no childhood Burkitt lymphomas in our study. The next common lymphoma seen in children were diffuse large B cell lymphoma of primary CNS type (121,122). Extranodal site was the predominant primary site of involvement constituting 54%.

6.7d. Hodgkin lymphoma:

Hodgkin lymphoma constituted a significant proportion of HIV associated lymphoma (n=9; 12%). However this was significantly low as compared to the HIV negative population (25.6%) {p value 0.0111}. This is in contrast to literature, where it has been quoted that the incidence of Hodgkin lymphoma in HIV positive population was higher (~7 times) than that in the negative population (91,124). Although it has been mentioned in literature that extranodal involvement is the unique feature of Hodgkin lymphoma in HIV positive population (18,40,91–93), all of our Hodgkin lymphomas had lymph node as the primary site of involvement. Almost all of our cases (88%; 8 of 9 cases) were of mixed cellularity type. This proportion was high when compared to that reported in the literature (66%) and in our HIV negative population (46%). Overall, the proportion of mixed cellularity in our population (both HIV+ and HIV-) is high as compared to the Western literature. The

comparison is shown in table 15. Most of our patients with Hodgkin lymphoma presented at advanced stage (III/IV - 89%) with B symptoms (89%) and had bone marrow involvement (87.5%), which is unique in an HIV positive population. The proportion of cases with bone marrow involvement was quite high in our study as compared to that mentioned in the literature (40-50%) (18,40,91–93). Prognostic scoring also showed that most of the patients were at poor risk (56%; 5 cases) and fair risk (44%; 4 cases) and none of them had good risk.

Table 15: Hodgkin lymphoma – subtype distribution in CMCH and Western countries among HIV+ and HIV-:

Morphological variant	**HIV+**		**HIV-**	
	CMCH	*R* (40,93)	*CMCH*	*R* (31)
Mixed cellularity	89	66	46	23
Nodular sclerosis	11	….	36	70

6.7e. PTCL NOS:

There were 3 cases of this type in our study and all these 3 were males. All had lymph node as the primary site of involvement, in contrast to literature where extranodal is most common site of T-NHL in HIV positive population (97). All of them presented with high stage (III/IV) disease and had B symptoms. This was high as compared to that reported in the literature (high stage – 75% and B symptoms – 66%) (99).

6.8. Prognosis:

Follow up was available in only about half of the patients (36 of 73). Among these 13 patients died (12 patients within 3 months and one at the 6th month). However 10 patients were in remission until the last follow up (5 years). The median survival observed in our study was 28 months. The survival curve is shown in Fig. 36. This was significantly high as compared to that reported in various studies so far and it ranged from 5.8 months to 12 months (27,84,89,99). Disease stage, IPI score and CD4 count did not correlate with the survival in our study population as the sample size was small for analyzing these prognostic factors.

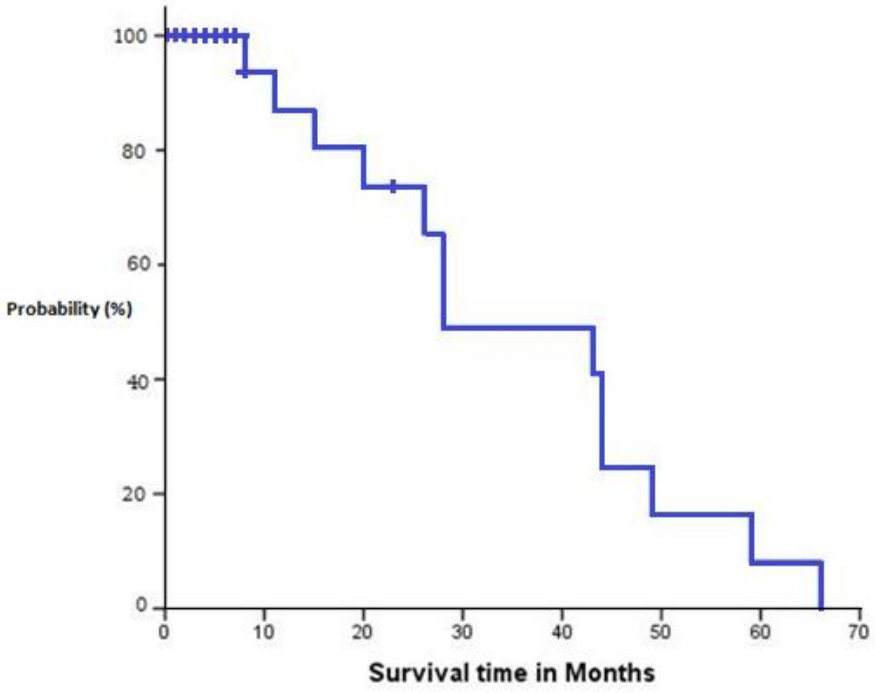


Figure 36: Survival curve for HIV associated lymphomas

7. CONCLUSION

I. This is the largest detailed clinicopathological study of HIV associated lymphomas from India.

II. DLBCL was the commonest type of HIV associated lymphoma followed by plasmablastic lymphoma, Burkitt lymphoma, Hodgkin lymphoma, PTCL NOS, ALCL, extranodal NK/T-cell lymphoma and extracavitary PEL.

III. Most cases were nodal, though there were a large number (44%) of extranodal primary sites involved.

IV. Most cases (69%) presented with high stage disease.

V. Survival was uniformly poor in 36% of the cases on whom the follow up was available with a median survival of 28 months.

VI. EBV association was seen in 47% of cases. Plasmablastic lymphomas and Hodgkin lymphoma were most often associated with EBV (90% and 100% respectively).

VII. There were significant differences between lymphomas in HIV positive individuals and lymphomas in HIV negative patients, the former being more aggressive types with high stage at presentation, more frequent extranodal involvement, EBV association and poor survival.

VIII. The striking differences from world literature included the higher frequency of plasmablastic lymphomas, lack of primary CNS lymphomas and low association with HHV8 (LANA immunohistochemistry) in our patients.

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Appendix 1:

Envision / Two step polymer procedure for immunohistochemistry:

1. 4µ tissue sections were cut and floated in slides coated with poly L-lysine.
2. Tissue sections were incubated overnight at 37˚C.
3. The sections were then deparaffinised using xylene (15 minutes) and then hydrated using different grades of alcohol (absolute – 90% - 80% - 70%).
4. Antigen retrieval was done using heat induced retrieval (HIR) method by Pascal pressure cooker to produce a temperature of 120˚C at 15psi pressure for 30 seconds in a retrieval solution/buffer (EDTA for EBV LMP1 and citrate for HHV8 LANA).
5. Washed with distilled water and TBS buffer (pH 7.6) and then covered with hydrogen peroxide for 10 minutes to block the endogenous peroxidase.
6. Washed with TBS buffer for 5 minutes (2 changes).
7. Primary antibody was added and incubated for 30 minutes at room temperature.
8. Washed with TBS buffer for 5 minutes (2 changes).
9. Incubated with secondary antibody (envision from DAKO) for 20minutes.
10. Washed with TBS buffer for 5 minutes (2 changes).
11. Covered with diaminobenzidine (DAB), a chromogen with substrate for 10 minutes.
12. Washed with running tap water.
13. Counterstained with Harris haematoxylin for 10 seconds.
14. Dehydrated using graded alcohol (70% - 80% - 90% - 100%).
15. Cleared and mounted in DPX.

Appendix 2:

EBER-ISH procedure:

1. The slides used for this procedure are positively charged silane coated slides on which 4μ thick sections were made.
2. The slides were loaded in the autostainer along with the detection kit, probe and the reagents.
3. Target sequences in the tissue were exposed by heating with the probe solution to denature the nucleic acids.
4. The reaction was then cooled to allow the probe to hybridize to its complementary nucleic acid sequence in the tissue.
5. This hybridized site was detected using the VENTANA ISH *i*VIEWBlue Detection Kit by the streptavidin-biotin technique.
6. This detection kit has the primary mouse anti-fluorescein antibody which binds to the fluorescein labeled hybridized site.
7. The biotinylated secondary goat anti-mouse IgG antibody binds to the primary antibody.
8. The streptavidin-alkaline phosphatase enzyme conjugate binds to the biotin residue in the secondary antibody.
9. This is visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (NBT) which produces a blue precipitate (nuclear staining).
10. This is readily visualized under light microscopy.

(All these steps were carried out through automation in Benchmark XT autostainer).

Appendix 3a (31,125)**:**

Ann Arbor staging system for non-Hodgkin lymphoma	
Stage I	Involvement of a single lymph node region or of a single extranodal site (IE).
Stage II	Involvement of two or more lymph node regions on the same side of the diaphragm; or localised involvement of an extranodal site or organ (IIE) and one or more lymph node regions on the same side of the diaphragm.
Stage III	Involvement of lymph node regions on both sides of the diaphragm, which may also be accompanied by localized involvement of an extranodal organ or site (IIIE), or spleen IIIS), or both (IIISE).
Stage IV	Diffuse or disseminated involvement of one or more distant extranodal organs with or without associated lymph node involvement.

Appendix 3b (31)**:**

Modified (Cotswold revision) Ann Arbor staging system for Hodgkin lymphoma	
Stage I	Involvement of a single lymph node region or lymphoid structure (e.g. spleen, thymus, Waldayer ring).
Stage II	Involvement of two or more lymph node regions on the same side of the diaphragm; the number of anatomic sites indicated by suffix.
Stage III	Involvement of lymph node regions or structures on both sides of the diaphragm. III_1 – with or without Splenic, hilar, celiac or portal nodes. III_2 – with paraaortic, iliac or mesenteric nodes.
Stage IV	Involvement of extranodal site(s) beyond those designated as E

A or *B:* The absence of constitutional symptoms is denoted by adding an "A" to the stage; the presence is denoted by adding a "B" to the stage. ("B symptoms" – Fever (>38˚C), drenching sweats, weight loss (10% body weight over 6 months)

E: Involvement of a single extranodal site, contiguous or proximal to known nodal site.

X: "Bulky disease" >10 cm large or mediastinum wider than 1/3 of the chest on a chest X-ray.

Appendix 4:

IPI scoring for aggressive non Hodgkin lymphoma:(126)

Risk Factor	
Age >60 yrs	1
Elevated serum LDH levels	1
Performance status ≥2(ECOG)	1
Ann Arbor stage III or IV	1
>1 site of extra nodal involvement	1

Risk: Low (0 or 1) / Low intermediate (2) / High intermediate (3) / High (4 or 5).

Performance status - Eastern Cooperative Oncology Group scale {ECOG}: (125,126)

Patient has no symptoms	0
Ambulatory patient with symptoms	1
Bedridden patient for less than half a day	2
Bedridden patient for half a day or more	3
Chronically bedridden with assistance required for daily activities	4

Appendix 5:

International Prognostic Score (IPS) for Hodgkin lymphoma:(127)

Albumin <4g/dL	1
Haemoglobin <10.5g/dL	1
Male	1
Age ≥45yrs	1
Stage IV disease	1
Leukocytosis – WBC>15,000/µL	1
Lymphopenia – lymphocyte differential count <8% and/or absolute lymphocyte count (ALC) <600 cells/ µL	1

Risk: Good risk = 0-1/ Fair risk = 2-3/ Poor risk = 4-7

Appendix 6:

Protocol for automated immunostaining:

1. Paraffin embedded tissue sections were cut at 4µ thickness and floated in poly L-lysine coated slides and incubated overnight at 37˚C.
2. These slides were then treated with 4% milk solution for 10 minutes to eliminate the hydrophobic effect and give positive charge to the slides.
3. Then the slide labels were bar coded and the labeled slides were loaded in Ventana Benchmark XT autostainer (a fully automated immunostainer).
4. Individual protocols have been designed in the software attached to the machine for each marker. Specific protocols were selected according to the marker.
5. A standard protocol was used for most of the markers with a minimal variation for few individual markers. The steps included in this protocol were as follows:
    a. Deparaffinization
    b. Liquid coverslip application.
    c. Heat induced antigen retrieval by treating with standard CC1 solution (pH patent with the company) for one hour at 90˚C.
    d. Then the primary antibody was added and incubated for 40 minutes @ 37˚C.
    e. Then the secondary antibody (Multimer) was added and incubated for 8 minutes.
    f. Finally the slides were counterstained with Haematoxylin and incubated for 8 minutes, followed by incubation with the bluing reagent for 4 minutes.

(From antigen retrieval till counterstaining, in between every step the slides were washed with reaction buffer. The whole process is automated).

6. Then the slides were brought to 80% alcohol (2 changes) to remove the liquid coverslip and then dried and mounted in DPX.

PROFORMA

Biopsy number: Age :

Name : Sex :

Clinical diagnosis : Hospital number:

Reference biopsies :

Site of Biopsy:

Nodal :

Extra nodal :

GIT: Stomach –

Small intestine –

Large intestine –

CNS:

Oral cavity:

Others:

Clinical details :

Fever : present/absent Malaise: present/absent

Loss of weight: present/absent Lymphadenopathy: Present / Absent

Hepatomegaly: mild/moderate/massive/absent Site: Cervical / Axillary / Abdominal / Inguinal

Splenomegaly: mild/moderate/massive /absent Marrow involvement: present / absent/not available

History of homosexuality: Yes / No / not available History of iv drug abuse: Yes / No / not available

Previous history of Kaposi's sarcoma: Yes / No/not available

Laboratory details:

Haemoglobin :

Total WBC :

Differential Count:

Platelet count :

Other blood borne virus :

Hypergammaglobulinemia:

LDH:

CD4 count:

PCR (HIV):

Serum creatinine:

Staging (Ann Arbor):

I (A/B)	II (A/B)	III (A/B)	IV (A/B)

IPI score :

Risk Factor	**Score**
Age >60 yrs	
Elevated serum LDH levels	
Performance status ≥2(ECOG) or ≤70 (Karnofsky)	
Ann Arbor stage III or IV	
>1 site of extra nodal involvement	

Risk: Low / Low intermediate / High intermediate / High

Morphology :

Effacement : Partial / Complete / Not applicable

Pattern : Nodular / Diffuse

Cell morphology :

I. Cell size: Small / medium / large

II. Cytoplasm:

a. Scant / moderate / abundant

b. Eosinophilic / amphophilic / clear

III. Nucleus:

a. Centroblastic

b. Immunoblastic

c. Plasmablastic

d. Plasmacytoid

e. Anaplastic

f. Reed Sternberg cell

IV. Mitotic index:

Stroma:

a. Vascularity: Unremarkable / increased

b. Necrosis : Present / absent

c. Sclerosis : Present / absent

Immunophenotype :

IHC marker	Positive	Negative	Reactive	Not done
LCA				
CD3				
CD20				
CD79a				
bcl 6				
CD 10				
bcl 2				
MIB 1				
CD15				
CD30				
PAX 5				
EBV- LMP 1				
Alk				
CD56				
VS38				
CD138				
KSHV-LANA				

Final diagnosis:

Treatment:

For lymphoma:

Drug regimen: CVP / CHOP / R-CHOP / MCP

For AIDS:

HAART – Given / not given

Follow up: Available / Not available

Period	Response to therapy			Disease Course			
	Complete	Partial	No response	Stable	Progressive	Relapsed	Expired
3 months							
6 months							
12 months							
18 months							
24 months							

Office of the Addl. Vice Principal (Research)

Christian Medical College,
Vellore 632 002

Ref: Res/01/2012

February 27, 2012

Dr. Rajalakshmi
PG Registrar
Department of Pathology
Christian Medical College
Vellore 632 002

Dear Dr. Rajalakshmi,

Sub: **FLUID Research grant project NEW PROPOSAL:**
HIV associated lymphomas – a five year clinicopathological study.
Dr. S. Rajalakshmi, PG Registrar, Pathology, Dr. Sheila Nair, Pathology, Dr. Vikram Mathews, Clinical Haematology, Dr. Auro Vishwabandya, Clinical Haematology, Dr. Rajesh Kannangai, Clinical Virology, Dr. Marie Therese Manipadam, Dr. Deepak Burad, Pathology.

Ref: IRB Min. No. 7740 dated 6.2.2012

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Yours sincerely,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board