DISSERTATION

on

EVALUATION OF PROGNOSTIC INDICATORS IN NON HODGKIN LYMPHOMAS: THE ROLE OF APOPTOTIC AND PROLIFERATIVE INDICES

submitted in partial fulfillment of the requirements for the degree of

Doctor of Medicine(BRANCH-III)

M.D. PATHOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY CHENNAI

TIRUNELVELI MEDICAL COLLEGE
TIRUNELVELI
APRIL 2017

CERTIFICATE

This is to certify that the dissertation titled **"EVALUATION OF PROGNOSTIC INDICATORS IN NON HODGKIN LYMPHOMAS: THE ROLE OF APOPTOTIC AND PROLIFERATIVE INDICES",** is a bonafide work done by **Dr.R.CHANDHRU MARI**, Post Graduate Student, Department of Pathology, Tirunelveli Medical College, Tirunelveli – 627011, in partial fulfilment of the university rules and regulations for the award of MD DEGREE in PATHOLOGY BRANCH-III, under my guidance and supervision, during the academic period from 2014 to 2017.

Prof. SITHY ATHIYA MUNAVARAH, MD,

Dean,
Tirunelveli Medical College,
Tirunelveli- 627011.

CERTIFICATE

I hereby certify that this dissertation entitled "**EVALUATION OF PROGNOSTIC INDICATORS IN NON HODGKIN LYMPHOMAS: THE ROLE OF APOPTOTIC AND PROLIFERATIVE INDICES"** is a record of work done by **Dr.R.CHANDHRU MARI**, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during his postgraduate degree course period from 2014- 2017. This work has not formed the basis for previous award of any degree.

Prof. K. SHANTARAMAN. MD,
Department of pathology,
Tirunelveli Medical College,
Tirunelveli- 627011.

Prof. K. SHANTARAMAN.MD,
Professor and Head,
Department of Pathology,
Tirunelveli Medical College
Tirunelveli- 627011.

DECLARATION

I solemnly declare that the dissertation titled **"EVALUATION OF PROGNOSTIC INDICATORS IN NON HODGKIN LYMPHOMAS: THE ROLE OF APOPTOTIC AND PROLIFERATIVE INDICES"** was done by me at Tirunelveli Medical College, Tirunelveli– 627011, during the period 2014 to 2017 under the guidance and supervision of **Prof.K.SHANTARAMAN, MD**, to be submitted to The Tamil Nadu Dr. M.G.R. Medical University towards the partial fulfilment of requirements for the award of MD DEGREE in PATHOLOGY BRANCH-III.

Place : Tirunelveli

Date :

Dr.R.CHANDHRU MARI
Post Graduate Student,
Department of Pathology
Tirunelveli Medical College,
Tirunelveli – 627011.

TIRUNELVELI MEDICAL COLLEGE

INSTITUTIONAL RESEARCH ETHICS COMMITTEE
TIRUNELVELI, STATE OF TAMILNADU, SOUTH INDIA PIN 627011
91-462-2572733-EXT; 91-462-2572944; 91-462-2579785; 91-462-2572611-16
online@tvmc.ac.in, tirec@tvmc.ac.in; www.tvmc.ac.in

CERTIFICATE OF REGISTRATION & APPROVAL OF THE TIREC REF NO: 648/PATHO /2015

PROTOCOL TITLE: EVALUATION OF PROGNOSTIC INDICATOR IN NON HODGKINS LYMPHOMA – A ROLE OF APOPTOTIC AND PROLIFERATIVE INDICES.

PRINCIPAL INVESTIGATOR: Dr.R,CHANDHRU MARI, MBBS,

DESIGNATION OF PRINCIPAL INVESTIGATOR: POST GRADUATE IN PATHOLOGY
DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE , TIRUNELVELI

Dear , Dr. R.Chandhrumari, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 09.02.15.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	**Clinical Trial Agreement (CTA)**	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented.
   d. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IEC, only then can they be implemented.
   e. Approval for amendment changes must be obtained prior to implementation of changes.
   f. The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g. Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

Dr.K.Shantaraman MD
Registrar, TIREC
Tirunelveli Medical College, Tirunelveli – 627011
State of Tamilnadu, South India

Dr.V.Ramasubramanian MD DM
Member Secretary, TIREC
Tirunelveli Medical College, Tirunelveli – 627011
State of Tamilnadu, South India

ACKNOWLEDGEMENT

I thank **Professor Dr. SITHY ATHIA MUNAVARAH, M.D**, Dean, Tirunelveli Medical College, for having permitted me to conduct the study and use the hospital resources in the study.

I express my heartfelt gratitude to **Professor Dr. SHANTARAMAN. K. MD**, Professor and Head, Department of Pathology, for his inspiration, advice and guidance in making this work complete.

I am extremely thankful to **Professors Dr. VALLIMANALAN. S. M.D, DR. SWAMINATHAN .K. M.D., DR.SURESH DURAI. J. M.D., DR.ARASI RAJESH, M.D, DR.VASUKI, M.D,** Additional Professors, Department of Pathology, for guiding me during the period of study.

I am extremely thankful to Assistant Professors**,** Dr.JohnsyMerla M.D, Dr.HidhayaFathima M.D, Dr.Sindhuja M.D, Dr.Mahalakshmi M.D, Department of Pathology, for guiding me academically and professionally during the period of study.

I also thank all the lab technicians and my fellow postgraduates for their cooperation which enormously helped me in the study. Without their humble cooperation, this study would not have been possible.

I thank **GOD AND MY PARENTS**, for blessing me not only in this study, but in all endeavours of my life.

ABBREVIATIONS

AIDS	-	Acquired Immune Deficiency Syndrome
ALK	-	Anaplastic Lymphoma Kinase
Bcl	-	b cell lymphoma gene
CD	-	Cluster of differentiation
CLL	-	Chronic Lymphocytic Leukemia
DNA	-	Deoxyribo Nucleic Acid
DLBCL	-	Diffuse Large B Cell Lymphoma
EBV	-	Ebstein Barr Virus
ELISA	-	Enzyme Linked Immunosorbent Assay
EDTA	-	Ethylene diamine tetra acetic acid
HL	-	Hodgkin Lymphoma
HHV	-	Human Herpes Virus
HTLV	-	Human T cell Lymphotrophic Virus
H&E	-	Haematoxylin and Eosin
IHC	-	Immunohistochemistry
Ig	-	Immunoglobulin

NHL	-	Non Hodgkin Lymphoma
PTLPD	-	Post Transplant Lymphoproliferative Disorder
PCR	-	Polymerase Chain Reaction
SLL	-	Small Lymphocytic Lymphoma
SLE	-	Systemic Lupus Erythematosus
Tris	-	Trisodium
WHO	-	World Health Organisation.

DISSERTATION

on

EVALUATION OF PROGNOSTIC INDICATORS IN NON HODGKIN LYMPHOMAS: THE ROLE OF APOPTOTIC AND PROLIFERATIVE INDICES

70

submitted in partial fulfillment of the requirements for the degree of

Doctor of Medicine (BRANCH-III)

M.D. PATHOLOGY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY CHENNAI

CONTENTS

AIMS AND OBJECTIVES

1. To estimate the role of apoptoic indices and proliferative indices in varying subtypes of non Hodgkin lymphomas
2. Evaluating the role of these indices to predict the prognosis of the patients with non Hodgkin lymphomas
3. To assess the trustworthiness of the working formulation classification of Non Hodgkin lymphoma

INTRODUCTION

Lymphoid neoplasms include various group of tumors of T-cell, B-cell, and NK-cell origin. In many circumstances the neoplastic cell phenotype resembles closely to that of a particular stage of normal lymphocyte differentiation. Worldwide lymphomas rank 6th among all cancers. [(1)]. Non Hodgkin lymphoma is the fifth most common cancer in the world with an upsurge of incidence in India. In past 4 decades the incidence of lymphoma has been doubled and it continues to peak.

Non-Hodgkin lymphoma (NHL) is a collective term for a heterogeneous group of lymphoproliferative malignancies with differing patterns of behaviour and responses to treatment. NHL in comparison to Hodgkin disease has a far greater predilection to disseminate to extranodal sites and much less predictable The prognosis depends on the stage, histologic type, and treatment.

Immunohistochemistry (IHC) is a method for localising specific antigens in tissues or cells based on antigen-antibody recognition. It exploits the specificity provided by the binding of an antibody with its antigen at a light microscopic level. Immunohistochemistry is applied in the following circumstances: to screen reactive tissue for a subtle abnormal population of cells, to completely phenotype the abnormal population of cells,and to further classify the abnormal population identified by flow cytometry . In lymphomas, immunohistochemistry is not only used in the above mentioned circumstances, but also in classifying, sub-classifying and in predicting the prognosis of several classes of lymphomas.

For Non Hodgkin's lymphomas there are many different classifications. The classification of lymphoma has always been a source of frustration for Clinicians and

pathologists. Recent WHO classification of lymphomas (2008)classifies NHL into B and T cell lymphomas and for this a panel of immunohistochemical markers are to be used Since, no single antigen is lineage or lymphoma specific, these panel of immunohistochemical markers are cost effective.

So in a case series study done by Lorenzo leoncini et al [(2)] quoted that working formulation classification of lymphomas still holds a greater significance. But to date there is no single classification that can predict how a particular tumour in a individual patient might behave. So a need to look at other prognostic indicators becomes necessary. Hence in this study we took apoptotic and proliferative indices as prognostic indicators[(3)] and we tried to evaluate the role of these indices in various subtypes of non Hodgkin lymphomas classified by working formulations.

REVIEW OF LITERATURE

LYMPH NODE

Lymphoid organs are one of the most important component of our body. They are classified into peripheral and central lymphoid organs. The lymphoid cells are primarily produced in the central lymphoid tissue while they differentiate, mature and processes the antigen in the peripheral lymphoid organs. The central lymphoid organs are bone marrow and thymus from which the lymphoid cells (B-lymphocytes and T-lymphocytes) are produced. The peripheral lymphoid organs are Lymph nodes, spleen, Mucosa associated lymphoid tissue (MALT).

Among all the other peripheral lymphoid organs, lymph nodes are considered the most important because they actively participate in immune response. Lymph nodes are composed of lymphoid cells which are arranged in follicles. They are covered by a capsule and located in regions where they drain the lymphatic vessels. These include axillary, cervical, abdominal, pelvic and inguinal group of lymph nodes. They are bean shaped organs composed of dense collection of lymphoid cells. Normally, the lymph node measures about 2 to 20 mm with an average of 15mm in its longest dimension.[4,5]

Afferent and efferent lymphatic vessels connects the lymph nodes to the general circulation . Normally, lymph nodes are grey-pink, soft, homogenous and are usually non palpable. They become palpable only as a result of intense immune response, neoplastic transformation or metastatic deposits. The diameter of lymph node greater than 3cm with nodular whitish cut surface and firm consistency are the features suggestive of neoplastic transformation or metastatic deposit.

The architecture of lymph node varies according to age , site and based upon the exposure of lymphoid cells to different antigens. Normally ,the cervical axillary lymph nodes and lymph nodes draining the head and neck region show abundant lymphoid follicles with reactive germinal centres which are due to secondary antigenic stimulation. But, lymph nodes such as andominal, pelvic, mesenteric nodes possess wide medullary cords and sinuses. The count of peripheral lymph nodes also varies significantly with no nodes in newborn, numerous in younger age groups and more numerous in the elderly[(6)].

Lymph nodes possess a complex variety of architecture where a variety of cell population which are arranged in distinct compartments. This environment provides a favourable factor for interaction and processing of foreign antigens resulting in effective response of immune system. Hence, lymph nodes are not only involved in mechanical filtration of foreign bodies through the lymphatic vessels but also play a very important role in recognition and processing of antigen.

The anatomy of lymph node includes cortex, paracortex and medulla which are composed predominantly by lymphocytes and plasma cells in different stages of maturation. The supporting framework is formed by the capsule, trabeculae, reticulin fibres and network of vesicular cells. The capsule is constituted by fibroblasts along with smooth muscle cells, Schwann cells nerve bundles, and blood vessels .these capsule extends into the underlying parenchyma as trabeculae.

The reticulin fibres which originate from reticulin cells are fine type 3 collagen fibrils, which form the main supporting framework of the lymph node. Reticulin fibres surround individual lymphoid cells, hence are not visualised under H&E stain. It can be well demonstrated by using silver impregnation technique (Gomori's technique).

CORTEX:

The lymph node cortex is primarily composed of lymphoid follicles. Lymphoid follicles can either be primary or secondary. Primary follicles are those which are usually a collection of dark staining naive B cells and are round to ovoid in shape. They transform into secondary follicles after antigenic stimulation. They are arranged with their long axis perpendicular to the lymph node [(5)].

The secondary follicles, which are transformed primary follicles, are composed of pale staining central germinal centre, a mantle zone and a marginal zone. Germinal centres are formed by the mixture of centroblasts, centrocytes dendritic reticulum cells, small lymphocytes which are primarily B-cells and tingible body macrophages with few scattered T lymphocytes.

The germinal centres are cuffed off by the mantle zone composed of small lymphocytes. The peripheral portion of the mantle zone is composed of cells that are loosely packed forming the marginal zone. These marginal zones are usually distinctly made out in the reactive follicles of spleen and they are not usually so evident in the lymph nodes [(7)].

PARACORTEX:

Paracortex are located deep beneath the cortex extending in between the lymphoid follicles and are composed of the T-cell packed zones in the lymph nodes . These areas are composed of T-cells in various stages of maturation [8], admixed with post capillary venules lined by high endothelial cells. In addition, there are interdigitating dendritic cells which serve as antigen presenting cells. They play an important role in evoking an immune response.

MEDULLARY AREA

This is located deep to the paracortex and is primarily composed of plasmacytoid lymphocytes, plasmablasts, mature plasma cells monocytes and macrophages and cords of lymphocytes. The plasma cells lose their surface markers and synthesize immunoglobulins of varying classes with kappa and lambda chain in the ratio of 2:1[9].

CELLULAR COMPOSITION:

The lymph node is formed by T-cell and B-cell zone containing cells in various stages of activation and differentiation. The paracortical area forms the T-cell zone whereas the germinal centres and lymphoid follicles form the B-cell zone. The medullary region is predominantly formed by the plasma cells.

The dark staining naive B cells that forms the primary follicles also accounts for the mantle zone of the secondary follicles[10]. These are small cells with scant cytoplasm and increased nuclear cytoplasmic ratio. Centroblasts are large cells that form the dark zone in the germinal centres and they express IgM on their surface. These centroblasts are differentiated into centrocytes which forms the light zone in the germinal centres.

The centroblasts that are transformed into plasma cells move into the medullary region while that differentiate into memory cells reside in the mantle zone. The histiocytes are the transformed monocytes in the tissues that accumulate in the paracortical areas and germinal centres secondary to immune activation. These are large cells that contain abundant neither strongly basophilic nor pyroninophilic cytoplasm. These can be differentiated from large lymphocytes with the help of ultrastructural and immunohistochemical study. The histiocytes that accumulate in the germinal centres form the tingible body macrophages. The tingible body macrophages contain numerous DNA fragments formed secondary to cell death after bcl-2 gene gets shut off.

NORMAL LYMPH NODE AND IHC:

In normal lymph node, the main application of immunohistochemistry is to study the differential expression of the same antibody in various regions of the lymph node and assess the cellular composition and clonality of the lymphoid cells. Studying the clonality among various lymphoid populations helps us to differentiate reactive hyperplasia from neoplastic transformation of the lymph node, more importantly Non Hodgkin lymphoma.

B cells in the lymphoid follicles usually express pan-B cell marker such as CD19, 20, 22 and 79a. In addition, centroblasts express CD10 and cells of the germinal centre show positive expression of Bcl-6 in the nucleus. Bcl-2 which is an anti-apoptotic protein is usually shut off in the germinal centre and shows positive expression in the mantle & marginal zone. In addition, positive expression of Bcl-2 in germinal centre is a feature of neoplastic transformation.[11,12,13] Naive B cells in the

primary follicle and mantle zone expresses CD5,surface IgM and IgD. [IgD is not expressed in the germinal centre].

The B-centroblast express IgM on their which shifts to IgD/IgA in the case of centrocytes. Immunoglobulin Kappa and lamda expression is usually seen in all the B lymphocytes but monoclonality points towards neoplastic transformation.

The small lymphocytes that are scattered throughout the germinal centre show positive expression for CD3, CD5, CD7, CD43 and CD4/8 that gives a clue that they are reactive T cells. Tingible body macrophages stain with CD11b, CD35 and CD68 whereas dendritic reticulum cells stain with CD21, 35, C3b, C3d. [14]

The cells in the paracortical region stain with pan T cell markers like CD2, CD3, CD5 and CD7, CD43 and HLA-DR. Interdigitating dendritic cells show positive expression for S100, CD24, HLA- DR. Plasma cells are CD138(Syndecan 1) positive. Macrophages stain for lyzozyme, α1-antitrypsin, S100 protein and CD68.[15,16]

LYMPHOMA:

Lymphomas and leukemias are the two challenging fields in medical investigation in which immunity and neoplasia interface. It is very important to identify the cases of lymphoma and differentiate it from various types of non neoplastic lymphadenopathies. Once the presence of lymphoma has been identified, it should be classified into Hodgkin and Non Hodgkin lymphoma.

The Non Hodgkin lymphoma should be further subclassified morphologically, immunologically and phenotypically so as to establish the exact diagnosis for the purpose of therapy and prognosis. There are many modes of investigations to diagnose malignant lymphomas which include cytomorphology, radiology, immunology and

molecular techniques. Hence, proper selection of the investigations will facilitate correct diagnosis at a reasonable expense to the patient.

NON-HODGKIN LYMPHOMA:

Non-Hodgkin lymphomas are clonal lymphoproliferative disorders characterised by proliferation of malignant lymphoid cells that forms discrete mass. This includes diverse group of tumor of B-cells, T-cells and natural killer cell origin. Majority of the cases of non Hodgkin lymphomas are of B-cell origin while the remainder of the cases have T-cell origin. Non-Hodgkin lymphoma very rarely involves natural killer cells. Most of the lymphoid neoplasms resemble some recognizable stage of B-cell or T-cell differentiation with neoplastic lymphoid cells recapitulating the behaviour of their normal counterpart.

In earlier days it was believed that lymphadenopathy was a secondary phenomenon occurring secondary to underlying infection or carcinoma. Thomas Hodgkin first recognised that lymph node enlargement could occur as a primary disorder rather than secondary to underlying infection or carcinomas[17]. Billroth was the first one who used the term malignant lymphoma for a collection of primary lymphoid disorders[18].

During eighteenth century Sternberg[19]and Reed[20] first identified the giant cells which are considered as the characteristic feature of Hodgkin lymphoma and introduced histopathological examination as the primary method of diagnosis and classification of lymphoproliferative disorders. Brill[21] and Symmers[22], in 1920, described the features of follicular lymphoma. In 1956, Rappaport through his classical

work classified Non-Hodgkin lymphoma based upon the morphology of the malignant lymphoid cells[23]. Roulet[24] in 1930 considered reticulum cell sarcomas as a synonym for malignant lymphomas.

In 1967, Good and Finstad demonstrated the relationship of B lymphocytes and T lymphocytes to the development of lymphoid neoplasms. Later Dameshek, through his work, suggested that lymphoproliferative disorders primarily occurs due to aberrations of immunologically competent cells and also lymphoid cells has the potential to get transformed into immunoblast like cells under appropriate antigenic stimulation. Immunological origin of malignant lymphocytes was confirmed in 1972 by the presence of immunoglobulins over the surface of B lymphocytes and by the formation of sheep erythrocyte rosette with neoplastic T lymphocytes [25, 26].

Lennert, Lukes and Collins at United states in 1974, classified Non-Hodgkin lymphoma based on cell of origin into B cell and T cell lymphomas **[27, 28].** Later in 1980, origin of lymphoid neoplasm was confirmed at molecular level after a detailed study on immunoglobulin gene over B lymphocytes and T cell receptors over T lymphocytes [29, 30]. Working formulation in 1982 classified Non-Hodgkin lymphoma based upon the histological grade and correlated it with the patient survival. From 1994, Revised European American Lymphoma classification came into use, which classified lymphoma based upon the clinical features, morphology, immunophenotyping and genetic data which were available[31]. Finally World Health Organisation has adopted the diagnostic principle of REAL classification and now WHO classification is used as schema for the diagnosis of all hematopoietic neoplasm.

Non Hodgkin Lymphoma and non modifiable risk factors

Non-Hodgkin lymphomas are the sixth most common cancer worldwide. Frequency of Non-Hodgkin lymphoma is age dependent and has variable distribution worldwide. Non-Hodgkin lymphomas are more common in males than females and hence show male preponderance. Malignant lymphomas are most common among adults and accounts for only 10% of all childhood malignancies in developed countries. Non-Hogkin lymphomas show a steady increase in incidence from childhood through age of 80 years[32].

Increased risk for development of Non Hodgkin lymphoma has been documented among close relatives. There is two to four fold increased incidence of Non-Hodgkin lymphoma among close relatives of patients with lymphoma[33]. Aggregations have been reported among siblings and male relatives [34, 35]. Earlier age of onset in subsequent generation has been recorded in Non-Hodgkin lymphoma[36]. Increase in incidence of lymphoproliferative disorders is seen in association with autoimmune lymphoproliferative syndrome which includes autoimmune features, splenomegaly and chronic lymphadenopathy[37]. Malignant lymphomas are more common among people with family history of lymphoma[38].

Young age and Non Hodgkin Lymphoma

Malignant lymphomas are the third most common cause of childhood neoplasms next to brain tumours and acute lymphoblastic leukaemias[39,40]. There is increased risk for Hodgkin lymphoma among the children less than 18 years, whereas Non Hodgkin lymphomas accounts for majority of the cases below 15 years. There

occur many differences in the manifestations of Non-Hodgkin lymphomas of adults and children. Lymphomas among the children are high grade with frequent extranodal manifestations, which in adults are primarily nodal and usually of low grade and intermediate grade.

Non-Hodgkin lymphomas in children are more common in boys when compared to girls and occur more frequently in blacks than whites. The three most important Non Hodgkin lymphomas occurring among children are Burkitt's lymphoma, lymphoblastic lymphomas and large cell lymphomas[41]. Large cell lymphomas are characterised by neoplastic transformation of lymphoid cells which are larger than the diameter of the histiocytic nucleus.

This includes Diffuse large B-cell lymphomas, Anaplastic large cell lymphomas and peripheral T-cell lymphomas among which Anaplastic large cell lymphomas are most common followed by Diffuse large B-cell lymphomas. But overall, lymphoblastic lymphomas forms bulk of the pediatric lymph node malignancies. Follicular lymphomas and marginal zone lymphomas occurs rarely and are considered uncommon neoplasms in the childhood.

Infection and Non Hodgkin Lymphoma

Infections play a major role in lymphomagenesis. The development of lymphoma depends on various factors including environment, geography and host factors[42]. Infectious agents cause lymphoma through direct lymphocyte transformation. Most of the infectious agents that cause lymphomas are found to be viruses which includes EBV, HTLV-1, HHV8. Chronic infection by hepatitis-C virus

can result in B-cell Non-Hodgkin lymphomas[43]. Indolent B cell lymphoma, lymphoplasmacytic lymphoma and marginal zone lymphoma are the most common types of lymphomas that are associated with hepatitis-C virus.

Helicobacter pylori is a gram negative rod that was discovered by Warren and Marshall in 1983 and was shown to be associated with peptic ulcer disease, carcinoma of stomach and Non Hodgkin lymphoma [44,45]. Later on Personett recognised that Helicobacter pylori infection preceded the development of lymphoma[46] and Wotherspoon observed that there is regression of lymphoma in most of the patients after treatment with antibiotics[47]. Other infectious agents includes, campylobacter jejuni which causes small intestinal immunoproliferative diseases, Borrelia burgdoferi which causes primary cutaneous B cell lymphoma and Chlamydia psittaci which is the causative agent for ocular adnexal MALTomas[42].

AIDS and transplant related Non Hodgkin Lymphoma

Lymphomas both Hodgkin and Non Hodgkin can be secondary to infection by Human immunodeficiency virus. Non-Hodgkin lymphomas can occur in setting of underlying and congenital and acquired immunodeficiencies[48]. After Kaposi sarcoma, lymphomas are the most AIDS related cancer [49]. NHL occurs in 3% of the patients suffering from AIDS [50], with sixty time greater risk of acquiring lymphomas when compared to persons without infection. Most of the Non-Hodgkin lymphoma represents B-cell type, among which 70% is constituted by diffuse large B-cell lymphomas and the remaining being Burkitt's lymphoma. Among patients of lymphomas found in the general population of young patients in which Hodgkin lymphomas are more common,

lymphomas in AIDS patients are mostly Non Hodgkin type with frequent extranodal manifestations [51, 52]. The occurrence of lymphomas in AIDS can be attributed to florid activation of lymphoid cells which provides opportunity for translocation, mutation and deletion of tumor suppressor gene [53, 54].

Post transplant lymphoproliferative disorders include both polymorphic and monomorphic proliferation of lymphoid cells. PTLPD are most common during first year after solid organ and bone marrow transplant. The persons with multiple organ transplants are at increased risk of developing post transplant lymphoproliferative disorders. Lymphoid cell proliferation can occur both in patients with and without EBV infection. The main cause for occurrence of both polymorphic and monomorphic proliferation can be attributed to mutation in the variable region of immunoglobulin[55]. These mutations have been found in about 75% of polymorphic PTLPD and 90% of monomorphic proliferation. Among monomorphic proliferation Burkitt's lymphomas constitute the bulk of the lymphomas, remaining being cases of Diffuse large B-cell lymphomas.

Autoimmune and other disorders

Chronic inflammation, immune hyperactivity and immunosuppression are considered to be important elements that predispose patients to lymphoma [56]. Non-Hodgkin lymphomas associated with Sjogren's syndrome and Hashimoto's thyroiditis are of B-cell origin and they usually occur in elderly females. In 1963, Bunin and Talal[57] reported Non-Hodgkin lymphoma in a case of Sjogren's syndrome. In 1957, Lindsay and Dailey[58] demonstrated the association of lymphoma with Hashimoto's

thyroiditis. There is 60 to 80 fold increase in thyroid lymphomas among the cases of thyroiditis with lifetime risk of 1-2%[59]. Non-Hodgkin lymphoma has been associated with rheumatoid arthritis, SLE, and dermatomyositis.

Non-Hodgkin lymphoma has been observed to originate from treated cases of Hodgkin lymphoma. Krikorian[60] reported 6 cases of Non-Hodgkin lymphoma arising from Hodgkin lymphoma. First case of Non-Hodgkin lymphoma associated with AIDS has been reported in 1982 [61]**.** Majority of the lymphomas arising from AIDS patients are of B-cell origin. Unique presentation of AIDS related lymphomas include plasmablastic lymphomas of oral cavity[62] and primary effusion lymphomas [63].

Non Hodgkin Lymphoma and presentation

Most of the cases of Non-Hodgkin lymphoma presents as painless lymphadenopathy in cervical and supraclavicular region [64]. However, 40% of patients have extranodal presentation. Gastro intestinal tract is the most common extra nodal site of presentation, in which stomach is the commonest site followed by small intestine, colon and esophagus, in that order. Rarely, patients with rectal involvement present with altered bowel habits [65]. Epistaxis and nasal obstruction are the modes of presentation in cases of Non-Hodgkin lymphoma of nasal cavity. Hepatosplenomegaly have been observed in cases of low grade B-cell lymphomas such as small B-lymphocytic lymphomas and marginal zone lymphomas. Skin is another most common site of Non-Hodgkin lymphoma presentation. Non-Hodgkin lymphoma can primarily involve CNS[66]in which diffuse large B-cell lymphomas are the most common. Other rare sites involved are eye,extraocular spaceincluding conjunctiva,eyelids &lacrimal

glands [67] ,testis,kidney and breast. Breast lymphomas associated with pregnancies and lactation often has diffuse involvement[68] . Primary cardiac lymphomas are extremely rare and usually occur in immunodeficient state [69] .

GRAY ZONE LYMPHOMAS

Malignant lymphomas are classified into Hodgkin and NHL based on morphologic pattern,cell type and more importantly based on analysis of antigenic expression. Both should be differentiated as they require distinct treatment for regression. The new modes of investigations like immunophenotyping and genetic analysis have revealed that there exist certain lymphoid neoplasms that can neither be classified as Hodgkin lymphoma nor as NHL. These were given the name, gray zone lymphomas [70, 71]. This includes nodular lymphocyte predominant Hodgkin lymphoma, T cell rich large B cell lymphoma, Anaplastic large cell lymphoma, peripheral T-cell lymphoma and primary mediastinal large B-cell lymphoma.

Gray zone lymphomas should be separated from conventional lymphomas as they are aggressive and require distinct treatment [72, 73].

CLASSIFICATION OF NON-HODGKIN LYMPHOMA

Currently, lymphoma diagnosis is based upon the classification of World Health Organisation, which relies upon the morphology of the node including pattern, cell size and shape, radiological features, flow cytometry and IHC for evaluating the antigenic expression and finally cytogenetics. Based upon these, lymphoma has been classified by WHO into Hodgkin and Non Hodgkin lymphoma. The Hodgkin Lymphoma has been broadly classified into nodular lymphocytic predominant HL and classic Hodgkin

lymphoma. The Non Hodgkin lymphoma has been further classified into B and T cell lymphomas which are sub-classified as precursor and peripheral (mature) B cell and Tcell Non Hodgkin lymphoma, respectively. The precursor B and T cell lymphomas represent the neoplastic transformation of the progenitor cells which are not exposed to antigens, hence are in an inactivated and undifferentiated stage. The peripheral lymphomas arise from the rest of the lymphoid cells in various stages of maturation.

Mature B cell neoplasm constitutes about 90% of NHL worldwide [(74)]. B cell represent all follicular lymphomas and diffuse lymphomas in North America and Europe, whereas T cell lymphomas are most common in Asia [(75)]. The nomenclature, classification and differentiation of various types of lymphomas depend upon various factors including the cell of origin eg; mantle cell lymphomas, clinicopathological name Eg: Burkitt's lymphoma, their location Eg: mediastinal large B cell lymphomas and presumed fuction Eg; MALTomas.

The grade and prognosis of Non Hodgkin lymphomas depend upon the morphological pattern, size and shape of the cell, mitosis and invasiveness. The pattern of neoplastic transformation is divided into nodular and diffuse in which the nodular growth pattern represents the differentiation of malignant lymphomas towards lymphoid follicles. The malignant lymphoma cells are classified into small, medium and large sized by comparing the nucleus of the neoplastic cells to the adjacent histiocytes or endothelial cells. On the basis of shape, it is classified into cleaved and non-cleaved in which the cleaved cells represent non-dividing cells and non-cleaved represent the dividing cells. In general, lymphomas are considered aggressive when the

cells are large, non-cleaved with a diffuse pattern of growth showing increased mitosis and invasion.

T cell neoplasms are less common when compared to B cell lymphomas in western countries, representing 20% of the Non Hodgkin lymphoma [(76)]. T cell lymphoma along with NK cell lymphomas account for 12% of all the NHLs [(77)]. They are classified similar to B cell neoplasms into precursor (or) thymic T cell lymphoma and mature (or) peripheral T cell lymphomas. The largest group which represents peripheral T cell lymphomas are the T cell lymphoma NOS type, accounting for more than 50% of the cases of T cell lymphomas. Generally, the T cell lymphomas are aggressive with a 5 year survival rate only 20-30% which are characterised by diffuse growth pattern, with mixed population of small and large cells in an inflammatory background. More frequently, epitheloid cells and eosinophils are also made out.

MODIFIED KIEL CLASSIFICATION [78]

KIEL CLASSIFICATION	Nearest correspondence to categories of Rappaport classification	Nearest correspondence to categories of Lukes-Collins classification
LYMPHOMAS OF LOW GRADE MALIGNANCY		
Lymphocytic lymphomas		
1. Chronic lymphocytic leukemia (B-cell type)	Well-differentiated lymphocytic lymphoma	B cell lymphoma of small lymphocytes
2.Hairy cell leukemia	-	-
3.Mycosis fungoides and Sezary's syndrome	-	T-cell lymphomas:.Mycosis fungoides and Sezary's syndrome
4.Chronic lymphocytic leukemia (T- cell type)	-	-
Lymphomas of immunoglobulin - secreting cells	Apart from lymphomas-'proliferative diseases with dysprotinemia'	
1.Lymphoplasmacytic/lymphoplasmacytoid lymphoma	-	B-cell lymphoma of plasmacytoid lymphocytes
2.Plasmacytic lymphoma	-	-
Lymphomas of germinal centre cells		

1.Centrocytic lymphoma	well –differentiated & poorly differentiated lymphocytic lymphomas; Mixed cell(lymphocytic- Histiocytic) lymphoma;Histiocytic lymphoma	B-cell lymphoma of follicular centre cells with cloven nucleus (diffuse type)
2.Centroblastic /centrocytic lymphoma follicular follicular and diffuse diffuse with or without sclerosis	well –differentiated & poorly differentiated lymphocytic lymphomas; Mixed cell(lymphocytic- Histiocytic) lymphoma;	B-cell lymphoma of follicular centre cells with cloven nucleus
LYMPHOMAS OF HIGH GRADE MALIGNANCY		
Centroblastic lymphomas	Histiocytic lymphoma; undifferentiated lymphoma	B-cell lymphoma of large follicular centre cells with non-cloven nucleus
1.Primary		
2.Secondary		
Lymphoblastic lymphomas (lymphoblastic sarcomas, including acute lymphoblastic leukemia)	Undifferentiated lymphoma; poorly differentiated lymphocytic lymphoma	B-cell lymphoma of small follicular centre cells with non-cloven nucleus
1.Burkitt lymphoma	Undifferentiated lymphoma	As above

2.Lymphoma of convoluted cells [i.e., cells with convoluted nucleus]	Undifferentiated lymphoma; poorly differentiated lymphocytic lymphoma	T-cell lymphoma of convoluted lymphocytes
3.Unclassified lymphoma	poorly differentiated lymphocytic lymphoma	U-cell ('undefined cell') lymphoma; unclassifiable lymphomas.
Immunoblastic lymphomas	Histiocytic lymphoma	Immunoblastic sarcoma of B cells; immunoblastic sarcoma of T cells
With plasmablastic/ plasmacytic differentiation (derived from B cells)		
Without plasmablastic/plasmacytic differentiation (derived from B cell or T cell)		
(a) Leukemic variant (immunoblastic leukemia)		

[This table represents Kiel classification modified by Lennert et al in 1977 [(79)]. The Kiel classification and Lukes-Collins classification relates to the functional characteristics of the cells that relate to the tumors.]

A WORKING FORMULATION OF NON-HODGKIN LYMPHOMAS [(79)]

This classification was introduced in 1982 by National cancer institute,

United states [(79)], as an attempt to provide a morphologic classification scheme that had a prognostic relevance [(83)].

LOW- GRADE
Malignant lymphoma
Small lymphocytic
Consistent with chronic lymphocytic leukemia
Plasmacytoid
Malignant lymphoma, follicular
Predominantly small cleaved
Diffuse areas;sclerosis
Malignant lymphoma, follicular
Mixed, small cleaved and large cells
Diffuse areas;Sclerosis
INTERMEDIATE-GRADE
Malignant lymphoma, Follicular
Predominantly large cell
Diffuse areas; Sclerosis
Malignant lymphoma, Diffuse
Small cleaved
Sclerosis
Malignant lymphoma, Diffuse
Mixed, small and large cell
Sclerosis; Epithelioid cell component
Malignant lymphoma, Diffuse
Large cell
Cleaved, non cleaved; Sclerosis
HIGH GRADE
Malignant lymphoma
Large-cell, Immunoblastic
Plasmacytoid; clear cell; polymorphic; epithelioid cell component
Malignant lymphoma
Lymphoblastic
Convoluted; non convoluted
Malignant lymphomas
Small noncleaved
Burkitt; Follicular areas
MISCELLANEOUS
Composite
Mycosis fungoides
Histiocytic
Extramedullary plasmacytoma
Unclassifiable
Others

WORLD HEALTH ORGANISATION CLASSIFICATION OF LYMPHOID NEOPLASMS (INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, LYON, 2008) [80]

This classification is based on the collaborative project of European Association for Haematopathology and society for Hematology [81]. This classification is based on principles initially defined in the "Revised European-American Classification of Lymphoid Neoplasms" (REAL), from the International Lymphoma Study Group (ILSG)[82].

PRECURSOR B- AND T-CELL NEOPLASMS

Precursor B-lymphoblastic leukaemia/lymphoblastic lymphoma
Precursor T-lymphoblastic leukaemia/lymphoblastic lymphoma

MATURE B-CELL NEOPLASM

Chronic lymphocytic leukaemia/ Small lymphocytic lymphoma
B-cell prolymphocytic leukaemia
Lymphoplasmacytic lymphoma
Splenic b-cell marginal zone lymphoma
Hairy cell leukaemia
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Heavy chain disease
MALT-lymphoma
Nodal marginal zone B-cell lymphoma
Follicular lymphoma
Mantle cell lymphoma
Diffuse large B-cell lymphoma, NOS
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Mediastinal large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK positive large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
Burkitt lymphoma
B-cell lymphoma, unclassifiable with features intermediate between DLBCL & Burkitt lymphoma
-cell lymphoma, unclassifiable with features intermediate between DLBCL and classic Hodgkin lymphoma

MATURE T-CELL AND NK-CELL NEOPLASM

T-cell prolymphocytic leukaemia
T-cell large granular lymphocytic leukaemia
Aggressive NK-cell leukaemia
Adult T-cell leukaemia/lymphoma
Systemic EBV positive T-cell lymphoproliferative disease of childhood
Hydroa vacciniforme-like lymphoma
Mycosis fungoides
Sezary syndrome
Primary cutaneous anaplastic large cell lymphoma
Primary cutaneous CD30 positive T-cell lymphoproliferative disorder
Primary cutaneous gamma-delta T-cell lymphoma

Lymphomatoid papulosis
Extranodal NK/T-cell lymphoma, nasal type
Enteropathy-type T-cell lymphoma
Hepatosplenic T-cell lymphoma
Subcutaneous panniculitis- like T-cell lymphoma
Angioimmunoblastic T-cell lymphoma
Peripheral T-cell lymphoma
Anaplastic large cell lymphoma

HODGKIN LYMPHOMA

Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin type
- Nodular sclerosis classical Hodgkin lymphoma
- Mixed cellularity classical Hodgkin lymphoma
- Lymphocyte rich classical Hodgkin lymphoma
- Lymphocyte depleted classical Hodgkin lymphoma

CELLS OF ORIGIN OF NON HODGKIN LYMPHOMA

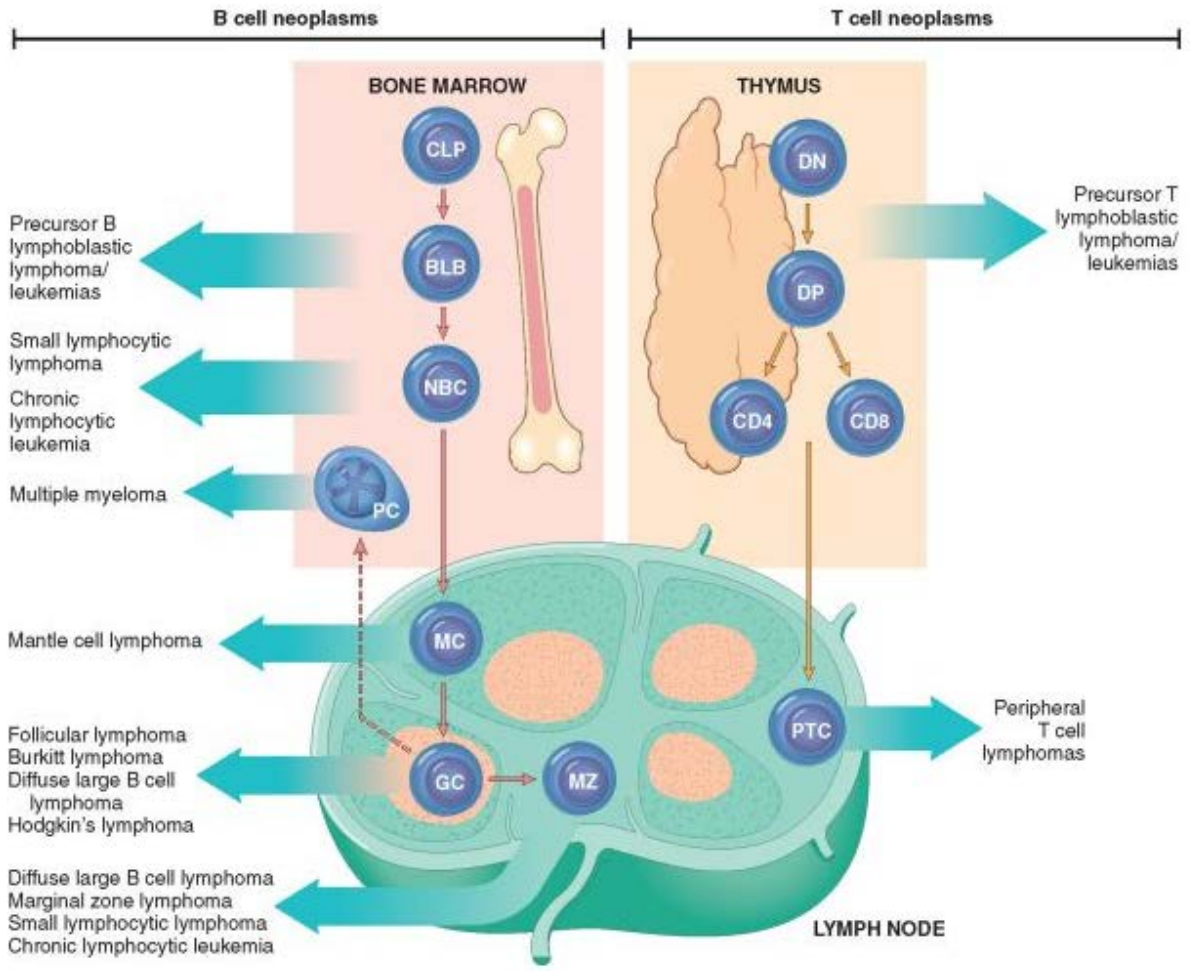

Figure 1[(81)]: Origin of lymphoid neoplasms. Stages of B- and T-cell differentiation from which specific lymphoid tumors emerge are seen.

As seen in the above illustration, the immature B- cells and T-cells derived from the bone marrow are the cells of origin for precursor B-cell and T-cell lymphomas/leukemias, respectively. The germinal centre B cell gives rise to burkitt lymphoma, follicular lymphoma and diffuse large B-cell lymphoma. DLBCL also arises from the postgerminal centre B-cells. The post germinal centre memory B-cells give rise to hairy cell leukemia , extranodal marginal zone lymphoma and CLL/SLL. The

peripheral T cell lymphomas of nodal origin arise from the T cells in the paracortical area of the lymph node.

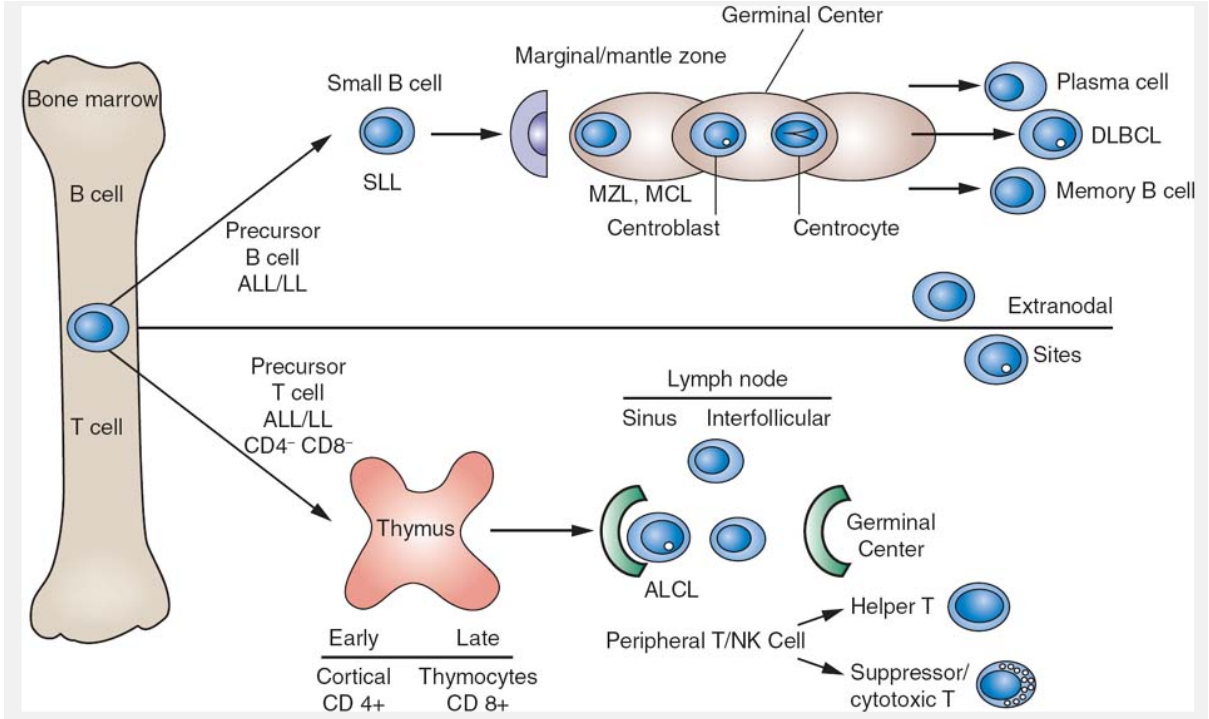

Figure 2[(82)]: cellular origin of Non Hodgkin Lymphoma based on B cell and T cell differentiation

The above illustration shows the different classes of lymphoma based on Lukes-Collin classification. The B cell lymphoma of small lymphocytes constitutes CLL/SLL, whereas the B-cell lymphoma of follicular centre cells with cloven nucleus (diffuse type) constitutes the centrocytic lymphoma. B-cell lymphoma of large follicular centre cells with non-cloven nucleus constitutes centroblastic lymphoma, whereas B-cell lymphoma of small follicular centre cells with non-cloven nucleus lymphoblastic lymphoma.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is atechnique which is used to detect the specific antigens over the tissues or cells based on the pattern of antigen antibody reactions. Coons [(83)] in 1940 introduced immunoflorescence to localise corresponding antigen in frozen section.the enzymatic label horseradish peroxidase developed by Avrameas [(84)] and by Nakane and colleagues[(85)] provided the new path for the visualisation of the labelled antibody by light microscopy in the presence of appropriate colorigenic substrate system.

The aims of immunohistochemistry are akin to those of histochemistry. Indeed, immunohistochemistry builds on the foundations of histochemistry. Immunohistochemistry does not replace histochemistry but rather serves as the valuable adjunct that greatly extends the variety of tissue components that can be demonstrated specifically with tissue sections. The main aim of performing IHC is to recognise microchemically the existence and distribution of substances which we have been made aware of macrochemically[(86)]. Immunohistochemistry can provide a tissue based immunoassay with the reproducibility and quantitative characteristics of an ELISA test.

Demonstration of antigen in routinely processed formalin-fixed paraffin embedded tissues[(87)] was done for the first time in 1974 by Taylor and Burns in Oxford.Then as the immunohistochemistry evolved, its utilisation in diagnostic pathology steadily expanded such that more than one immunohistochemistry markers were used in routine surgical pathology especially with respect to tumour diagnosis and classification. The introduction of hybridoma technique[(88)] facilitated in the

development of immunohistochemistry and the manufacture of many highly specific monoclonal antibodies.

The phenomenon of enzyme digestion was then introduced by Huang as the pretreatment to immunohistochemistry staining to unmask some antigens that had been altered by formalin fixation[89]. The main disadvantage of using enzyme digestion was that it proved difficult to control the optimal digestion conditions for different tissue sections when stained with different antibodies. Hence, to overcome the difficulties encountered in enzyme digestion Shi and his associates in 1991[90, 91, 92, 93, 94, 95] introduced antigen retrieval technique, based on series of studies by Fraenkel and co-workers [96, 97, 98]. Various modifications of antigen retrieval has been described, among which majority of those used different buffer solution in the place of metal salt solution, which may have serious toxic effect[99,100].

Antigen and antibody reaction

An antibody is a molecule that has the property of combining specifically with the antigen. Antigen-antibody recognition is based on three dimensional structure of the protein. Antibodies are immunoglobulin molecules containing pair of light chain and pair of heavy chain. An epitope is an antigenic determinant on which the antibody combines. An epitope is an antigenic determinant on which antibody combines. For a protein, the term epitope corresponds to a cluster of aminoacid residues that binds specifically to the paratope of an antibody[101].

Antigen-antibody reaction depends on two factors that are related to antibody which includes sensitivity and specificity. Antibodies are primarily of two

types monoclonal and polyclonal. Comparitive studies on sensitivity and specificity among monoclonal and polyclonal antibodies proves that polyclonal antibodies are more sensitive and less specific than monoclonal antibodies. The reason may be polyclonal antibody which is a composite of many antibodies may recognise several binding epitope whereas monoclonal antibody recognise single type of epitope.

Most of the monoclonal antibodies in current use are derived from murine clones. Recently a number of rabbit derived monoclonal antibodies have appeared on the market. Some of the rabbit derived antibodies offer advantage over murine clones for the detection of antigens by immunohistochemistry.

Enzyme labels in immunohistochemistry

Enzymes are the widely used labels used in immunohistochemistry. Incubation with the chromogen using proper histochemical method produces stable colour reaction end product that can be easily read by using light microscopy. Variety of enzymes and chromogens are available which produces many number of coloured end products. Horseradish peroxidase is the most commonly used enzyme label. When used in conjunction with most favoured chromogen like Diaminobenzidine tetrachloride yield insoluble, stable brown coloured end product. Horseradish peroxidase is most preferred as they are very small in size, and can be very easily obtained in pure form. It is very stable and does not undergo alteration during manufacture, storage and during application.

Various other chromogens are also used based on the choice of end product coloured reaction. Graham in 1965 used 3-amino 9-ethyl carbazole which

produces red colour. Later in 1968, Nakane used 4-chloro 1-napthol produces blue colour. Hanker in 1977, used Hanker- Yates reagent, that produces a dark blue colour. Commercially chromogens are available in Kit form such as vector laboratories which used vector red and vector blue. Alternatives to Diaminobenzidine tetrachloride are more commonly used in multilabelling technique. Rarely colloid metal label such as gold conjugates are used. When used alone it appears pink under microscope. A silver precipitation reaction can be used to amplify the visibility of gold conjugates. The use of radioisotopes as the tracer requires autoradiographic facilities.

Antigen retrieval

Antigen retrieval is the process of reversal of chemical reaction that has occurred between formalin and protein. Heat induced antigen retrieval technique is now widely used in pathology[(102)]. It has certainly brought a very good improvement in the quality of immunohistochemistry. The optimal result of antigen retrieval in immunohistochemistry depends on heating temperature, period of heating and P_H of antigen retrieval solution.

Various methods are used in the process of antigen retrieval which includes microwave antigen retrieval, pressure cooker antigen retrieval and retrieval using streamer, water-bath, autoclave and combined microwave and trypsin digestion antigen retrieval. The use of conventional heating at 100^0c achieves results similar to those obtained by microwave. Distilled water could be used as the antigen retrieval solution with slight less effect[(103)].

Shi et al in 1991 introduced the use of microwave in the technique of antigen retrieval, but the use of heavy metals posed significant difficulties and problems. Later in 1992 Gerdes performed antigen retrieval with non toxic citrate buffer. Cottoretti in 1993 argued that microwave antigen retrieval is an alternative to proteolytic enzymes. Various problems including uneven heating and hot spot production are the important problems with the use of microwave[(103)].

In 1994, Norton suggested the use of pressuer cooker method for antigen retrieval. It is considered as one of the suitable method as it produces even heating and has no disadvantages which includes hot spot and cold spot production which often occur with the use of microwave. Steamer antigen retrieval is less efficient when compared to microwave and pressure cooker. Kawai in 1994 showed that water bath set at 90^0C is adequate for antigen retrieval. However, quality of antigen retrieval improves with increase in the temperature to 95-98^0C.

Evers and Nylings[(103)] in his study found that antigen retrieval depends on both p_H and temperature. They concluded that it is not important what kind of solution is used as long as the p_H is appropriate. Chemical composition and the molarity of the antigen retrieval solution may act as a cofactor for effectiveness of antigen retrieval and hence immunohistochemistry.

Non specific background staining

Non specific staining in immunohistochemistry is attributed to non specific antibody binding and action of endogenous enzymes. Non specific staining is more common with usage of polyclonal antibody because of multiple unwanted

antibodies. Blocking of endogenous enzymes activity is important. The degree of susceptibility of an enzyme to denaturation and inactivation varies from tissues to tissues. Any residual activity of endogenous enzymes must be abolished. Endogenous peroxidase activity is seen in neoplastic cells, erythrocytes, neutrophils, eosinophils, and hepatocytes. Usage of various substances for blocking endogenous peroxidase is suggested.

Some investigators thought that use of methanol-hydrogen peroxide may cause denaturation of antigen. Strans[104] advocated the use of phenylhydrazine by combination of phenyl hydrazine, nascent hydrogen peroxide and sodium azide. Mixture of hydrogen peroxide and sodium azide was found to be very effective[105]. More recently cyclopropane hydrate was shown to inhibit endogenous activity without adverse effect[105]. Endogenous alkaline phosphatase activity is usually blocked by addition of Levamisole to the substrate. Levamisole selectively inhibits certain types of alkaline phosphatase but not intestinal and placental. 20% glacial acetic acid is a better alternative to levamisole.

IMMUNOHISTOCHEMISTRY AND NHL

The use of antibody panels in immunohistochemistry is well accepted. Single antibody directed against the malignant cells will not serve for the diagnosis and is insufficient. We need to use panel of markers to differentiate one form of Non-Hodgkin lymphoma from the others [106,107]. Much of the early work in diagnostic hematopathology required frozen section or acetone fixed frozen section as available

antibodies failed to recognize the antigens fixed by cross linking fixatives. Partial enzyme digestion gives good result[108].

Understanding of the development, maturation and migration of lymphocytes provides rationale for immunophenotyping because stages in lymphocyte maturation are defined in part by the macromolecules they produce. Malignant cells expresses antigen that resembles the antigens of lymphocytes in different developmental stages. Lymphomas are thought of as being composed of cells arrested in development of certain stages[109]. Hence the panel of markers directed against the malignant cells will be of more help.

Immunohistochemical markers are selected based upon the morphology. Morphological assessment takes into account the architectural alteration in B-cell zone and T-cell zone. The markers include leucocyte common antigen, B-cell markers such as CD20 and CD79a, T-cell markers like CD3 and CD5 and other markers including CD23, bcl-2, CD10, cyclinD1, CD15, CD30, ALK-1 and CD138. Currently there are more than 50 antibodies which are used in the diagnosis of Non-Hodgkin lymphoma. Explosion of antigens and reagents to detect them needs judicious use of selected panel of markers. It should always be noted that no antigens is totally specific and therefore needs careful selection of panel of markers to arrive at proper diagnosis.

LYMPHOMA DIAGNOSIS

The diagnosis of lymphoma is based on the World health organisation classification [110](2008). Further classification is based on the stage of maturation and cell of origin. Other features which help in the diagnosis are anatomic architectural alteration in the lymphoid compartment, determination of cell size, nuclear character, and character of nuclei.

B-cell markers

CD20 is the most common antibody used as the pan-B cell marker and is expressed by naive B cell until the final stages of maturation just prior to plasmacytic differentiation. Chronic lymphocytic lymphoma/small lymphocytic lymphoma rarely shows weak positivity[111] to CD20. In cases of prior rituximab therapy, CD79a should be added. Early stage including naive cellsand other B-cell expresses Pax-5 but not the plasma cells[112]. Few cases of precursor T-cell acute lymphoblastic lymphoma expresses CD79a and rare cases of AML and merkel cell carcinoma shows positive expression of Pax-5.

When B-cell follicles are not well made under histopathology the immunohistochemical markers like CD21, CD23 and CD35 may be helpful in highlighting follicular dendritic cells. Staining the sections for kappa and lambda light chains may be useful in detecting the abnormal clonal population, especially plasma cells. Immunohistochemistry although less sensitive in the detection of immunoglobulin expression, can be more valuable when used along with histopathology. Plasma cells and immunoblast shows strong expression of immunoglobulins which can be detected

by paraffin immunoperoxidase method. Calorimetric insitu hybridisation is an alternate and most sensitive method used in the detection of immunoglobulin.

T-cell markers

CD3 is the most commonly used pan-T cell marker used to detect the antigenic expression over the malignant and normal T-cell at the second stage of thymic differentiation and beyond. Sometimes there may be loss of pan-T cell marker over the malignant lymphoid cells as in case of anaplastic large cell lymphoma[(113)]. T cells are divided into CD4 and CD8 subsets. Antibodies directed against these antigens are used as basic panels to detect abnormal distribution of lymphoid cells. CD5 is another pan-Tcell marker which is lineage non-specific and is not expressed on Natural killer cells. CD5 is present over small subset of B-cells especially among the cases of chronic lymphocyte is lymphoma, mantle cell lymphoma.

Atypical cells and Lymphoma

Histopathological examination of some lymphomas may show many large atypical lymphoid cells admixed with other similar looking lymphoma cells. When there is presence of large atypical cells, the possibilities of carcinomas, Hodgkin lymphoma and anaplastic large cell lymphoma should be ruled out. This is done by using a panel of markers including CD45, CD15 CD30 and HMB45. The usage of antibody directed against ALK-1 may prove more valuable.

Other antibodies

Several other markers have found application in the diagnosis of Non-Hodgkin lymphoma. These include CD10, Bcl-6, cyclin D1, Bcl-2, CD56 and CD57. CD10 and Bcl-6 are the markers that show positive expression in normal and neoplastic follicular centres. CD10 also show positive expression on granulocytes, T-cells in angioimmunoblastic lymphadenopathy, precursor B-cells and T-cells, epithelial cell neoplasms like renal cell carcinomas, hepatocellular carcinomas and endometrial stromal cells. Bcl-6 is expressed in follicular centres and few number of T-cells. The presence of CD10 positive lymphocytes along with Bcl-6 positive cells outside the follicles strongly suggests neoplastic proliferation. But rare cases of follicular lymphoma which shows negative expression of CD10 have been recorded.

Bcl-2 is an antiapoptotic molecule which is normally expressed over pre-B cells, and resting B-cells of normal mantle zone and in rare cases with proliferating B-cells. Most of the neoplasms composed of small lymphoid cells usually show positive expression of Bcl-2[(114)] and expression of Bcl-2 is downregulated in normal follicles. B-cells in reactive follicles show negative expression of Bcl-2 and there is strong expression of Bcl-2 in lymphoid cells of mantle zone and cells of primary follicle[(115)]. The interpretation of Bcl-2 should be carried out in conjunction with CD3 staining for T-cells as T-cells normally show positive expression of Bcl-2. Most of the cases of marginal zone lymphoma shows Bcl-2 expression but reactive hyperplastic marginal zones of spleen and lymph nodes of abdomen also shows Bcl-2 positivity. CyclinD1 is another immunohistochemical marker which has gained its importance in the diagnosis of Mantle cell lymphoma. Cyclin D1 is normally expressed

in endothelium, nucleus of histiocytes, and fibroblast. It is usually expressed in the nucleus of lymphoid cells of mantle cell lymphoma. Cytoplasmic expression is considered non diagnostic. Although nuclear expression of CyclinD1 is almost diagnostic of mantle cell lymphoma many cases of CyclinD1 negative mantle cell lymphomas are on the records and even CyclinD1expression has been recorded in many cases of small lymphocytic lymphoma. Immunohistochemical markers like CD56 and CD57 play important role in the diagnosis of natural killer cell proliferation and neoplasms arising from natural killer cells[(115)].

PANEL OF MARKERS FOR NON HODGKIN LYMPHOMA

Lynette K Tumwine et al [(116)], studied the expression of B cell antigens in 119 Non Hodgkin lymphoma patients using a panel of CD3,5,10,20,23,30,38,79a,138, bcl-2, MUM1/IRF4, cyclinD1 and Ki-67/Mib1 antibodies. They found 100% positivity for CD20 in DLBCL and Burkitt lymphoma patients. Among DLBCL patients, 10.5% positivity was seen for bcl6, 22.2% positivity for MUM1/IRF4 and 66.7% for CD138. According to I.Satish Rao[(117)] distinction of monomorphic small B cell lymphomas are done by basic panel of markers such as CD5, 23, 10, cyclin D1 and bcl2, intermediate cells with diffuse pattern by markers such as CD20,10, Tdt, CD99 and Mib1 and large cells with diffuse pattern by CD20, CD79a, CD138 and EBER. Nirmala Ajit Jambhekar et al [(118)] suggests a panel of markers for Non Hodgkin lymphoma diagnosis which includes CD20 which is a B-cell marker showing membrane staining, CD3 is a T-cell marker, CD21/CD35 stains follicular dendritic cells and hence are useful to demonstrate germinal centers within lymphoid the proliferation; CD5 which detects B-cell CLL/SLL and mantle cell lymphomas. CD23 shows positivity for B-CLL/SLL,

whereas it is negative in mantle cell lymphoma. Mantle cell lymphoma shows immunoreactivity to Cyclin D1. Alk-1 is specific for anaplastic large cell lymphomas (ALCL) which also shows CD30 positivity and focal EMA positivity. Bcl-2 is useful in distinguishing reactive follicles (negative) from follicles of follicular NHL (positive) whereas CD10 is positive in Burkitts lymphoma.

Kwakiw and Wilson M [(119)] in their study found out that among all the cases of DLBCL, 65% to 85% are of B cell type and 15%-35% are T cell type. T cell rich variant of DLBCL may show membranous staining of pan T cell marker (CD3). Hans et al [(120)] in their study on 152 cases of DLBCL demonstrated the expression of CD10 in 28% of cases, bcl-6 in 56%, MUM1 in 47%, bcl-2 in 50%, cyclin D2 in 13% and FOXP1 in 61% of cases. Ting Li et al [(121)] studied immunohistochemical features in 63 cases which resulted expression of CD10 in 30% of cases, bcl6 in 35% and MUM1 in 51% of cases.

Strauchen JA and Mandeli JP [(122)] in their study of 345 cases of B cell NHL found Ig expression in 59% cases of large cell lymphoma and 100% of small cell lymphoma. Picker LJ et al [(123)] documented that immunoblastic variant especially those with plasmablastic differentiation showed cytoplasmic Ig expression. Piris m et al [(124)] through their study suggested that most of the anaplastic variant showed positive membrane staining for CD30. Fang JM et al [(125)] found that 20-30% of DLBCL showed CD10 positivity.

Follicular lymphoma cells show positive membrane staining for CD 19, 20, 22 & 79a with typical surface monoclonal Ig expression. Jaffe ES et al [(125)] through their study found out that follicular lymphoma cells do not express CD3, 5 and 23. Both

follicular and inter follicular zones contain κ or λ clonally restricted B cell. Lai R et al [126] in their study found that malignant cells were negative for CD43. Gaulard P et al [127] demonstrated overexpression of nuclear positivity for bcl-2 in germinal centre among the cases of low grade follicular lymphoma.

Robert R. Lorsbach et al [128] through their study on 19 paediatric follicular lymphoma patients showed CD20 and bcl6 positivity in all cases and CD10 positivity in 80% of cases. Andrew J Davies et al [129] observed through their study that among the follicular lymphoma patients assessed for transformation to DLBCL, 89% showed germinal centre phenotype with $CD10^{+}$, $bcl6^{+}$, and $MUM1^{-}$. Follicular lymphoma in addition to mimicking the growth pattern of reactive follicles, contain non neoplastic T cells and follicular dendritic reticulum cells. According to Said JW et al [130] follicular dendritic reticulum cells in follicular lymphoma and reactive follicular hyperplasia express CD21 and CD35 whereas follicular dendritic reticulum cells in follicular lymphoma but not in reactive follicular hyperplasia, lack the expression of fascin.

In mantle cell lymphoma, the neoplastic cells show membranous positive expression of CD19, 20, 22, 79a and CD5. Vasef MA [131] and Cheuk W [132] through their study showed that almost all the cases of mantle cell lymphoma showed strong nuclear expression for cyclin D1. In case of CLL/SLL, the cells are B cells with positivity for surface Ig light chain, IgM, IgD, pan B cell antigens, CD5 and 23 antigens.

According to Williamze R et al [133] the cells of mycosis fungoides and Sezary syndrome have a mature T cell phenotype and are of T helper cell lineage. These cells

express pan T cell antigens CD2,3,5 and CD45 RO. Picker LJ et al [134] in their study showed that PTCL cells express CD2,3,CD5 and 7,43 and CD45RO but are negative for B cell antigens. Through their study Rudiger et al [135] showed that AITL express antigens like CD10, bcl6, CXCL13, SAP, programmed death-1 and activation induced cytidine deaminase. In cases of anaplastic large cell lymphoma, ALK^+ anaplastic large cell lymphoma by definition shows ALK positivity and CD30 expression in a distinctive pattern along with expression of T cell antigens.

OTHER MODALITIES OF DIAGNOSIS OF LYMPHOMA

Ultrastructural examination is not routinely done in the identification of lymph node lesions, but it is inexpensive to fix the sample in glutaraldehyde which can be used later if needed. Electron microscopy is useful in rare disorder which include storage disorder like Gaucher's disease,lymphoblastic lymphoma, mycosis fungoides and amyloidosis. Immune electron microscopy can be useful to study the cell surface markers and immunoglobulins after applying peroxidase labelled antibodies. Ultrastructure is more useful in identifying metastatic deposits in lymph nodes especially in the cases of malignant melanomas demonstrating melanosomes[136]. Ewing's sarcoma with abundant glycogen[137] and small cell carcinomas with neurosecretory granules[138].

Chronic lymphocytic lymphomas are charectrized by increased synthesis of immunoglobulin light chain when compared to heavy chain.ultrastuctural examination in such cases reveals Ig light chain in both perinuclear space and rough endoplasmicreticulum whereas heavy chains are limited rough to rough endoplasmic reticulum

Lmphoblastic lymphoma under electron microscope show round cells with a high nuclear to cytoplasmic ratio with convoluted nucleas showing deep indentation and fine chromatin with peripheral nucleolus[139].The examination of angioimmunoblastic lymphoma shows nuclear and indentation,spekeled hetrochromatin and prominent nucleolus[140].

The diagnosis of lymphoma as proposed by WHO,now primarily depends on imunophenotyping.Imunophenotyping is usually performed by using IHC and flow cytometry .Flow cytometry has many advantages over IHC and is usually performed in higher centers.It is a qualitative analysis of antigen expression which determines the density of more than one antigenic expression simultaneously by using many fluorochromes per test tube.Hence,it can be easily standardised and also has a rapid turnover time.The main disadvantage of the flow cytometry is it can be performed only in the fresh living tissues .

Molecular diagnosis is considered as gold standard [141,142]in assessing monoclonality and chromosomal translocations.Unlike immunohistochemistry which detect proteins,molecular diagnosis detects DNA of genes encoding immunoglobulins and Tcell receptor.Various techniques used in the routine practice are conventional cytogenetics,southernblot technique,polymerase chain reaction and flourecence insitu hybridisation .Polymerase chain reaction is versatile technique used in the analysis of clonality,chromosomal translocation,genetic mutation,infectious agents and minimal residual disease.The main disadvantage is the occurance of the false positivity because of its high sensitivity.

Chromosomal translocation plays an important role in the occurance of many lymphomas and hence identification of such translocation proves the diagnosis.This is carried out often using conventional cytogenesis or the flourececnce insitu hybridisation technique. This can identify wide range of translocationswhich includes myc transloction in Burkitt's lymphoma[143], t(11;14) involving cyclinD1 in mantle cell lymphoma[144] ,t(14;18)involving Bcl2 gene in follicular lymphoma,Bcl2 in case of diffuse large B-cell lymphoma and subset of follicular lymphoma[145],ALK translocation as in case of anaplastic large cell lymphoma,t(1;14) (11;18)(14;18) in MALT lymphomas and t(11;14)(4;14) as in cace of myeloma.

CONDITIONS MIMICKING LYMPHOMA:

Reactive lymphoid hyperplastia is defined as begging reversible process charecterised by proliferation of reactive lymphoid cells in various pattern secondary to antigen stimulation. Clinically, this condition is manifested as enlarged lumph nodes in the multiple sites includes cervical nodes which are often affected in infectious mononucleosis posterior cervical group as in the case of toxoplasmosis, axillary node in cat-scratch disease, parotid, submaxillary and epitrochellar group as in the case of HIV infection. when there is involvement of supracavicular lymphnodes careful evaluation of the patient including age,sex,family history,symptoms and signs are performed. This is because these nodes are associated with either primary or metastatic malignancy in 25% of patients younger than 40 years and 90% of patients older than 40 years.[146]

Reactive lymphoid hyperplasia can be caused by various bacterial and viral infections,exposure to chemical substances and environmental pollution,drugs and numerous allergens.Among the drugs phenytoin,penicillins,gold and quinidine plays a

major role[147].Reactive lymphoid hyperplasia,occur most commonly in children and younger age group.Hyperplastic nodes are less common among the elderly because of the decreased humoral immune response[148] and hence the proliferation of germinal centre is less apparent than in young patients[149].

Most of the hyperplastic nodes shows non specific pattern.But charecteristic morphological pattern can be seen in the reactive follicular hyperplasia secondary to activated humoral immune response,diffuse paracortical hyperplasia as in the case of viral lymphadenitis and sinus hystiocytosis.Histopathology of reactive follicular hyperplasia is charectrised by numerous reactive secondary follicles of varying size and shape distributed in the cortex and medullary region,with well demarcated mantle cell zone.The germinal centre comprises mixture of small and large lymphoid cells.

The presence of numerous follicles throughout the cortical and medularry zones makes morphological distinction of exaggerated follicular hyperplasia from follicular lymphoma.However according to Nathwani[150],numerous back to back follicles with even distribution of the cortex and medulla with scant interfollicular area in addition with cytological feautures helps in the distinction. But in more difficult cases,immunohistochemistry may prove useful.

Diffuse paracortical hyperplasia is charecterised by the expansion of paracortical area due to increase in number of reactive T –cells containing mixture of small cells and activated large cells,the immunoblast.immunoblasts are larger cells with scant cytoplasm,large nucleus and prominent nucleoli resembling Hodgkin and RS cell [151] and rarely metastatic deposit from amelanotic melanoma. In difficult cases like this,immunohistochemistry solves the problem.

A typical lymphoid hyperplasia is a group of disorder that is neither benign nor malignant which occurs in cases of primary immunodeficiency. This disorder occupies an intermediate position between reactive lymphoid hyperplasia and malignant lymphoma. The morphology is characterised by nodular or diffuse proliferation of lymphoid follicles admixed with many atypical large cells with nucleus and prominent nucleoli. These atypical cells can be either singly scattered or in sheets,and when so can cause difficulty in differentiating it from malignant lymphomas and metastatic deposits. In such case immunostaining to demonstrate monoclonal light chain (either kappa or lambda) is necessary.

Some of the drugs are used for therapy either in shorter term or after a long period of time can produce generalised lymph node enlargement mimicking malignant lymphoma. But these can be differentiated from true cases of malignant lymphoma by the regression of lymphnodes after discontinuation of the drugs. This is called as anticonvulsant hypersensitivity syndrome as such cases can occur after the use of phenytoin and carbamazepine. This syndrome is found to have genetic predisposition which can be attributed to inherited defects in the epoxide dehydrolase[(152)] which detoxifies epoxide hydrolase formed as a result of metabolism of phenytoin and carbamazepine by cytochromeP450 .

DRUGS CAUSING LYMPHADENOPATHY

PHENYTOIN	PRIMIDONE
CARBAMAZEPINE	PHENOBARBITOL
QUINIDINE	GABAPENTIN
TETRACYCLINE	LAMOTRIGINE
PENICILLIN	HALOTHANE
ABACAVIR	IRON DEXTRAN
ALLOPURINOL	GENTAMYCIN
PHENYLBUTAZONE	IVERMECTIN

LYMPHNODE AND NECROSIS

Necrosis in the lymphnode can be focal or diffuse replacing the nodal architecture. It can be of any type which includes coagulative,caseous and fibrinoid type. Caseous necrosis most commonly occurs in the infections like tuberculosis in which the necrotic area appear granular and eosinophilic surrounded by epitheloid cell granulomas,lymphocytes and fibroblasts. In addition, the disease causing organisms can be demonstrated in such kind of lesions.

Fibrinoid necrosis can occur in the cases of vasculitis involving the lymph node. Apart from this,histiocytic necrotising lymphadenitis also called Kikuchi-fujimotos lymphadenopathy is characterised by patchy or confluent areas of fibrinoid material with necrotis debris surrounded by histocytic proliferation. Some cases rarely show scattered cells with karyorrehexis and pyknosis[(155)]. The periphery of the necrosis

is characterised by thrombosed vessels, and nests of plasma cells,monocytes and immunoblast. Some of these cells are atypical can be mistaken for lymphoma. The histiocytes which phagocytosed under debris have peripherally placed crescentic nucleus and hence can be mistaken for signet ring cell carcinoma deposit. But importantly,absence of neutrophils and eoisonophils are the distinct morphological feature of Kikuchi-fujimoto disease.

Lymph nodes are organs with rich vascularity and hence rarely infracted. However, cases of coagulative necrosis involving most of the nodal parenchyma, sparing only a narrow subcapsular rim have been recorded[156,157].

The causes include occlusive vascular thrombosis,thrombophelibitis,localized trauma secondary to fine needle aspiration and as a secondary complication to surgical procedure. Coagulative necrosis can occur secondary to malignant lymphomas. This may be due expansion of lymphomas causing pressure effects over the vessels or due to direct invasion of the hilar vessels. Hence massive infarction should always be viewed with a suspicion of underlying malignancy. The demonstration of preserved antigen in the necrotic area through immune histochemistry is useful in such situations.

Castleman's disease is defined as the large benign asymptomatc lymphadenopathy usually involving mediastinal groups. The cause for Castleman lymohadenopathy is not known, however HHV-8 is considered to be a known etilogical agent . primarily two variance has been described namely hyaline vascular and plasma cell variant which can be unicentric or multicentric. The hyaline vascular variant is by for the most common variant[158,159]. Histologically it is characterised by changes in the follicular and inter follicular area. With in the follicles there may be twinning of

germinal centers with reduced number of small lymphoid cells. The blood vessels are arranged radially and there may be deposition of hyaline material in the center of the vessels. The expansion of the mantle zone is observed with small lymphoid cells arranged concentrically in an onion-skin fashion. The interfollicular area shows mixed population of cells.

The plasma cell variant is characterised by sheets of plasmablasts and mature plasma cells expanding the interfollicullar area. Immunohistochemical evaluation is needed when there is florid proliferation of thesecells leading to confusion with lymphocytic lymphoma and plasma cell neoplasm.

PROGNOSTIC FACTORS

In non Hodgkin lymphoma there is no such classification in which how a specific subtype of tumour behave, so a role of prognostic factors like proliferative and apoptotic indices are considered into account. Cell growth =cell proliferation minus cell death.in this study we calculated cell death by calculating apoptotic bodies in haematoxylin and eosin stain. Similarly cell proliferation is calculated by immunostaining with ki67 and measuring the positive nuclear staining .

APOPTOTIC INDEX

Cell doubling time is slowed by many factors, among which the most important are the death of some tumor cells by apoptosis (programmed cell death), ischemic, or hemorrhagic events, and the presence of a pool of quiescent cells that do not enter the mitotic cycle.[160]

Apoptosis is a pathway of cell death that is induced by a tightly regulated suicidal program in which the cells destined to die activate intrinsic enzymes that degrade cell's own DNA,nuclear and cytoplasmic proteins. Apoptosis is a physiologic form of cell death that serves as one aspect of tissue growth and size regulation [161] .This is an active process requiring protein synthesis and specific endonucleolytic digestion of cellular DNA.[162].These apoptotic cells breakup into fragments called apoptotic bodies which contain portions of cytoplasm and nucleus.the plasma membrane of apoptotic cell and apoptotic bodies remains intact but its structure altered in such a way that it becomes a tasty target for phagocytes.

TheDNA fragmentation that ensues is one hallmark of apoptotic cell death. The inhibition of apoptosis is believed to play a role in carcinogenesis in one of two ways:[163] it may allow for the unchecked accumulation of genetic alterations [164, 165] and it may lead to unbalanced proliferation of tumor[166]. The morphologic criteria for identifying cells undergoing apoptosis are well established and include cytoplasmic condensation, loss of cell– cell contact,and cell shrinkage. This is separate and distinct from necrosis, an alternate form of cell death that involves cell swelling and rupture with associated surrounding tissue damage [167,168,169]. Cells undergoing apoptosis can be identified using these morphologic criteria on a standard hematoxylin and eosin (H&E) slide.

PROCESSING OF TISSUES FOR THIN SECTIONS

In as much as apoptotic bodies are often difficult to identify in conventional paraffin sections but can be recognized with great confidence in thin sections.[170] Sections are 3 microns thick, stained with haematoxylin and eosin.

Apoptotic bodies are counted

COUNTING PROCEDURE FOR ASSESSMENT OF APOPTOTIC INDICES

The apoptotic index was determined by counting a total of at least 1000 neoplastic nuclei subdivided in 10 fields chosen randomly at 100x magnification. Apoptotic cells were identified by morphologicalchanges, such as cell shrinkage, membrane blebbing, and chromatin condensation, to distinguish apoptotic cells and apoptotic bodies from necrotic cells,which were not considered as apoptotic cells.

Based on the AI, cells were divided into four grades [171]

GRADE	APOPTOTIC INDICES
grade I (-)	<0.3%
grade II (+)	0.4%-2.4%%
grade III (++)	2.5%~5.4%
grade IV(+++)	5.5%-8.8%

PROLIFERATIVE INDICES:

Proliferative indices (Pls) of malignant non-Hodgkin's lymphomas (NHLs) are useful prognostic indicators and provide information independent of other histological and clinicalvariables[172]. Even more attention is now being given to monoclonal antibodies (MAbs) that recognize epitopes of antigens expressed by proliferatingcells,[173] in particular the MAbs Ki-67 and PC [174,175,176] Assessment of the growth fraction can be accomplished by determining the percentage of cells expressing the Ki-67 antigen, a nuclear protein of unknown function expressed only in cycling cells [177] Recent data suggest, eg, that a high ki67 index, which can be assessed on formaldehyde fixed paraffin sections, may be an adverse prognostic factor in primary gastrointestinal lymphomas [178] Ki-67 is a molecule that can be easily detected in growing cells in order to gain an understanding of the rate at which the cells within a tumor are growing. The **Ki-67** protein (also known as **MKI67**) is a cellular marker for proliferation[179]It is strictly associated with cell proliferation.It was first identified after immunization with hodgkin's disease

During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent from resting cells (G_0) [180] The Ki-67 labeling index (LI), based on the proportion of tumor-positive cells, has usually been used as an indication for evaluation,and many reports have shown its clinical significance in a variety of cancers regardless of whether the tumor origin is epithelial or nonepithelial . Increase in antigenic expression during cell cycle in both benign and malignant cell lines assessing their proliferative status has also been shown [181] . Ki-67 score is now used to predict the prognosis**,** survival, and even the recurrences. A high level of Ki-67 proliferative index (PI) is associated with aggressive tumoral behavior and metastasis[182,183,184]

COUNTING :

To count the number of Ki-67-positive cells, 10 representative areas were chosen. A representative area was defined not to contain residual germinal centers, hot spots of proliferation or proliferating Tcells. Hot spots of proliferation are areas of tumor cells (not germinal center residues) of less than two high-power fields in size (HPF, field of vision at ×40 magnification), which proliferate higher than the rest of the tumor. In each area, the positive cells were counted using an in a 40 Xmagnification. The Ki-67 index was calculated as the percentage of positive cells by averaging the values obtained from 10 high power fields

GRADING :

Based on the Ki67 positivity ,proliferative indices are scored as follows [185],

SCORE	Ki67(nuclear staining)
Negative	0-5% cells
Score 1	6-40%
Score 2	41-80% cells
Score 3	81-100%

MATERIALS AND METHODS

Study material includes cases of Non Hodgkin lymphoma diagnosed in the Department of Pathology of Tirunelveli Medical College from 2010 to 2016

INCLUSION CRITERIA

(1) All cases that were diagnosed as non Hodgkin lymphoma using haematoxylin and eosin are included

(2) Both nodal and extranodal non Hodgkin lymphoma cases are included

(3) The blocks and slides of the respective cases are collected

EXCLUSION CRITERIA

(1) Inadequate lymph node sample

(2) Poorly processed material

(3) All cases of Hodgkin lymphoma

(4) Autolysed specimen

(5) Cases with dense tissue necrosis

MATERIALS REQUIRED

(1) Donor blocks which contains formalin fixed paraffin embedded tissue obtained from all the cases of Non-Hodgkin lymphoma.

(2) Hematoxylin and eosin stained tissue sections made from the donor blocks.

(3) Postively charged slides for holding tissue sections for IHC

(4) Chemicals for preparing antigen retrieval solutions and for wash buffers

(5) Pressure cooker for antigen retrieval.

(6) Kit for performing immunohistochemistry which includes primary antibody (Ki67) and universal kit.

(7) Microscope used for grading of IHC slides and for counting apoptotic indices

METHODOLOGY

COLLECTION OF DONOR BLOCKS AND SLIDES

The haematoxylin and eosin stained sections which were prepared from formalin fixed paraffin embedded blocks of all cases of non Hodgkin lymphoma were collected. The following cases were selected.

- Slides which contain full sections and complete lymphnode with capsule
- Cases with no tissue necrosis

The corresponding formalin fixed paraffin embedded tissues were also obtained which constituted the donor blocks.

PREPARATION OF HAEMATOXYLIN AND EOSIN SLIDES

Apoptotic bodies were better appreciated in thin sectioned slides.So a section of 3-4 microns thickness were made. Slides were stained with routine H&E stains. Apoptotic bodies were counted and graded.

All cases were classified by **working formulations** into

- HIGH GRADE OF LYMPHOMA
- INTERMEDIATE GRADE OF LYMPHOMA
- LOW GRADE OF LYMPHOMA

IMMUNOHISTOCHEMISTRY

Section cutting

Sections were taken at 5microns thickness on the surface of the APES (3-aminopropyltriethoxysilane) coated slides. This was followed by incubation of slides at 58-60^{0}c for one hour.

Antigen retrieval solution

We used antigen retrieval solution and a wash buffer as prescribed by the manufacturer (PATH INSITU).

1. Tris EDTA at a P_H of 9 for Ki67.

2. Tris wash buffer at P_H of 7.6.

Antigen retrieval

Many methods have been used for antigen retrieval which includes Microwave method, and water bath, autoclave, proteolytic enzyme and pressure cooker method. In our institution we followed antigen retrieval by using pressure cooker as it produces even heating with lesser disadvantages as compared to other methods.

Procedure for immunohistochemistry as given by manufacturer

1. Section cutting and incubation is followed by Xylene wash (3 changes) for 10minutes each.

2. Rehydrated in graded alchohol containing 100%, 80%, 70% for five minutes each.

3. Rinsed in distilled water for 2minutes.

4. Antigen retrieval for 15-20 minutes in Tris-EDTA buffer.

5. Cooling for 15minutes.

6. Washed in TBS wash buffer- 3changes 5minutes each.

7. Treated with endogeneous peroxide block for 7-10minutes.

8. Washed in TBS wash buffer- 3changes 10minutes each.

9. Application of primary antibody (Ki67) – 30 mins.

10. Washed in TBS wash buffer- 3changes 10minutes each.

11. Add Target binder for 15 mins

12. Washed in TBS wash buffer- 3changes 10minutes each

13. Application of HRP POLYMERASE for 15 mins.

14. Washed in TBS wash buffer- 3changes 10minutes each.

15. Application of Diamino-benzidine tetrachloride(DAB) chromogen (1 drop)and DAB buffer (1ml) for 5 mins.

16. Washed in distilled water – 2 changes.

17. Counterstaining with Harris Hematoxylin – 1dip/30seconds to impart background staining.

18. Wash in running tap water.

19. Place in xylene – 2 changes 5 minutes each.

20. Dehydrate in 100% alcohol – 5minutes.

21. Mount the section with Dextrene phthalate xylene

22. Observation and grading under light microscope.

GRADING OF IHC STAINED SECTIONS:

After immunohistochemistry was done, the slides were examined under all the magnification with the help of light microscopy and grading was done. This is done by following the method as opted by Adi Broyde, Olga Boycov [185] in his study on "ROLE OF PROGNOSTIC SIGNIFICANCE OF THE Ki67 IN NON HODGKIN LYMPHOMA". First the tumour cells were observed for positive nuclear staining pattern after which it is scored based upon the percentage of tumour cells which shows positive expression of antibody .

After scoring of Ki67 apoptotic indices were calculated by examining H&E slides for apoptotic bodies – cells with intensely eosinophilic cytoplasm with fragments of dense nuclear chromatin and are then graded. This was done by the method opted by " Y. Soini, P. Pa¨a¨kko¨ , and V-P. Lehto" in their study of Histopathological evaluvation of apoptosis in cancer.[171]

OBSERVATION AND RESULTS

TABLE 1:

Sl.no	Block number	Age	Sex	Site	classification by working formulation	Proliferative indices	Apoptotic indices	Observation using correlation of these indices
1	491/13	31YRS	MALE	Cervical node	low grade -small lymphocytic	60%	1.60%	Intermediate
2	441A2/15	5YRS	MALE	Cervical node	low grade -small lymphocytic	10%	2.40%	Low
3	797A1/13	11YRS	MALE	Axillary node	low grade -small lymphocytic	20%	1.30%	Low
4	58 A2/13	50YRS	MALE	Cervical node	low grade -small lymphocytic	10%	1.10%	Low
5	1726R2/16	80YRS	FEMALE	Cervical node	low grade-small lymphocytic	10%	0.70%	Low
6	1567A11	64YRS	FEMALE	Inguinal node	low grade-small lymphocytic	20%	1.50%	Low
7	491/13	31YRS	MALE	Cervical node	low grade-small lymphocytic	60%	1.60%	Intermediate
8	2316R4/13	57YRS	FEMALE	Inguinal node	low grade-small lymphocytic	25%	1.40%	Low
9	1427/10	40YRS	MALE	Cervical node	low grade-small lymphocytic	25%	2.40%	Low
10	3790B1/15	60YRS	FEMALE	Cervical node	low grade-small lymphocytic	10%	0.70%	Low
11	1813/11	65YRS	FEMALE	Cervical node	low grade-small lymphocytic	45%	0.90%	Intermediate
12	2426/11	78YRS	MALE	Inguinal node	low grade-small lymphocytic	8%	2.30%	Low
13	1593N3/11	62YRS	FEMALE	Axillary node	low grade-small lymphocytic	40%	2.70%	Low
14	394/12	65YRS	MALE	Inguinal node	low grade-small lymphocytic	10%	0.20%	Low
15	2444A/13	62YRS	MALE	Inguinal node	low grade-small lymphocytic	10%	0.30%	Low
16	2857R3/11	15YRS	MALE	submandibular node	low grade-follicular predominant small cleaved	10%	1.40%	Low
17	3130B/15	57YRS	MALE	Cervical node	low grade-follicular predominant small cleaved	15%	1.50%	Low
18	2607/13	45YRS	FEMALE	Cervical node	low grade-follicular predominant small cleaved	15%	1.60%	Low

19	1217/13	60YRS	MALE	tonsillar growth	low grade-follicular predominant small cleaved	10%	1.80%	Low
20	1304A2/12	39YRS	MALE	Inguinal node	low grade-follicular diffuse areas	7%	2.50%	Low
21	2072B/15	48YRS	MALE	Cervical node	low grade-follicular diffuse areas	72%	2.90%	Intermediate
22	189A3/15	71YRS	MALE	Cervical node	intermediate grade-diffuse small cleaved	50%	2%	Intermediate
23	190/13	4YRS	MALE	Cervical node	intermediate grade-diffuse small cleaved	60%	2.50%	Intermediate
24	1117A3/12	9YRS	FEMALE	Inguinal node	intermediate grade -diffuse small cleaved	60%	3.60%	Intermediate
25	273A2/14	7YRS	MALE	Inguinal node	intermediate grade-diffuse small cleaved	30%	3.10%	Intermediate
26	1146A1/14	5YRS	MALE	Axillary node	intermediate grade-diffuse small cleaved	70%	2.90%	Intermediate
27	614A2/15	8YRS	MALE	Cervical node	intermediate grade-diffuse small cleaved	50%	1.90%	Intermediate
28	5938/15	35YRS	MALE	Cervical node	intermediate grade -diffuse small cleaved	62%	1.80%	Intermediate
29	607F/15	45YRS	FEMALE	Cervical node	intermediate grade-diffuse small cleaved	50%	4.60%	Intermediate
30	2218/10	47YRS	MALE	Cervical node	intermediate grade-diffuse small cleaved	50%	1.70%	Intermediate
31	1158/10	70YRS	MALE	Cervical node	intermediate grade-diffuse small cleaved	45%	1.20%	Intermediate
32	211/11	8YRS	MALE	Axillary node	intermediate grade-diffuse small cleaved	70%	4.10%	Intermediate

33	2551B/12	60YRS	FEMALE	Inguinal node	intermediate grade-diffuse large cleaved	84%	2.50%	High
34	768A/14	60YRS	MALE	Cervical node	intermediate grade-diffuse large cleaved	70%	2.50%	Intermediate
35	539/14	5YRS	MALE	Cervical node	intermediate grade-diffuse large cleaved	15%	2.70%	Low
36	615A2/14	5YRS	FEMALE	Inguinal node	intermediate grade-diffuse large cleaved	95%	1.70%	High
37	59r10/15	5YRS	MALE	Mesentric node	intermediate grade-large non cleaved	10%	1.20%	Low
38	680A2/15	10 YRS	FEMALE	Mesentric node	intermediate grade-large non cleaved	90%	2.10%	High
39	3413/15	50YRS	FEMALE	submandibular node	intermediate grade-diffuse mixed small and large cell	30%	4.50%	Low
40	3550A/15	11YRS	MALE	submandibular node	intermediate grade-diffuse mixed small and large cell	85%	3.80%	High
41	1341/10	57YRS	MALE	Cervical node	intermediate grade-diffuse mixed small and large cell	35%	1.20%	Low
42	681A2/14	10YRS	FEMALE	Mesentric node	intermediate grade-diffuse mixed small and large cell	65%	4%	Intermediate
43	576/10	14YRS	MALE	supraclavicular node	intermediate grade-diffuse mixed small and large cell	83%	0.40%	High
44	2408A/16	13YRS	MALE	Cervical node	intermediate grade-diffuse mixed small and large cell	50%	2%	Intermediate
45	771C/14	10YRS	FEMALE	Cervical node	high grade-burkitt's	85%	5.60%	High
46	2139/16	20YRS	MALE	supraclavicular node	high grade -burkitts like	90%	6.50%	High

47	1998/14	65YRS	MALE	Inguinal node	HIGH GRADE-BURKITT'S LIKE	95%	5.50%	High
48	1761/14	32YRS	FEMALE	Axillary node	HIGH GRADE -BURKITTS LIKE	90%	4.90%	High
49	1104A/14	65YRS	MALE	Inguinal node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	85%	1.70%	High
50	1567/14	47YRS	MALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	90%	5.10%	High
51	1148/14	4YRS	MALE	Cervical node	HIGH GRADE- LARGE CELL IMMUNOBLASTIC	95%	3.40%	High
52	71A2/15	12YRS	FEMALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	85%	4.10%	High
53	2111B/16	55YRS	FEMALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	90%	3.30%	High
54	478/16	63YRS	MALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	82%	4.60%	High
55	2928B/13	60YRS	FEMALE	Cervical node	HIGH GRADE LYMPHOBLASTIC LARGE CELL	84%	0.80%	High
56	119B/16	52YRS	MALE	Cervical node	HIGH GRADE LYMPHOBLASTIC LARGE CELL	85%	2.50%	High
57	980/14	53YRS	FEMALE	Cutaneous lymphoma	HIGH GRADE-CUTANEOUS T CELL LYMPHOMA	90%	0.60%	High

TABLE 2 : DISTRIBUTION OF CASES

CASES	NUMBER OF CASES	PERCENTAGE OF CASES
Total number of cases	57	100%
Low grade	21	36.84%
Intermediate grade	23	40.35%
High grade	13	22.80%

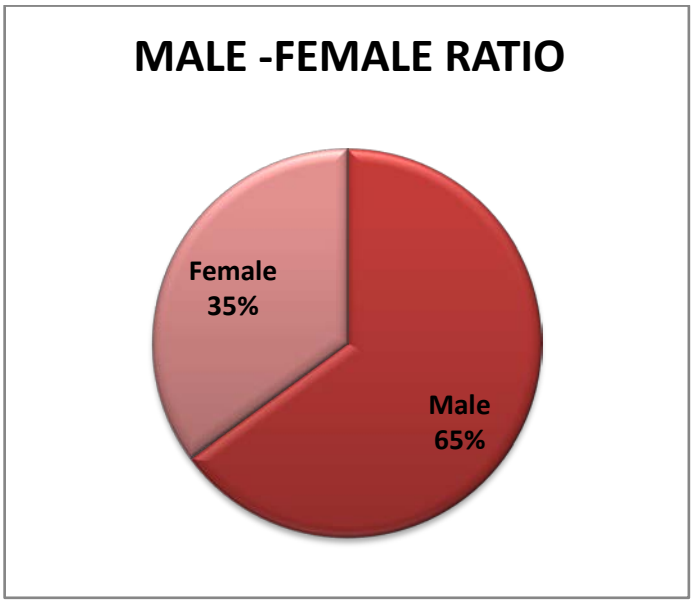

CHART 1 : Out of the total 57 cases there is increase in male predominance showing 65%incidence in males and 35% incidence in females.

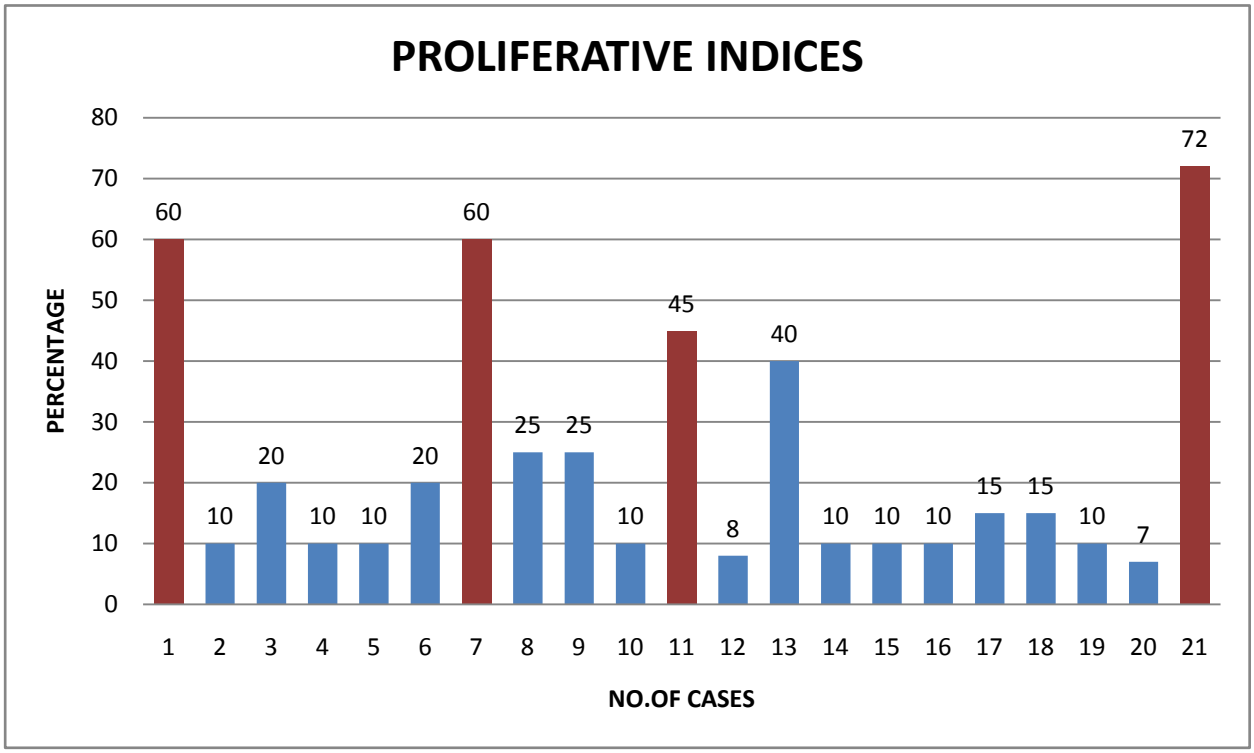

CHART 2 :LOW GRADE LYMPHOMA

Out of the 21 cases of low grade lymphomas 17 cases show proliferation rate of less than 40% while the rest of the cases show proliferation rate of greater than 45%. Among these 4 cases, 3 cases are of low grade small lymphocytic type and 1 case is of low grade diffuse follicular areas.

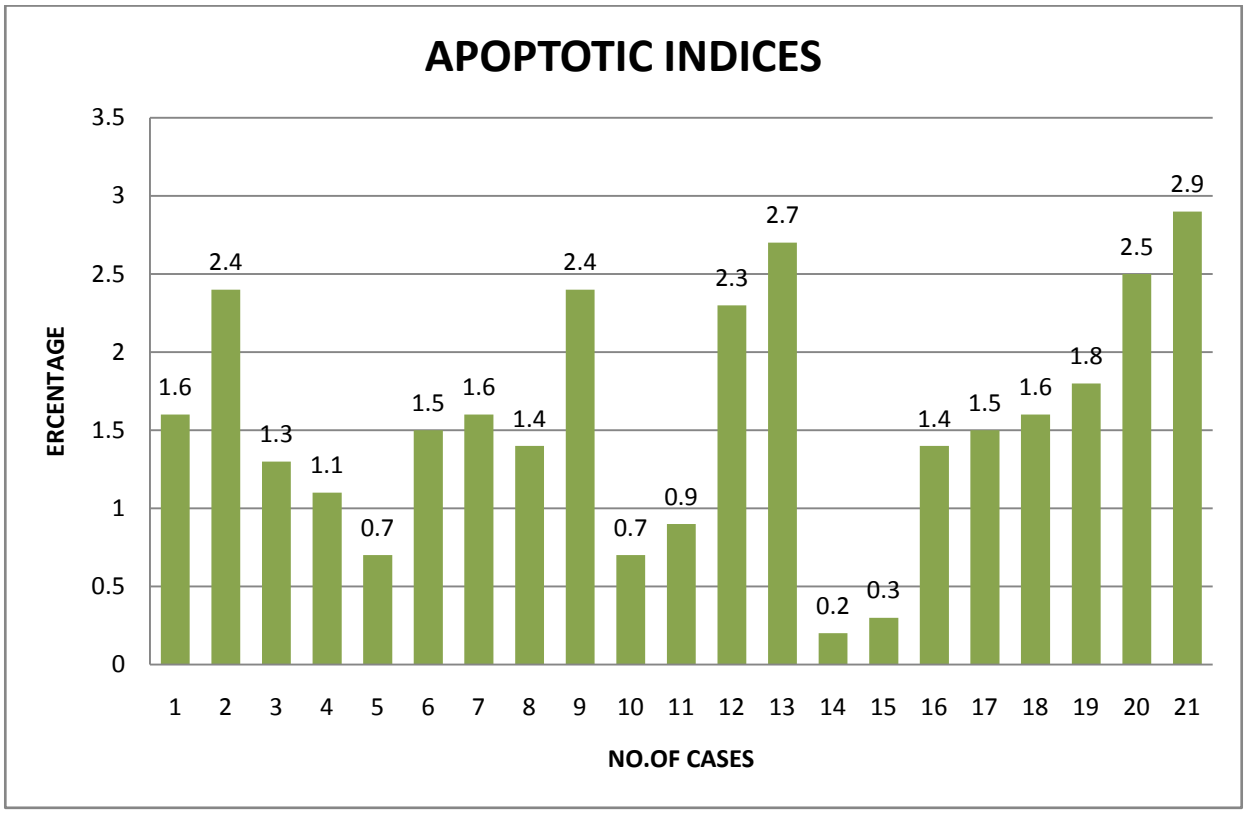

CHART 3 :LOW GRADE LYMPHOMA

Out of the 21 cases of low grade lymphomas 18 cases show a low apoptotic indices of less than 2.4% while the rest of the cases show apoptotic indices greater than 2.4%

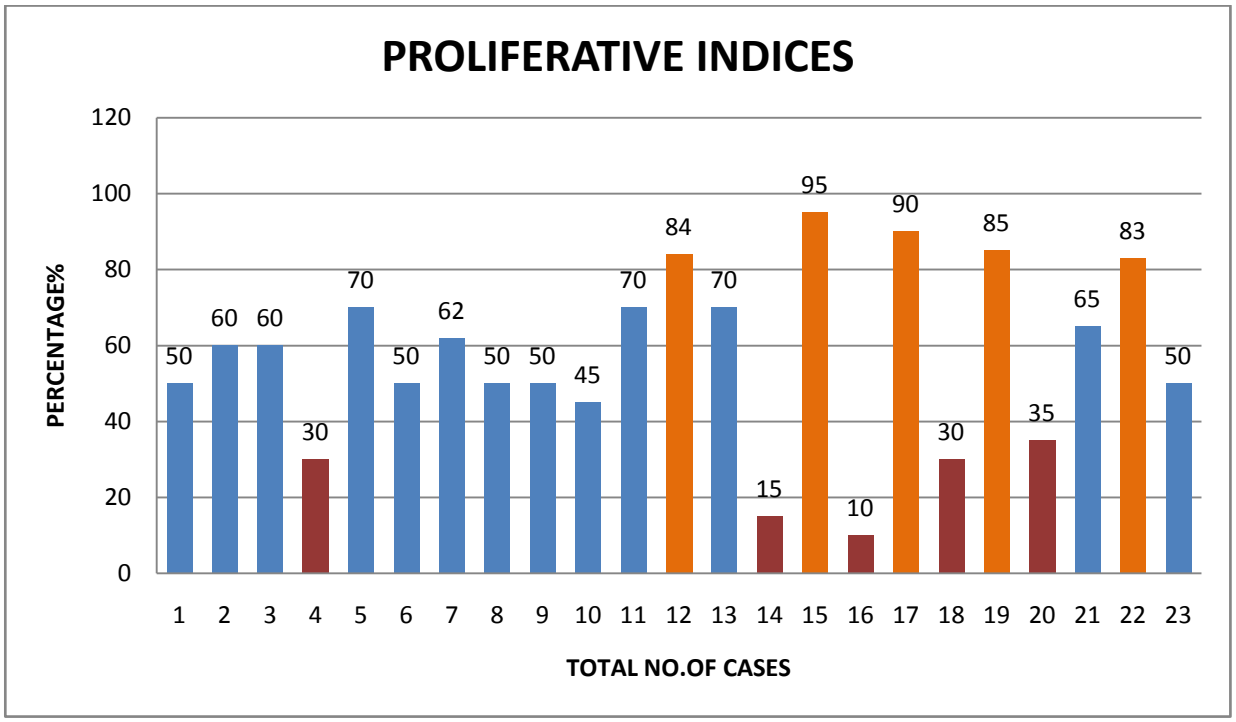

CHART 4 : INTERMEDIATE GRADE OF LYMPHOMAS

Out of the 23 cases of intermediate grade lymphomas 13 cases show proliferation rate between 40-75%while among the rest of the 10 cases 5 cases show proliferation rate of less than 40 % and the other 5 cases show proliferation rate of greater than 80%.

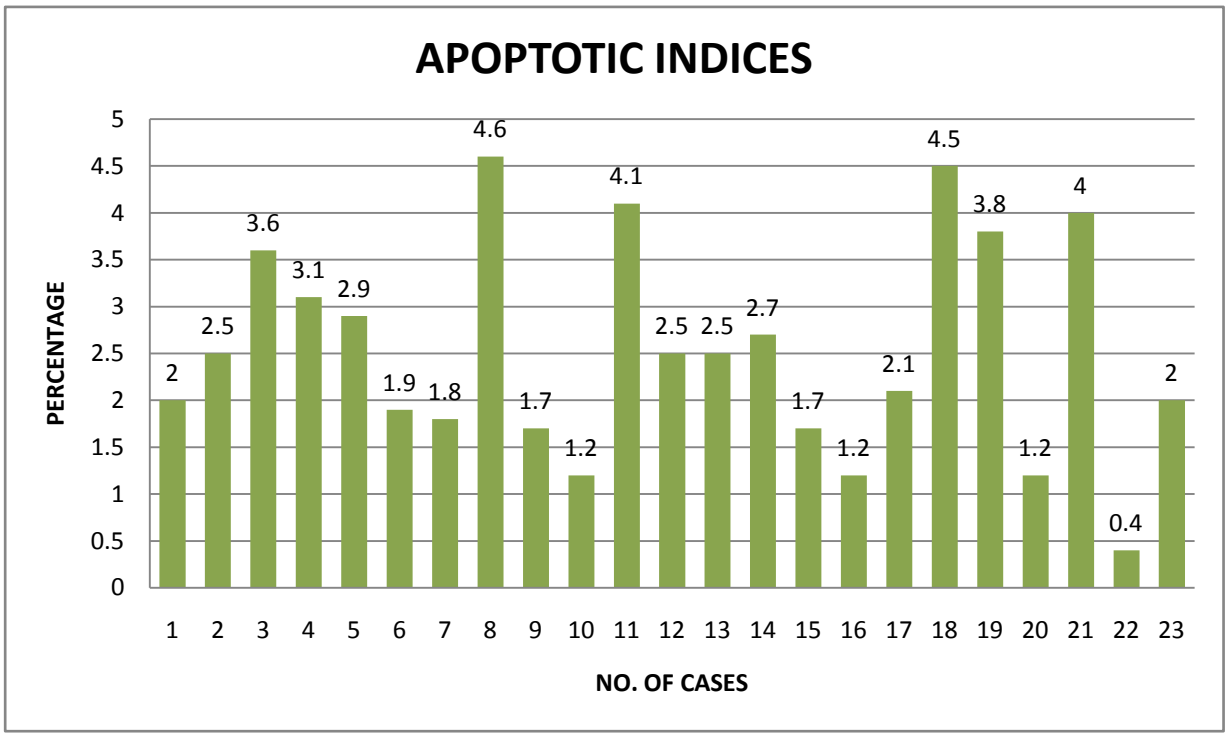

CHART 5 : INTERMEDIATE GRADE OF LYMPHOMA

There is a great variation in apoptotic indices of intermediate grade lymphomas. Out of the 23 cases 12 cases show apoptotic indices of greater than 2.4% while rest of the 11 cases show apoptotic indices of less than 2.4%

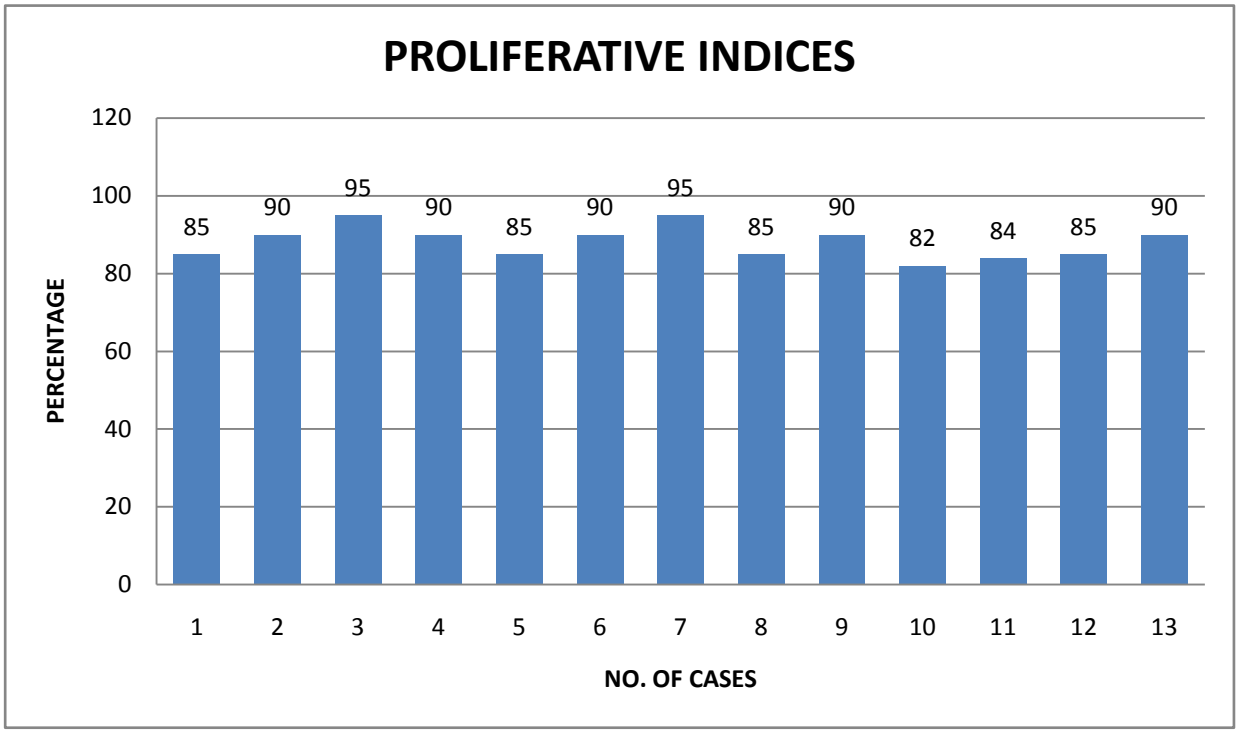

CHART 6 : HIGH GRADE LYMPHOMAS

Out of the 13 cases of high grade lymphomas all the cases show very high proliferation rate of greater than 80%

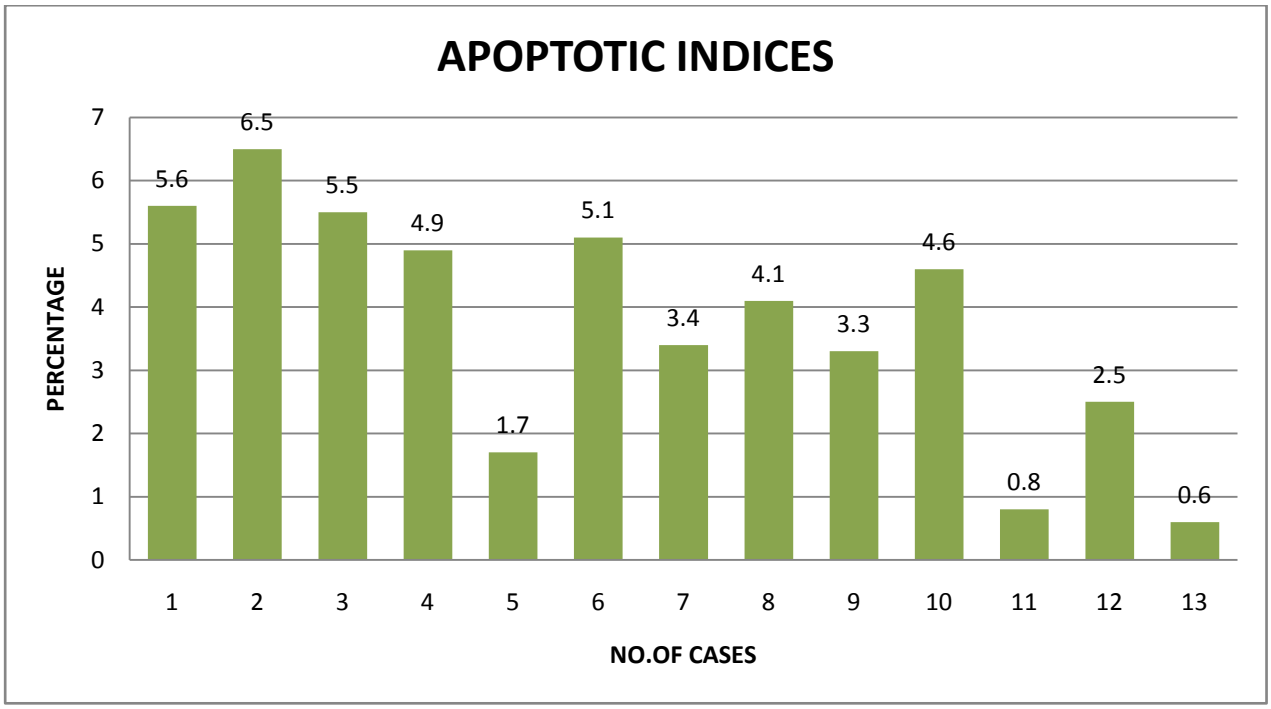

CHART 7 : HIGH GRADE LYMPHOMAS

Among the 13 cases of high grade lymphomas 10 cases show high apoptotic indices of greater than 2.4% while rest of the 3 cases show lower apoptotic indices of less than 2.4%

TABLE 3 : AVERAGE VALUES OF PROLIFERATIVE AND APOPTOTIC INDICES

WORKING FORMULATION	PROLIFERATIVE INDICES (MEAN VALUE)	STANDARD DEVIATION	APOPTOTIC INDICES (MEAN VALUE)	STANDARD DEVIATION
Low grade	23.43	19.87	2.91	2.68
Intermediate	56.91	23.00	2.22	2.41
High grade	88.15	4.14	0.56	0.15

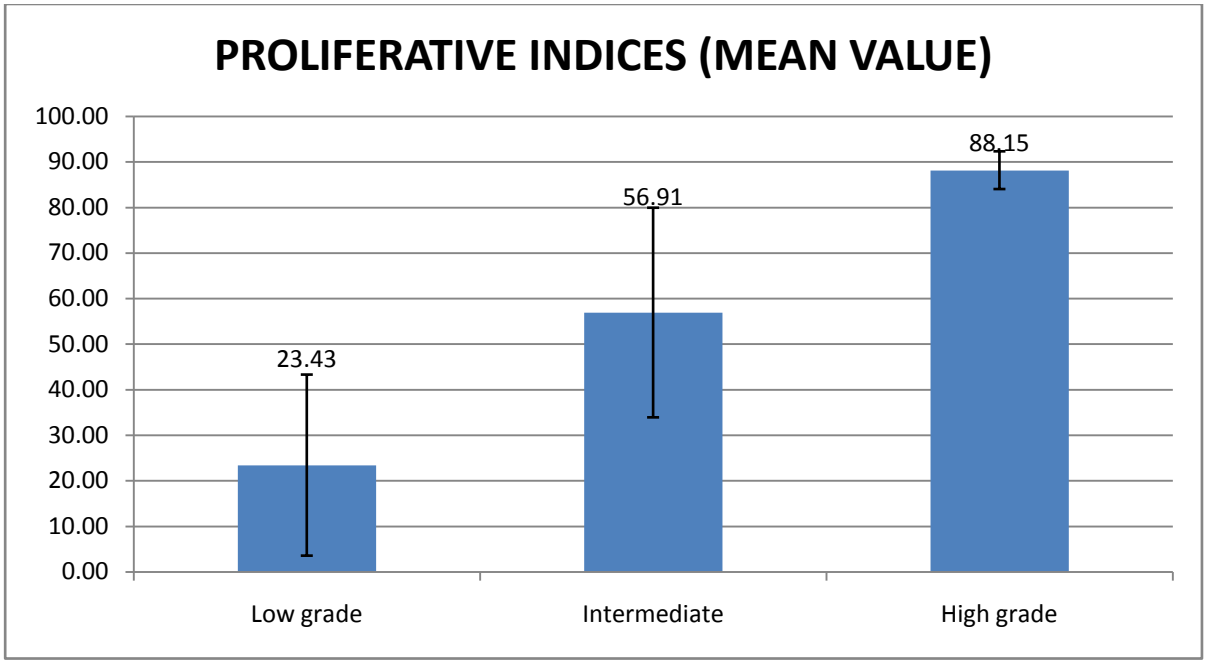

CHART 8: In this chart low grade lymphomas shows an average proliferation rate of 23.43% while intermediate and high grade lymphomas shows an average of 56.91% and 88.15% respectively.

TABLE 4:SUBTYPES OF LOW GRADE LYMPHOMAS

LOW GRADE LYMPHOMAS	TOTAL NO.OF CASES	LOW GRADE	PROGRESSION INTO INTERMEDIATE GRADE
LOW GRADE -SMALL LYMPHOCYTIC	15	12	3
LOW GRADE-FOLLICULAR PREDOMINANT SMALL CLEAVED	4	4	0
LOW GRADE-FOLLICULAR DIFFUSE AREAS	2	1	1
TOTAL	21	17	4

This **table 4** showing that out of 15 cases which was classified as low grade small lymphocytic lymphoma by working formulations 3 cases show progression into intermediate grade lymphoma. And out of 2 cases of low grade diffuse follicular areas one case show progression into intermediate grade lymphoma.

TABLE 5:SUBTYPES OF HIGH GRADE LYMPHOMAS

HIGH GRADE LYMPHOMAS	TOTAL NO.OF CASES	HIGH GRADE
HIGH GRADE -BURKITTS LIKE	4	4
HIGH GRADE IMMUNOBLASTIC LARGE CELL	6	6
HIGH GRADE LYMPHOBLASTIC LARGE CELL	2	2
HIGH GRADE-CUTANEOUS T CELL LYMPHOMA	1	1
TOTAL	13	13

TABLE 6: SUBTYPES OF INTERMEDIATE GRADE LYMPHOMAS

INTERMEDIATE GRADE LYMPHOMAS	TOTAL NO.OF CASES	INTERMEDIATE GRADE	REGRESSION INTO LOW GRADE	PROGRESSION INTO HIGH GRADE
INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	11	10	1	0
INTERMEDIATE GRADE-DIFFUSE LARGE CLEAVED	4	1	1	2
INTERMEDIATE GRADE-LARGE NON CLEAVED	2	0	1	1
INTERMEDIATE GRADE-DIFFUSE MIXED SMALL AND LARGE CELL	6	2	2	2
TOTAL	23	13	5	5

This table 6 shows out of the 23 cases of intermediate grade lymphomas 4 cases were intermediate large cell diffuse cleaved type by working formulation classification. Among the 4 cases 2 cases show progression to high grade lymphoma and 1 case shows regression into low grade lymphoma after evaluvating with prognostic indicators. Similarly out of the 6 cases classified as intermediate grade diffuse mixed small and large cell cleaved type 2 cases show progression into high grade and 2 cases show regression into low grade lymphomas. Out of the 2 cases classified as intermediate grade large non cleaved one case shows progression into high grade lymphoma.

TABLE 7 :OBSERVATION

		OBSERVATION			
		Low	Intermediate	High	Total
WORKING FORMULATION CLASSIFICATION	Low grade	17	4	0	21
	Intermediate grade	4	14	5	23
	High grade	0	0	13	13
	Total	21	18	18	57

Thus among the total 57 cases of NHL by working formulation classification 21 cases are of low grade,23 cases are intermediate grade and 13 cases are high grade. And then by applying prognostic indicators like proliferation and apoptotic indices out of the 21 cases of low grade lymphomas 4 cases show higher proliferative capacity and these low grade lymphomas may behave as intermediate grade.

Similarly among the 23 cases of intermediate grade lymphomas 5 cases show lower proliferative capacity and these may behave as a low grade with good survival and other 5 cases show a higher proliferative capacity of >80% and these intermediate grade lymphomas may behave as a higher grade with poor survival.

Among the 13 cases of high grade lymphoma all the cases shows a very higher proliferative capacity with very high apoptotic indices and these all may behave as a really very aggressive tumour with a poorer survival rate.

DISCUSSION

Cell proliferation rate and death rate is an important factor for grading human neoplasms and predicting their clinical behavior and outcome.This study aimed at the examination of the prognostic significance of Ki-67 expression and apoptotic indices in malignant non-Hodgkin's lymphomas

. Though there are numerous classification for non Hodgkin lymphomas were available no single classification determines how a tumour behave in a particular patient. Though WHO CLASSIFICATION holds good a minimum of 6 immunohistochemical markers are required which is expensive. So in this study we classified NHL by working formulations and assessed the trustworthiness of this classification by applying prognostic indicators like proliferative and apoptotic indices.

Scholzen and Gerdes [187] generated a Ki-67 monoclonal antibody which undergoes expression exclusively in proliferating cells. They are synthesized at the beginning of the cell proliferation process, it is indispensable for cell division and it is effectively degraded at the end of the proliferative cycle. . The protein is encoded by a gene localized to 10q25-qter.

Immunohistochemical reaction with anti-Ki-67 allows proliferating cells to be distinguished from cells in the G0-phase of the cell cycle. We demonstrated the Ki-67 expression in tumour cells by calculation of the proportion of immunopositive cells. This allows the determination of the proliferative activity of the tumour. In this study ,we demonstrated a level of expression of Ki-67 and apoptotic indices in varying grades of NHL .

Adi broyde et al[185] in his study mentioned that low /indolent grade lymphomas show a Ki67 proliferation rate at an average of about 27%. In my study out of 21 low grade lymphomas 17 cases show Ki-67 proliferation rate at less than 40 %. Only 4 cases of low grade lymphoma show proliferation rate between 45-75%.

Zeggai et al[186] in his study mentioned that aggressive /intermediate grade lymphomas show a Ki67 proliferation rate ranges between 40-75%. In my study 23 cases were of intermediate grade lymphomas. Among that 13 cases show proliferation rate between 45-75% while 5 cases show a proliferation rate greater than 80%. Remaining 5 cases show a proliferation rate of less than 35 %.

Olga boycov et al [188] in his study mentioned that very aggressive /high grade lymphomas show a proliferation rate of > 80%. In my study all high grade lymphomas show proliferation rate of > 80%.

Leoncini et al [189]in his study mentioned that low grade lymphomas show a lower apoptotic indices < 2.4% In my study out of 21 low grade lymphomas 18 cases show apoptotic indices of less than 2.4% and 3 cases show apoptotic indices greater than 2.4%

Soini et al[190] in his study on "histopathological evaluvation of apoptosis in cancer" mentioned that there is variation in apoptotic indices in varying grades of lymphomas. He also stated that high grade lymphomas and intermediate grade lymphomas show wide range of apoptotic indices from 2.5-8.8. leoncini et al in his study also mentioned that there was a tendency for greater dispersion towards higher Apoptotic indices values in high grade than in intermediate grade NHLs. In my study out of 23 cases of intermediate grade lymphomas 12 cases show apoptotic indices

value greater than 2.4% and the remaining 11 cases show a value less than 2.4%.Roser et al. (191) in his study mentioned that apoptotic index has shown an direct correlation with the proliferative index, corresponding to a more aggressive clinical course of the disease. In my study out of 13 cases of high grade lymphomas only 3 cases show apoptotic indices value of less than 2.4 % and the remaining 10 cases show apoptotic indices value ranging from 2.5-6.5%

Gerdes et al in his study have indicated a relationship between expression levels of Ki-67 on one hand and the histological type of the tumour, which affects the degree of malignancy, and survival time in NHL on the other (192,193,194). Tiemann et al. (195), who have shown that in patients with diagnosed high grade lymphoma with high proliferation index has correlated with shorter survival time. Martin et al. (196), examining low grade lymphomas, also found that a lower proliferative index was associated with longer survival time was observed.

NHL affects all the age group, commonly involving the elderly individual. The median age of incidence of NHL in Asian countries is significantly lower compared to the population based registration in Western countries. On the basis of data collected by Smith et al (197), the median age of the patients was reported as 68 years by the Hematological Malignancy Research Network. However, the median age of the patient is 54 years in Asia, 54 years in Taiwan (Lee,Tan, Feng, 2005) , 52 years in Korea (Y-H Ko et al, 1998) 54.5 years in Japan (Aozasa, et al, 1985) , 55 years in Iran (Mozaheb , Farzad, Aledavod,2011)

In the present study, average age of low grade lymphomas were over 40 years of age which constitutes for about 36.84% of total NHL cases and average age of intermediate grade lymphomas were 27 years of age which constitutes 40.35% of total cases. There were about 13 cases of high grade lymphomas of which average year was over 41 which constitutes 22.80 % of total cases. .

According to Manzoor Ahmed, Amir Hussain Kahn, Sami Saeed (198), 78.78% of cases of NHL presented with lymph node enlargement. Among these, 40.5% had generalised lymphadenopathy whereas 24% of patients had cervical lymph node enlargement. Sudipta chakravarthi, Supriya Sarkar (199) in their study documented that peripheral lymphadenopathy was noted in 94.7% of cases of NHL with associated symptoms of anemia in 60.5% of the cases. The size of the lymph node also plays an important role in the diagnosis of lymphoma. A diameter larger than 3cm, firm consistency, white, nodular cut surface are the features suggestive of neoplastic transformation.

In current study the patients of NHL presented with lymph node enlargement with one of the case showing additional lesion in the tonsil and other case in the skin. Maximum number of patients showed cervical group of enlarged lymph nodes which constituted for 52.63% (30cases). Other nodes involved are inguinal node comprising 21% (12 cases),submandibular node and axillary node comprising 7% each(4 cases),mesenteric node comprising 3 cases(5%),submandibular node constituting 2 cases (3.5%).

Leoncini et al also stated in his study that there is increased incidence of NHL in males . In my study there is increased incidence in males 65% compared to females. The maximum size of enlarged node was found to be 6cm with minimum size of 0.3cm.

SUMMARY AND CONCLUSION

This study was conducted in the department of pathology,Tirunelveli Medical College in which the slides were prepared from cases diagnosed as cases of NON HODGKIN LYMPHOMA. In this study all the cases diagnosed as NON HODGKIN LYMPHOMA are subjected and classified by working formulation classification into LOW,INTERMEDIATE,HIGH grade of lymphomas. Then to assess the prognostic value of the proliferation and apoptotic indices Ki67 –a proliferative marker was applied and the score was calculated for varying grades of lymphoma. Similarly all the cases with the help of light microscopy apoptotic bodies are calculated and graded.

All the high grade lymphoma that were classified by working formulation shows a very high proliferative indices and very high apoptotic indices indicating the aggressiveness of the tumour. So working formulations holds good for high grade lymphomas.

And among the the low grade lymphomas that were classified by working formulation 17 cases shows a low proliferation rate whereas the rest of the 4 cases shows a higher proliferative indices. This indicating a shortcoming in the working formulation classification. Though these 4 cases were classified as low grade there is a high chance for this tumour to behave as intermediate to high grade with poorer survival comparing to that of low grade lymphomas.

And among the 23 cases that were diagnosed as a case of intermediate grade lymphomas only 13 cases show proliferative capacity of intermediate grade.5 cases shows a very high proliferative rate higher than 80% so these cases though classified as intermediate grade lymphoma has the ability to behave as a very aggressive tumour

with poor prognosis and poorer survival outcome. And the rest of 5 cases though classified as intermediate grade lymphomas has a lower proliferative capacity. So these cases has an increased chance of this tumour behaving as a lower grade with good prognosis and a survival outcome.

Thus by concluding though working formulation classification of Non Hodgkin lymphoma holds good for many cases it has its drawbacks in some cases. So by the use of these inexpensive and an effective prognostic indicators along with the working formulation classification it may throw some light on the how a particular subtypes of Non hodgkin lymphoma in a particular patient might behave.

-COLOUR PLATES

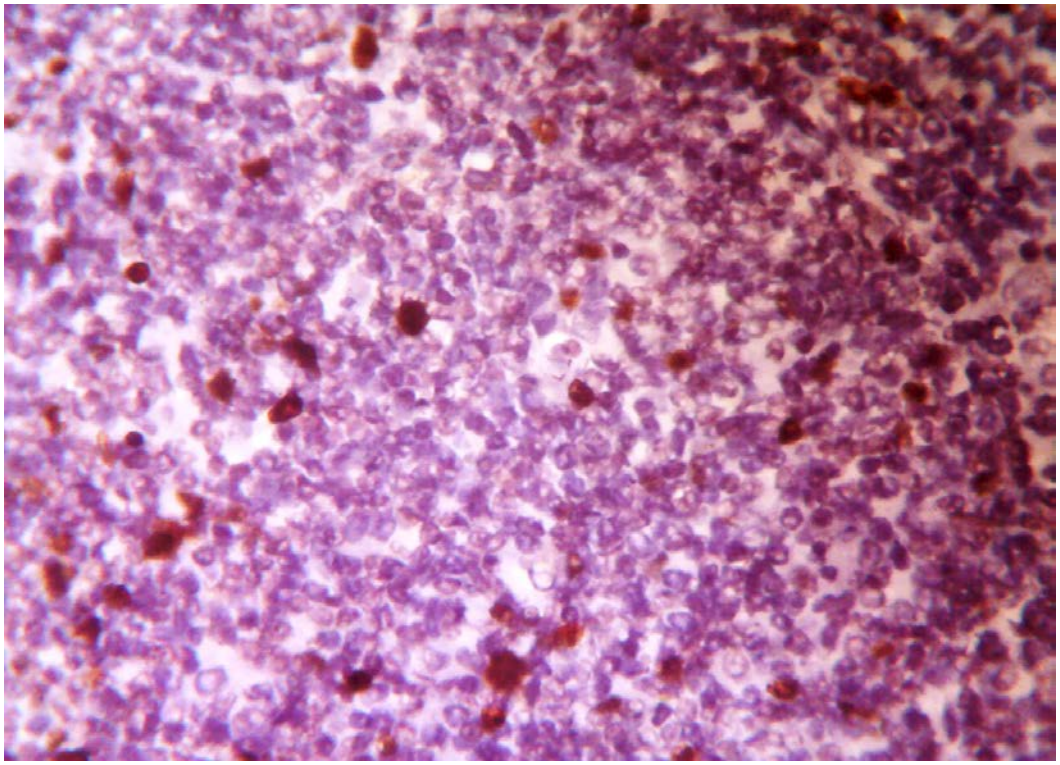

FIGURE 1: A case of low grade lymphoma with low proliferative indices –Ki67 (x 40)

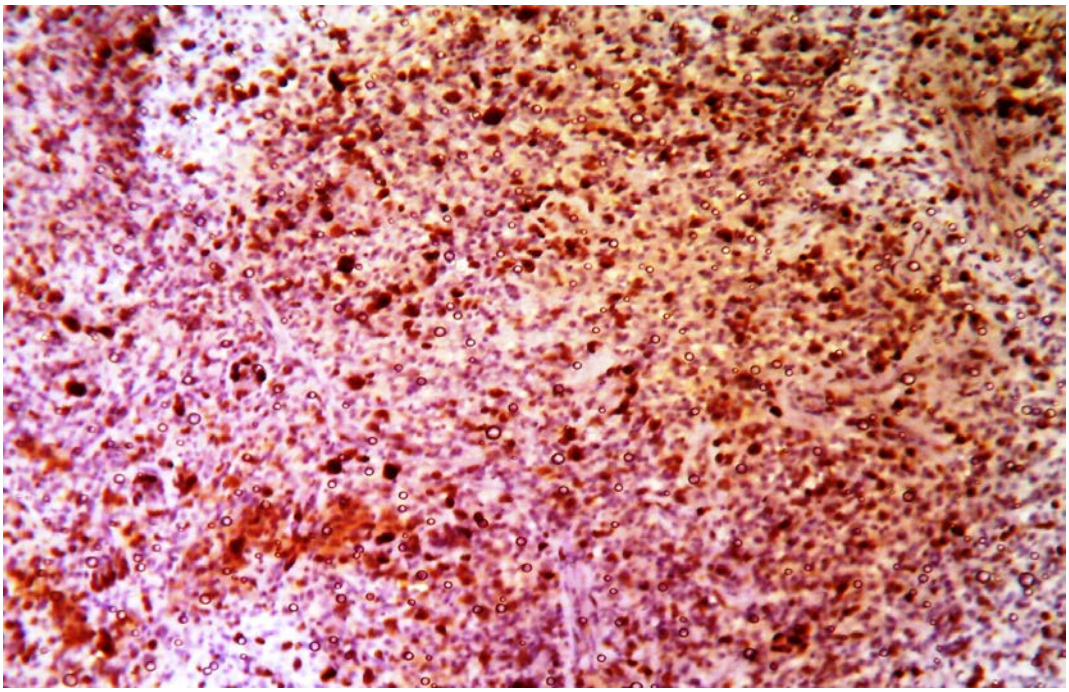

FIGURE 2:A case of low grade lymphoma with intermediate proliferative indicesKi67(x10)

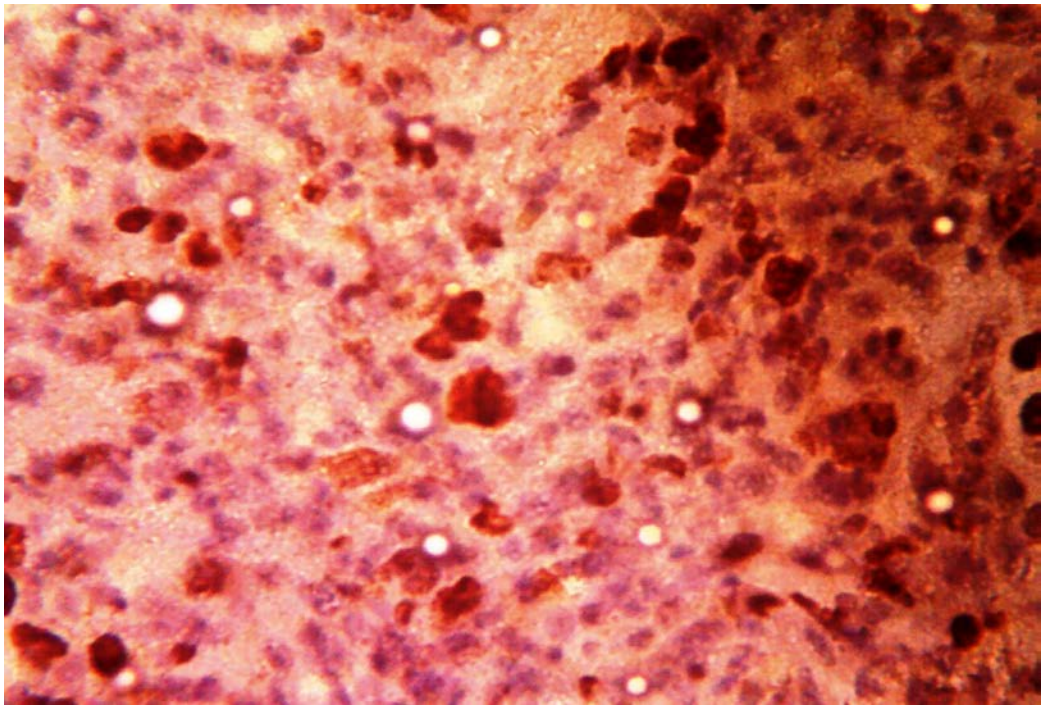

FIGURE 3: A case of low grade lymphoma with intermediate proliferative indices-Ki67(x40)

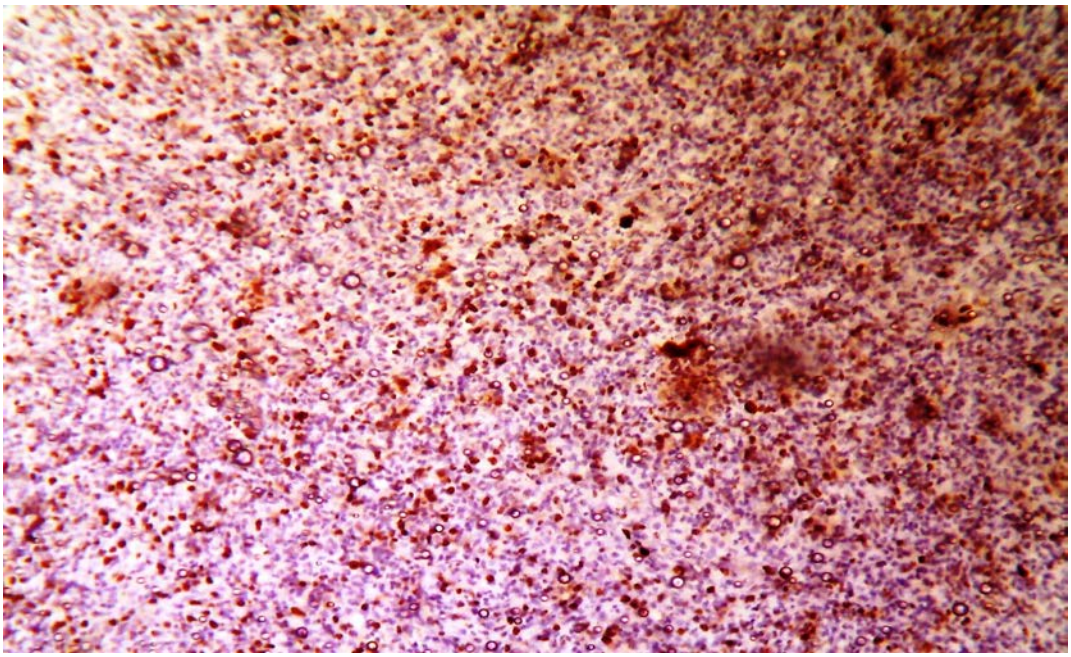

FIGURE 4:A case of intermediate grade lymphoma with intermediate proliferative indices-Ki67(x10)

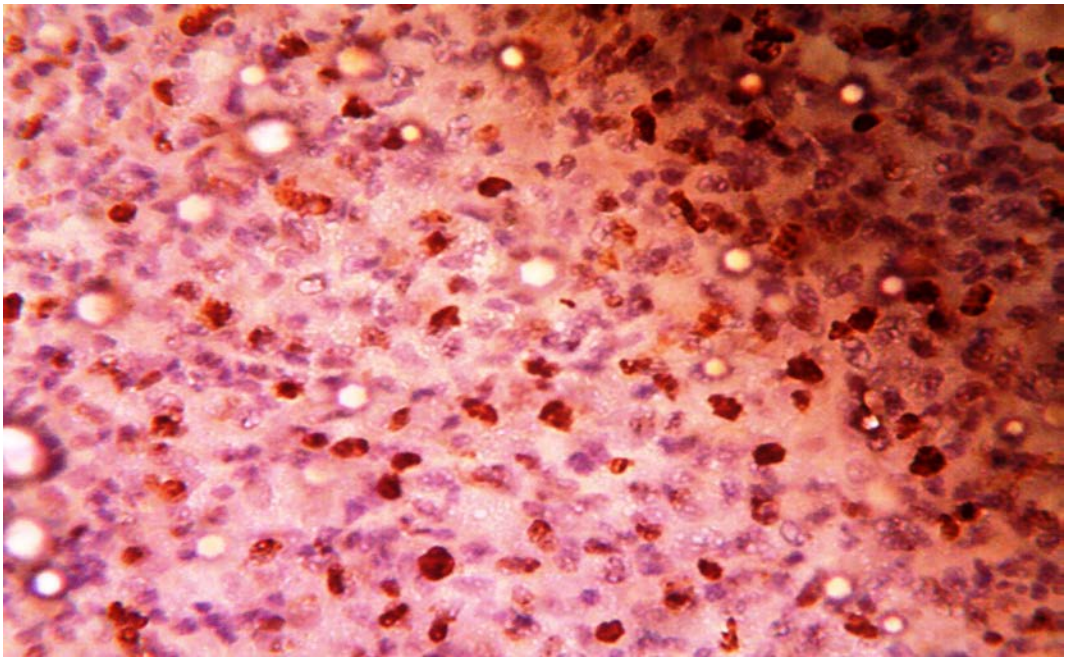

FIGURE 5: A case of intermediate grade lymphoma with intermediate proliferative indices- Ki67(x40)

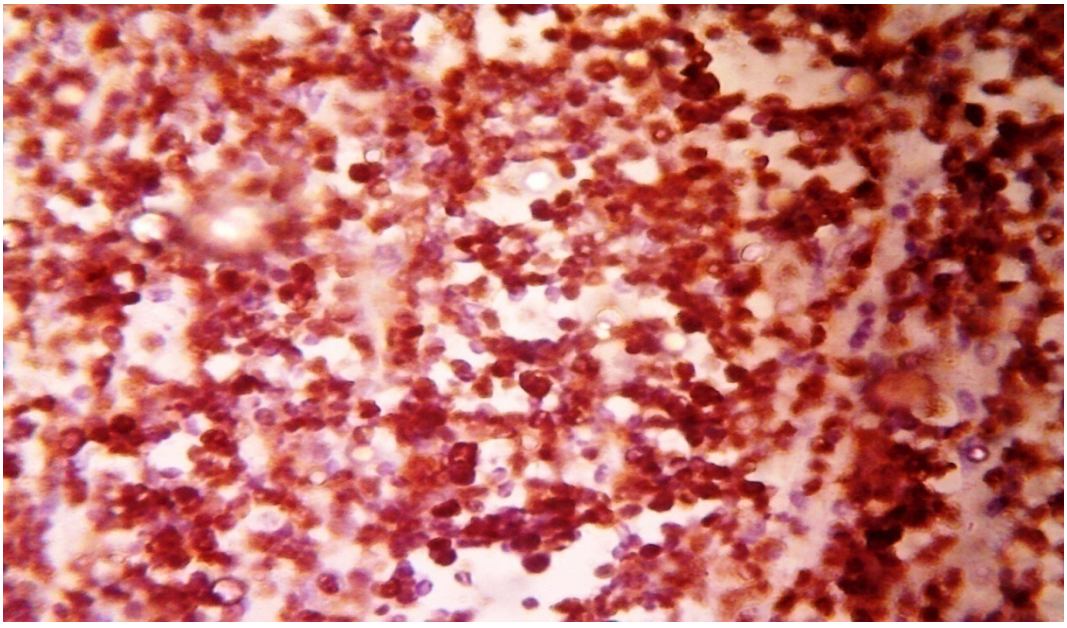

FIGURE 6: A case of intermediate grade lymphoma with high proliferative indices- Ki67(x40)

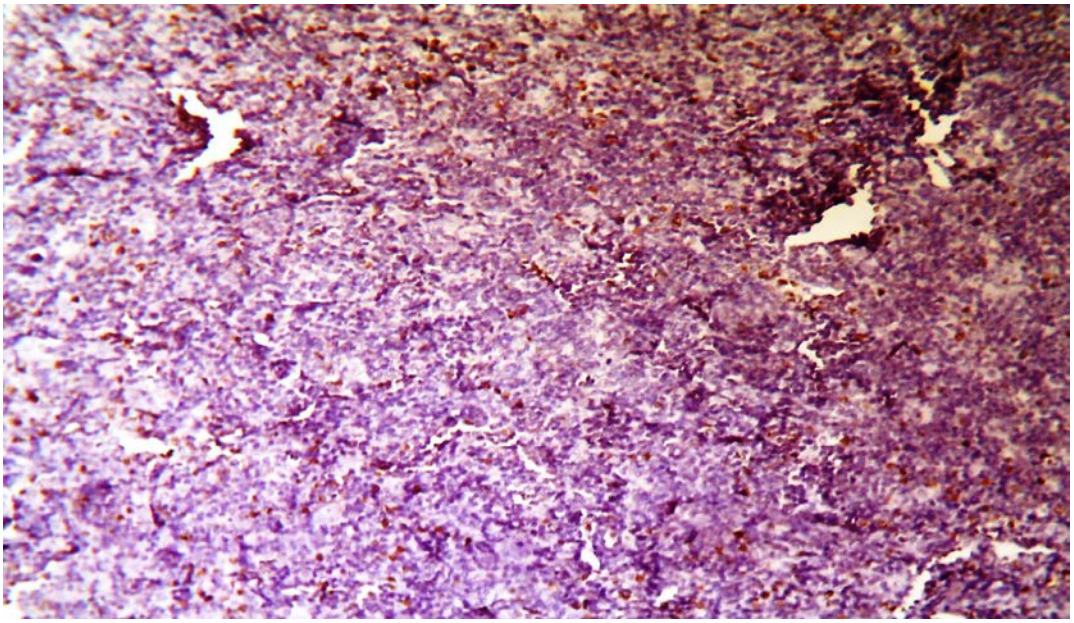

FIGURE 7: A case of intermediate grade lymphoma with low proliferative indices Ki67(x10)

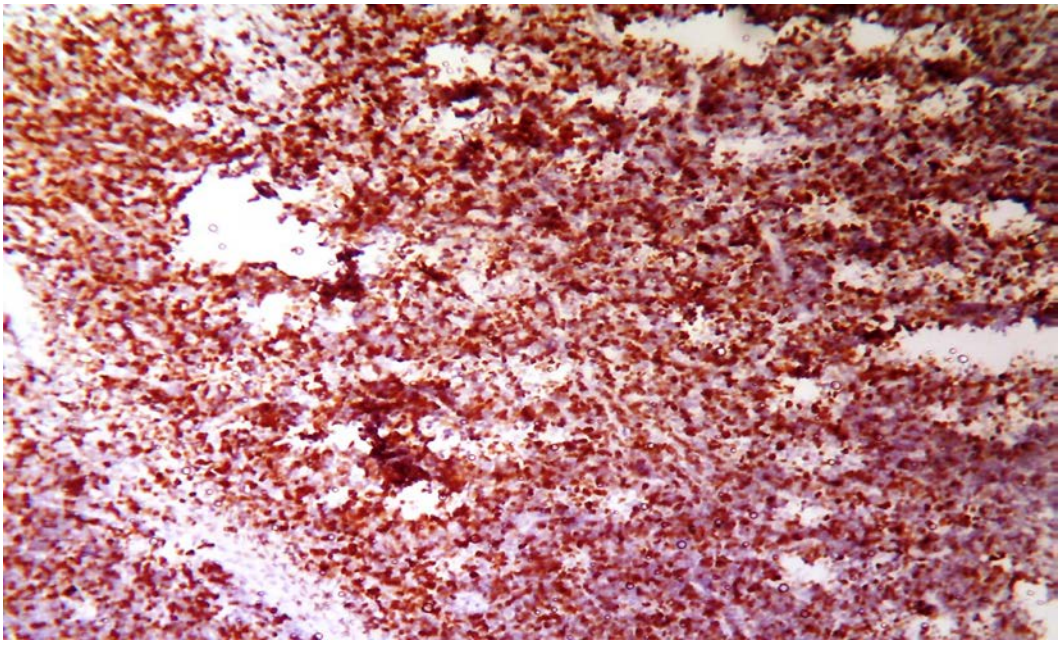

FIGURE 8: A case of high grade lymphoma with high proliferative indices-Ki67(x10)

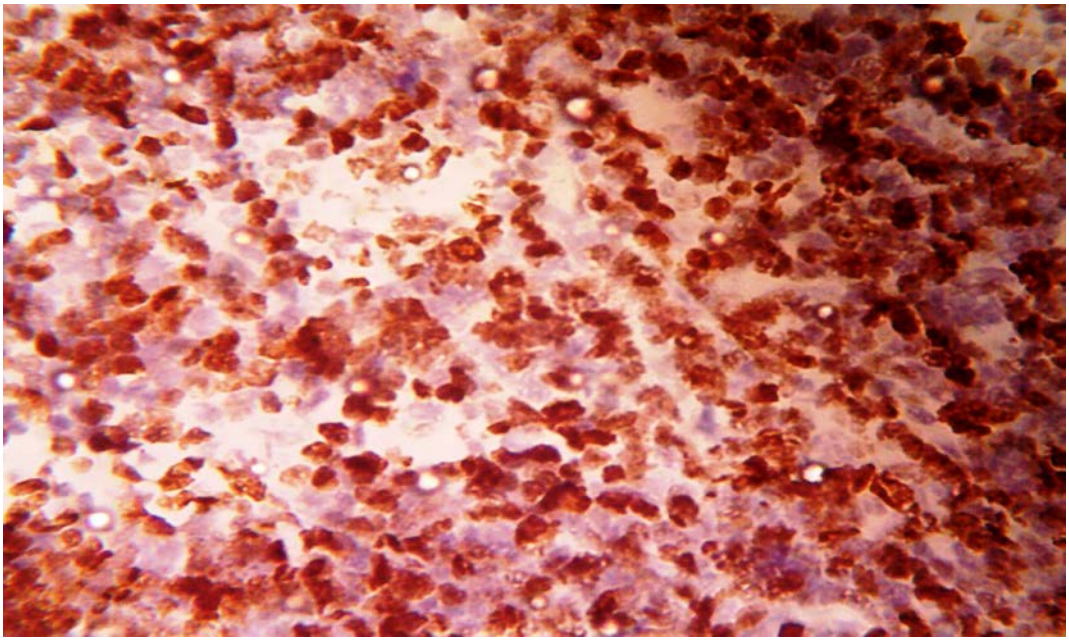

FIGURE 9: A case of high grade lymphoma with high proliferative indices-Ki67(x40)

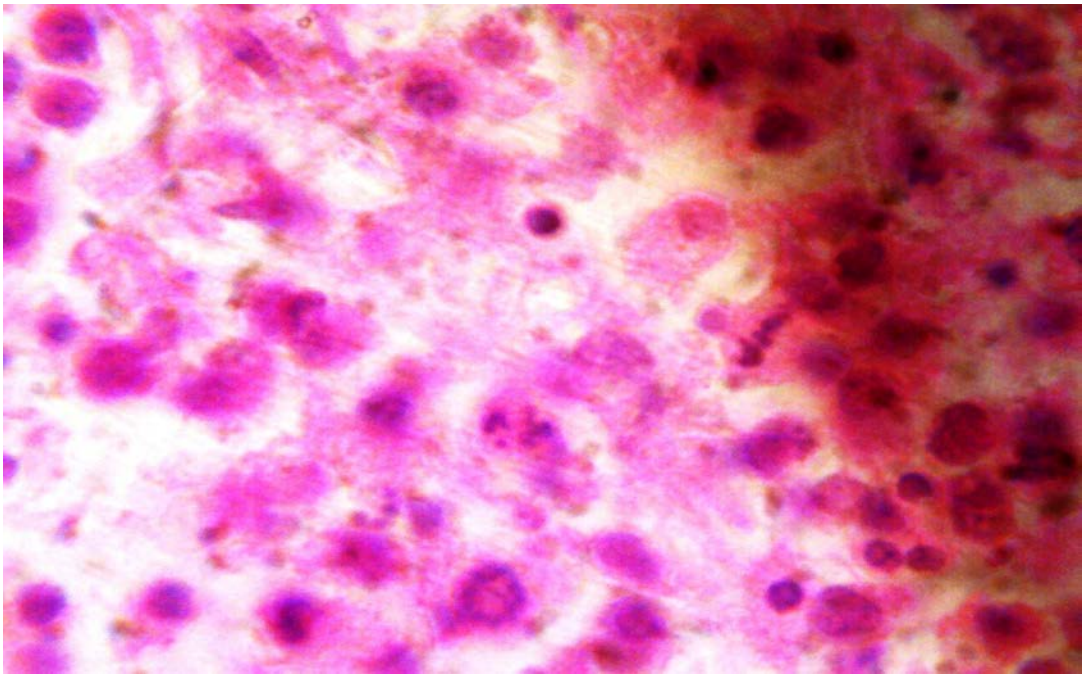

FIGURE 10: A high grade lymphoma showing apoptotic body (x100)

BIBILIOGRAPHY

BIBLIOGRAPHY

1. I. SatishRao. Role of immunohistochemistry in lymphoma, Indian J Med PaediatrOncol. 2010;31(4):145–147.
2. Del Vecchio MT, Leoncini L, Buerki K, Kraft R, Megha T, Barbini P, Tosi P, Cottier H: Diffuse centrocytic and/or centroblastic malignant non-Hodgkin's lymphomas: comparison of mitotic and pyknotic (apoptotic) indices.Int J Cancer 1991, 47:38-43
3. Hall PA, Woods AL: Immunohistochemical markers of cellular proliferation: achievements, problems and prospects. Cell Tissue Kinet 1990, 23:505-522
4. Rhodin JAG. Lymph nodes. In: Histology. New York: Oxford University Press, 1974:378–394.
5. Olah I, Röhlich P, Törö I. Lymph node. In: Ultrastructure of lymphoid organs. Philadelphia: JB Lippincott Co, 1975:216–255.
6. Cottier H, Turk J, Sobin L. A proposal for a standardized system of reporting human lymph node morphology in relation to immunological function. Bull World Health Organ 1972;47:375–408.
7. Roitt I. The anatomy of the immune response. In: Essential immunology. London: Blackwell Science, 1997:152–167.
8. Stein H. Lymphocyte differentiation. In: Mason DY, Harris NL, eds. Human lymphoma: clinical implications of the REAL classification. New York: Springer Verlag,1999.
9. van der Walk P, Meijer CJL. Reactive lymph nodes. In: Sternberg SS, ed. Histology for pathologists. Philadelphia: Lippincott-Raven, 1997:651–673.
10. Harris NL. Mature B-cell neoplasms. Introduction, Pathology and Genetics. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press, 2001;121–126.
11. Gaulard P, d'Agay M-F, Peuchmaur M, et al. Expression of the bcl-2 gene product in follicular lymphoma. Am J Pathol 1992;140:1089–1095.
12. Sheu L-F, Chen A, Meng C-L, et al. Analysis of bcl-2 expression in normal, inflamed, dysplastic nasopharyngeal epithelia and nasopharyngeal carcinoma: association with p53 expression. Hum Pathol 1997;28:556–562.
13. Torlakovic E, Cherwitz DL, Jessurun J, et al. B-cell gene rearrangement in benign and malignant lymphoid proliferations of mucosa-associated lymphoid tissue and lymph nodes. Hum Pathol 1997;28:166–173.
14. van der Valk P, van der Loor EM, Jansen J, et al. Analysis of lymphoid and dendritic cells in human lymph node, tonsil and spleen. A study using monoclonal and heterologous antibodies. Virchows Arch B Cell Pathol Mol Pathol 1984;45:169–185.
15. Papadimitriu CS, Stein H, Papacharalampoulos NS. Presence of a1-antichymotrypsin and a1-antitrypsin in hematopoietic and lymphoid tissue cells as revealed by the immunoperoxidase method. Pathol Res Pract 1980;169:287–297.

16. Crocker J, Williams M. An enzyme histochemical study of the sinuses of reactive lymph nodes. J Pathol 1984;142:31–38.
17. Hodgkin T. On some morbid appearances of the absorbent glands and spleen. Trans R Med Chir Soc Glasgow 1832;XVII:68–114.
18. Robb-Smith AHT, Taylor CR. Lymph node biopsy. New York: Oxford University Press, 1981.
19. Sternberg C. Uber eine Eigenartige unter dem Bilde der Pseudoleukamic verlaufende Tuberculose des lymphateschen Apparates. Ztschr f Heilkd 1898;xix:21–90.
20. Reed DM. On the pathological changes in Hodgkin's disease, with especial reference to its relation to tuberculosis. Johns Hopkins Hosp Red 1902;10:133–196.
21. Brill NF, Baehr G, Rosenthal N. Generalized giant lymph node hyperplasia of lymph nodes and spleen. A hitherto undescribed type. JAMA 1925;84:668–671.
22. Symmers D. Follicular lymphadenopathy with splenomegaly. A newly recognized disease of the lymphatic system. Arch Pathol Lab Med 1927;3:816–820.
23. Hicks EB, Rappaport H, Winter WJ. Follicular lymphoma: a re-evaluationtion of its position in the scheme of malignant lymphoma, based on a survey of 253 cases. Cancer 1956;9:792–821.
24. Epstein MA, Achong BG. Discovery and general biology of the virus. In: Epstein MA, Achong BG, eds. The Epstein-Barr virus. Berlin: Springer-Verlag, 1979:1–22.
25. Aisenberg AC, Bloch KJ. Immunoglobulins on the surface of neoplastic lymphocytes. N Engl J Med 1972;287:272–276.
26. Barcos MP, Lukes RJ. Malignant lymphomas of convoluted lymphocytes: a new entity of possible T-cell type. In: Sinks LF, Godden JO, eds. Conflicts in childhood cancer: an evaluation of current management. New York: Alan R. Liss,1975.
27. Lukes RJ, Collins RD. Immunologic characterization of human malignant lymphomas. Cancer 1974;34(suppl):1488–1503.
28. Lester JN, Fuller LM, Conrad FG, et al. The roles of staging laparotomy, chemotherapy, and radiotherapy in the management of localized diffuse large cell lymphoma: a study of 75 patients. Cancer 1982;49:1746–1753.
29. Korsmeyer SJ, Hieter PA, Ravetch JV, et al. Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B-cells. Proc Natl Acad Sci U S A 1981;78:7096–7100.
30. Waldmann TA, Davis MM, Bongiovanni KF, et al. Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms. N Engl J Med 1985;313:776–783.
31. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361–1392.
32. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov). SEER stat database: mortality—all COD, public-use with

state, total U.S. for expanded races/Hispanics (1990–2003), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006. Underlying mortality data provided by NCHS (www.cdc.gov/nchs), 2006.
33. Paltiel O, Schmit T, Adler B, et al. The incidence of lymphoma in first-degree relatives of patients with Hodgkin disease and non-Hodgkin lymphoma: results and limitations of a registry-linked study. Cancer 2000;88:2357–2366.
34. Chatterjee N, Hartge P, Cerhan JR, et al. Risk of non-Hodgkin's lymphoma and family history of lymphatic, hematologic, and other cancers. Cancer Epidemiol Biomarkers Prev 2004;13:1415–1421.
35. Wang SS, Slager SL, Brennan P, et al. Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10 211 cases and 11 905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 2007;109:3479–3488.
36. Wiernik PH, Wang SQ, Hu XP, et al. Age of onset evidence for anticipation in familial non-Hodgkin's lymphoma. Br J Haematol 2000;108:72–79.
37. Straus SE, Jaffe ES, Puck JM, et al. The development of lymphomas in families with autoimmune lymphoproliferative syndrome with germline Fas mutations and defective lymphocyte apoptosis. Blood 2001;98:194–200.
38. Linet MS, Pottern LM. Familial aggregation of hematopoietic malignancies and risk of non-Hodgkin's lymphoma. Cancer Res 1992;52:5468s–5473s.
39. Sandlund JT, Downing JR, Crist WM. Non-Hodgkin's lymphoma in childhood. N Engl J Med 1996;334:1238–1248.
40. Murphy SB, Fairclough DL, Hutchison RE, et al. Non-Hodgkin's lymphomas of childhood: an analysis of the histology, staging, and response to treatment of 338 cases at a single institution. J Clin Oncol 1989;7:186–193.
41. Jaffe ES, Harris NL, Stein H, et al. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon, France: IARC Press, 2001.
42. Suarez F, Lortholary O, Hermine O, et al. Infection-associated lymphomas derived from marginal zone B cells: a model of antigen-driven lymphoproliferation. Blood 2006;107:3034–3044.
43. Pozzato G, Mazzaro C, Crovatto M, et al. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. Blood 1994;84:3047–3053.
44. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;1:1311–1315.
45. Parsonnet J, Friedman GD, Vandersteen DP, et al. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–1131.
46. Parsonnet J, Hansen S, Rodriguez L, et al. Helicobacter pylori infection and gastric lymphoma. N Engl J Med 1994;330:1267–1271.

47. Wotherspoon AC, Doglioni C, Diss TC, et al. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. Lancet 1993;342:575–577.
48. Ioachim HL. Immune deficiency: opportunistic tumors. In Bertino JR, ed., Encyclopedia of Cancer, 2nd ed. San Diego: Academic Press, 2002;2:469–487.
49. Lim ST, Levine AM. Recent advances in acquired immunodeficiency syndrome (AIDS)-related lymphoma. CA Cancer J Clin 2005;55:229–241.
50. Centers for Disease Control. HIV/AIDS surveillance report, l-22. Atlanta, GA: CDC, 1990.
51. Ioachim HL, Cooper MC. Lymphomas of AIDS [letter]. Lancet 1986;1:96–97.
52. Ioachim HL, Dorsett B, Cronin W, et al. Acquired immunodeficiency syndrome-associated lymphomas: clinical, pathologic, immunologic, and viral characteristics of 111 cases. Hum Pathol 1991;22:659–673.
53. Gaidano G, Carbone F, Dalla-Favera R. Pathogenesis of AIDS-related lymphomas. Molecular and histogenetic heterogeneity. Am J Pathol 1998;152:623–630.
54. Pelicci PG, Knowles DM, Arlin Z, et al. Multiple monoclonal B-cell expansions and c-myc oncogene rearrangements in AIDS-related lymphoproliferative disorders: implications for lymphomagenesis. J Exp Med 1986; 164:2049–2060.
55. Capello D, Rossi D, Gaidano G. Post-transplant lymphoproliferative disorders: molecular basis of disease histogenesis and pathogenesis. Hematol Oncol 2005;23:61–67.
56. Isaacson P, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. Cancer 1983;52:1410–1416.
57. Bunim JJ, Talal N. The association of malignant lymphoma with Sjogren's syndrome. Trans Assoc Am Physicians 1963;76:45.
58. Lindsay S, Dailey ME. Malignant lymphoma of the thyroid gland and its relation to Hashimoto disease: a clinical and pathologic study of 8 patients. J Clin Endocrinol 1955;15:1332.
59. Holm LE, Blomgren H, Lowhagen T. Cancer risks in patients with chronic lymphocytic thyroiditis. N Engl J Med 1985;312:601–604.
60. Krikorian JG, Burke JS, Rosenberg SA, et al. Occurrence of non-Hodgkin's lymphoma after therapy for Hodgkin's disease. N Engl J Med 1979;300:452–458.
61. Doll DC, List AF. Burkitt's lymphoma in a homosexual. Lancet 1982;1:1026–1027.
62. Delecluse HJ, Anagnostopoulos I, Dallenbach F, et al. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. Blood 1997;89:1413–1420.
63. Cesarman E, Chang Y, Moore PS, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 1995;332:1186–1191.
64. Rosenberg SA, et al. Lymphosarcoma. Medicine 1961;40:31.

65. Bilsel Y, Balik E, Yamaner S, et al. Clinical and therapeutic considerations of rectal lymphoma: a case report and literature review. World J Gastroenterol 2005;11:460–461.
66. Batchelor T, Loeffler JS. Primary CNS lymphoma. J Clin Oncol 2006;24:1281–1288.
67. Shenkier TN, Connors JM. Primary extranodal non-Hodgkin's lymphomas. In: Canellos GP, Lister TA, Young BD, eds. The lymphomas, 2nd ed. Philadelphia: Saunders, Elsevier, 2006:325–347.
68. Hugh JC, Jackson FI, Hanson J, et al. Primary breast lymphoma. An immunohistologic study of 20 new cases. Cancer 1990;66:2602–2611.
69. Zaharia L, Gill PS. Primary cardiac lymphoma. Am J Clin Oncol 1991;14: 142–145.
70. Calvo KR, Traverse-Glehen A, Pittaluga S, et al. Molecular profiling provides evidence of primary mediastinal B-cell lymphoma as a distinct entity related to classic Hodgkin lymphoma: implications for mediastinal grey zone lymphomas as an intermediate form of B-cell lymphoma. Adv Anat Pathol 2004;11:227–238.
71. Stein H, Johrens K, Anagnostopoulos I. Non-mediastinal grey zone lymphomas and report from the workshop. Eur J Haematol 2005;75(Suppl 66):42–44.
72. Dogan A. Grey zone lymphomas. Hematology 2005;10(Suppl 1):190–192.
73. Traverse-Glehen A, Pittaluga S, Gaulard P, et al. Mediastinal grey zone lymphoma: the missing link between classic Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma. Am J Surg Pathol 2005;29:1411–1421.
74. Jaffe ES, Harris NL, Stein H, Vardiman JW. Tumours of haematopoietic and lymphoid tissues. Lyon, France: World Health Organization-IARC Press, 2001.
75. Siebert JD, Harvey LA, Fishkin PA, et al. Comparison of lymphoid neoplasm classification. A blinded study between a community and an academic setting. Am J Clin Pathol 2001;115:650–655.
76. Glaser SL, Dorfman RF, Clarke CA. Expert review of the diagnosis and histologic classification of Hodgkin disease in a population-based cancer registry: interobserver reliability and impact on incidence and survival rates. Cancer 2001;92:218–224.
77. The Non-Hodgkin's Lymphoma Classification Project. A clinical evaluation of the International Lymphoma Study Group. Classification of non-Hodgkin lymphoma. Blood 1997;89:3909–3918.
78. Lennert K, Mohri N, Stein H, Kaiserling E, Muller-Hermelink HK. Malignant Lymphomas other than Hodgkin's disease. Berlin, Heidelberg and New York, 1977 [in press].
79. Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. Cancer 1982;49:2112–2135.

80. Serdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW (eds) WHO classification of tumors of hematopoeitic and lymphoid tissues. IARC: Lyon 2008.
81. Aster JC. Diseases of white blood cells, lymph nodes, spleen and thymus. In Robbins and Cotran Pathologic Basis of Disease. Kumar V, Abbas AK, Fausto N (eds). Robbins and Cotran Pathologic Basis of Disease. 7th ed. Elsevier Saunders:Philadelphia, PA;2005. Figure 14-4, Origin of lymphoid neoplasms; p.669.
82. Greer JP, Williams ME. Non-Hodgkin Lymphoma in Adults. In Greer JP , Foerster J , Rodgers GM, Paraskevas F, Glader B, Arber DA, Means Jr RT (eds). Wintrobe's Clinical Hematology. 12th Edition. Lippincott Williams & Wilkins: Philadelphia, PA;2009.Figure 91.1, Cellular origins of non-Hodgkin lymphoma by B- and T-cell differentiation pathways; p.2145.
83. Coons AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. *Proc Soc Exp Biol Med*. 1941;47:200.
84. Avas S. Enzyme markers: Their linkage with proteins and use in immunohistochemistry. *Histochem J*. 1972;4:321.
85. Nakane PK, Pierce GBJ. Enzyme-labeled antibodies for the light and electron microscopic localization of tissue antigens. *J Cell Biol*. 1967;33:307.
86. Mann G, ed. *Physiologic histology*. Oxford: Oxford University Press; 1902.
87. Taylor CR, Burns J. The demonstration of plasma cells and other immunoglobulin containing cells in formalin-fixed,paraffin-embedded tissues using peroxidase labeled antibody. *J Clin Pathol*. 1974;27:14.
88. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975;256:495.
89. Huang SN. Immunohistochemical demonstration of hepatitis B core and surface antigens in paraffin sections. *Lab Invest*.1975;33:88.
90. Shi S-R, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: An enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem*. 1991;39:741.
91. Shi S-R, Tandon AK, Cote C, et al. S-100 protein in human inner ear: Use of a novel immunohistochemical technique on routinely processed, celloidin-embedded human temporal bone sections. *Laryngoscope*. 1992;102:734.
92. Shi S-R, Tandon AK, Haussmann RR, et al. Immunohistochemical study of intermediate filament proteins on routinely processed, celloidin-embedded human temporal bone sections by using a new technique for antigen retrieval. *Acta Otolaryngol (Stockh)*. 1993;113:48.
93. Shi S-R, Cote C, Kalra KL, et al. A technique for retrieving antigens in formalin-fixed, routinely acid-decalcified, celloidin-embedded human temporal bone sections for immunohistochemistry. *J Histochem Cytochem*. 1992;40:787.
94. Taylor CR, Shi S-R, Chen C, et al. Comparative study of antigen retrieval heating methods: Microwave, microwave and pressure cooker, autoclave, and steamer. *Biotech Histochem*. 1996;71:263.
95. Taylor CR, Shi S-R, Cote RJ. Antigen retrieval for immunohistochemistry: Status and need for greater standardization. *Appl Immunohistochem*. 1996;4:144.

96. Fraenkel-Conrat H, Brandon BA, Olcott HS. The reaction of formaldehyde with proteins. IV: Participation of indole groups. *J Biol Chem*. 1947;168:99.
97. Fraenkel-Conrat H, Olcott HS. The reaction of formaldehyde with proteins. V: Cross-linking between amino and primary amide or guanidyl groups. *J Am Chem Soc*. 1948;70:2673.
98. Fraenkel-Conrat H, Olcott HS. Reaction of formaldehyde with proteins. VI: Cross-linking of amino groups with phenol, imidazole, or indole groups. *J Biol Chem*. 1948;174:827.
99. Gown AM, de Wever N, Battifora H. Microwave-based antigenic unmasking: A revolutionary new technique for routine immunohistochemistry. *Appl Immunohistochem*. 1993;1:256.
100. Cattoretti G, Pileri S, Parravicini C, et al. Antigen unmasking on formalin- fixed, paraffin-embedded tissue sections. *J Pathol*. 1993;171:83.
101. van Regenmortel MHV. The recognition of proteins and peptides by antibodies. In: van Oss CJ, van Regenmortel MHV, eds. *Immunochemistry*. New York: Marcel Dekker; 1994:277-300.
102. Shi Y, Li G-D, Liu W-P. Recent advances of the antigen retrieval technique. *Linchuang yu Shiyan Binglixue Zazhi (J Clin Exp Pathol)*. 1997;13:265.
103. Evers P, Uylings HB. Microwave-stimulated antigen retrieval is pH and temperature dependent. *J Histochem Cytochem*. 1994;42:1555.
104. Li C-Y, Zeismer SC, Lacano-Villareal O. Use of azide and hydrogen peroxide as an inhibitor for endogenous peroxidise method. *J Histochem Cytochem*. 1987;35:1457.
105. Schmid KW, Hittmair A, Schmidhammer H, et al. Non-deleterious inhibition of endogenous peroxidase activity (EPA) by cyclopropanone hydrate: A definitive approach. *J Histochem Cytochem*. 1989;37:473.
106. Colvin RB, Bhan AK, McCluskey RT, eds. *Diagnostic Immunopathology*. 2nd ed. New York: Raven Press; 1995.
107. DeLellis RA, Sternberger LA, Mann RB, et al. Immunoperoxidase techniques in diagnostic pathology: Report of a workshop sponsored by the National Cancer Institute. *Am J Clin Pathol*. 1979;71:483.
108. Heras A, Roach CM, Key ME. Enhanced polymer detection system for immunohistochemistry. *Mod Pathol*. 1995;8:165A.
109. Taylor CR. A history of the Reed-Sternberg cell. *Biomedicine*. 1978;28:196.
110. Zola H, Swart B, Nicholson I, et al. CD molecules 2005: human cell differentiation molecules. *Blood.* 2005;106:3123–3126.
111. Chen CC, Raikow RB, Sonmez-Alpan E, Swerdlow SH. Classification of small B-cell lymphoid neoplasms using a paraffin section immunohistochemical panel. *Appl Immunohistochem Mol Morphol.* 2000;8:111.
112. Barberis A, Widenhorn K, Vitelli L, Busslinger M. A novel B-cell lineagespecific transcription factor present at early but not late stages of differentiation. *Genes Dev.* 1990;4:849–859.
113. Bonzheim I, Geissinger E, Roth S, et al. Anaplastic large cell lymphomas lack the expression of T-cell receptor molecules or molecules of proximal T-cell receptor signaling. *Blood.* 2004;104:3358–3360.

114. Wang T, Lasota J, Hanau CA, Miettinen M. Bcl-2 oncoprotein is widespread in lymphoid tissue and lymphomas but its differential expression in benign versus malignant follicles and monocytoid B-cell proliferations is of diagnostic value. *APMIS.* 1995;103:655–662.
115. Zutter M, Hockenbery D, Silverman GA, Korsmeyer SJ. Immunolocalization of the Bcl-2 protein within hematopoietic neoplasms. *Blood.* 1991;78:1062– 1068.
116. Tumwine LK, Agostinelli C, Campidelli C, et al. Immunohistochemical and other prognostic factors in B cell non Hodgkin lymphoma patients, Kampala, Uganda. BMC Clin Pathol. 2009;9(11)
117. Rao IS. Role of immunohistochemistry in lymphoma. Indian J Med Paediatr Oncol. 2010;31:145-7.
118. Jambhekar NA, Chaturvedi AC, Madur BP. Immunohistochemistry in surgical pathology practice: A current perspective of a simple, powerful, yet complex, tool. Ind J Pathol Microbiol 2008;51:2-11.
119. Kwak LW, Wilson M, Weiss L, et al. Clinical significance of morphologic subdivision in diffuse large cell lymphoma. Cancer 1991;68:1988–1993.
120. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood. 2004; 103: 275-282.
121. Li T, Medeiros LJ, Lin P, et al. Immunohistochemical Profile and Fluorescence In Situ Hybridization Analysis of Diffuse Large B-Cell Lymphoma in Northern China. Arch Pathol Lab Med. 2010;134:759-65.
122. Strauchen JA, Mandeli JP. Immunoglobulin expression in B-cell lymphoma. Immunohistochemical study of 345 cases. Am J Clin Pathol 1991;95:692–695.
123. Picker LJ, Weiss LM, Medeiros LJ, et al. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. Am J Pathol 1987;128:181–201.
124. Piris M, Brown DC, Gatter KC, et al. CD30 expression in non-Hodgkin's lymphoma. Histopathology 1990;17:211–218.
125. Fang JM, Finn WG, Hussong JW, et al. CD10 antigen expression correlates with the t(14;18)(q32;q21) major breakpoint region in diffuse large B-cell lymphoma. Mod Pathol 1999;12:295–300.
126. Lai R, Weiss LM, Chang KL, et al. Frequency of CD43 expression in non-Hodgkin lymphoma. A survey of 742 cases and further characterization of rare CD43+ follicular lymphomas. Am J Clin Pathol 1999;111:488–494.
127. Gaulard P, d'Agay M, Peuchmaur M, et al. Expression of the bcl-2 gene product in follicular lymphoma. Am J Pathol 1992;140:1089–1095.
128. Lorsbach RB, Shay-Seymoore D, Moore J, et al. Clinicopathologic analysis of follicular lymphoma occurring in children. Blood. 2002;99:1959-1964.
129. Davies AJ, Rosenwald A, Wright G, et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. Br J Haematol 2007;136(2):286-293.
130. Said JW, Pinkus JL, Shintaku IP, et al. Alterations in fascin-expressing germinal center dendritic cells in neoplastic follicles of B-cell lymphomas.

Mod Pathol 1998;11:1–5.
131. Vasef MA, Medeiros LJ, Koo C, et al. Cyclin D1 immunohistochemical staining is useful in distinguishing mantle cell lymphoma from other low-grade B-cell neoplasms in bone marrow. Am J Clin Pathol 1997;108:302–307.
132. Cheuk W, Wong KO, Wong CS, et al. Consistent immunostaining for cyclin D1 can be achieved on a routine basis using a newly available rabbit monoclonal antibody. Am J Surg Pathol 2004;28:801–807.
133. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. Blood 2005;105:3768–3785.
134. Picker LJ, Weiss LM, Medeiros LJ, et al. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. Am J Pathol 1987;128:181–201.
135. Rudiger T, Weisenburger DD, Anderson JR, et al. Peripheral T-cell lymphoma (excluding anaplastic large cell lymphoma):results from the Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 2002;13:140–149. 136. Mazur MT ,katzenstein AL,Metastatic melanoma: the spectrum of ultrastructural morphology. Ultrastruct Pathol 1980;1:337-356
137. Llombart-Bosch A,Blache R,Peydro-Olaya A. Ultrastructural study of 28 cases of Ewings sarcoma:typical and atypical forms. Cancer 1978;41:1362-137
138 .Gould VE. Neuroendocrinomas and neuroendocrine carcinomas:APUD cell system neoplasms and their aberrant secretory activities. Pathol Annu 1977;12:33-62
139.Ioachim HL,Finkbeiner JA.pseudonodular patterns of T-cell lymphoma. Cancer 1980 ;45:1370-1378

140. Caulet S. Audouin J, le Tourneau A, et al. Angio-immunoblastic lymphadenopathy (AIL) or T-cell :malignant lymphoma of AIL-type. A histopathologial, immunohistochemical and ultrastructural study of 8 cases. Pathol Res Pract 1988;183:724-734.
141. Serie Rappaport H. Tumors of the hematopoietic system. In: Atlas of tumor pathology. Series I, Fascicle 8. Washington , DC: Armed Forces Institute of Pathology, 1966.
142. Lukes RJ, Collins RD. New approaches to the classification of the lymphoma. Br J Cancer 1975;31(Suppl 2):1-28.
143.Greiner TC, Medeiros J, Jaffe ES.Non-Hodgkin’s lymphoma. Cancer 1995;75:370-380.

144. Krishnan J, Wallberg K, Frizzera G. T-cell rich large B-cell lymphoma:a study of 30 cases 1994;18:455-465
145, Hsi ED. The search for meaningful prognostic markers in diffuse large B-Cell lymphoma.Am J Clin Pathol 2001;115:481-483
146.Fijten GH, Blijham GH. Unexplained lymphadenopathy in family practice. An evaluation of the probability of malignant causes and the effectiveness of physician’s workup. J Fam Pract 1988;27:373:6.
147.Pangalis GA Vassilakopoulos TP, Boussiotis VA, et al. Clinical approach to lymphadenopathy.Semin Oncol 1993; 20:570s-582
148.Johnson JT Myers EN. Evaluation of cervical masses in the elderly Geriatrics

1983;38: 99-106
149.Osborne BM, Butler JJ. Clincal implications of nodal reactive follicular hyperplasia in the elderly patient with enlarged lymph nodes . Mod Pathol 1991; 4:24-30.
150..Nathwani BN Winberg CD, Diamond LW, et al. Morbhologic criteria for the differentiation of follicular lymphoma from florid reactive follicular hyperplasia: a study of 80 cases. Cancer 1981;48:1794-1806,
151. Abbondanzo SL . Epstein-Barr virus-associated lymphadentis: the differential diagnosis of diffuse paracortical lymphoid hyperplasia Pathol Case Rev 2004;9:192-198.
152. Shear NH, Spielberg SP. Anticonvulsant hypersensitivity syndrome: in vitro assessment of risk. J Clin Invests 1988;82:1826-1832.
153. Jeng YM, Tien HF, Su IJ. Phenytoin-induced pseudolymphoma:reevaluation using modern molecular biology techniques Epilepsia 1996;37:104-107.
154. Braddock SW, Harrington D, Vose J. Generalized nodular cutaneous pseudolymphoma associated with phenytoin therapy. Use of T-cells receptor gene rearrangement in diagnosis and clinical review of cutaneous reactions to phenytoin. J Am Acad Dermatol 1992;27:337-340.
155. Chamulak GA, Brynes RK, Nathwani BN. Kikuchi –Fujimoto disease mimicking malignant lymphoma.Am J surg pathol 1990;14:514-523
156. Davies JD,Stansfeld AG. Spontaneous infarction of superficial lymph nodes.J Clin pathol 1972;25:689-696
157. Elie H,Joubert B,Mandard JC. Infarctus ischemique spontane massif d'un ganglion lymphatique cervical.Ann pathol 1982:2:240-242
158. Casper C. The aetiology and management of castleman disease at 50years: translating pathophysiology tyo patient care. Br J Haematol 2005;129:3-17
159. Keller AR, Hochholzer L ,Castleman B. Hyaine-vascular and plasma cell types of giant lymph node hyperplasia of the mediastinum and other locations. Cancer 1972;29:670-683.
160. Landolt AM: Growth of pituitary adenomas, malignant adenomas. Pituitary Adenomas. Edited by Landolt AM, Vance ML, Reilly PL. Edinburgh, Churchill Livingstone, 1996, pp 73–82
161. Cummings MC, Winterford CM, Walker NI. Apoptosis. Am J Surg Pathol 1997;21:88–101
162. Kerr JFR, Winterford CM, Harmon BV: Apoptosis: its significance in cancer and cancer therapy. Cancer 1994, 73:2013–2026
163. Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ, Levin B. bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 1995;55:237–41.
164. Bedi A, Pasricha PJ, Akhtar AJ, Barber JP, Bedi GC, Giardiello FM, *et al.* Inhibition of apoptosis during development of colorectal cancer. Cancer Res 1995;55:1811–6.
165. Baretton GB, Diebold J, Christoforis G, Vogt M, Muller C, Dopfer K, *et al.* Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas. Cancer 1996;77:255–64.
166. Kerr JFR, Winterford CM, Harmon BV. Apoptosis: its significance

in cancer and cancer therapy. Cancer 1994;73:2013–26.
167. Willingham MC. Cytochemical methods for the detection of apoptosis. J Histochem Cytochem 1999;47:1101–10.
168. Leist M, Nicotera P. The shape of cell death. Biochem Biophys Res Commun 1997;236:1–9.
169. Sarraf CE, Bowen ID: Proportions of mitotic and apoptotic cells in a range of untreated experimental tumours. Cell Tissue Kinet 1988, 21:45-49
170. Determination of the apoptotic index in osteosarcoma tissue and its relationship with patients prognosis
171. Y. Soini, P. Pa¨a¨kko¨ , and V-P. Lehto: Histopathological Evaluation of Apoptosis in Cancer: *American Journal of Pathology, Vol. 153, No. 4, October 1998;1041-1053*
172. Donhuijsen K: Mitosis counts: reproducibility and significance in grading of malignancy. Hum Pathol 1986, 17:1122-1125
173. Hall PA, Woods AL: Immunohistochemical markers of cellular proliferation: achievements, problems and prospects. Cell Tissue Kinet 1990, 23:505-522
174. Gerdes J, Schwab U, Lemke H, Stein H: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983, 31:13-20
175. Weiss LM, Strickler JG, Medeiros W, Gerdes J, Stein H, Warnke RA: Proliferative rates of non-Hodgkin's lymphomas as assessed by Ki-67 antibody. Hum Pathol 1987, 18:1155-1159
176. Hall PA, Levison DA, Woods AL, Yu CCW, Kellock DB, Watkins JA, Barnes DM, Gillett CE, Camplejohn R, Dover R, Waseem NH, Lane DP: Proliferating Cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol 1990, 162:285-294
177. Woods AL, Hall PA, Shepherd NA, Hanby AM, Waseem NH, Lane DP, Levison DA: The assessment of proliferating cell nuclear antigen (PCNA) immunostaining in primary gastrointestinal lymphomas and its relationship to histological grade, S+G2+M phase fraction (flow cytometric analysis) and prognosis. Histopathology
178. Akerman M, Brandt L, Johnson A, Olsson H: Mitotic activity in non-Hodgkin's lymphoma. Relation to the Kiel classification and to prognosis. Br J Cancer 1987, 55:219-223
179. Brandt L, Johnson A, Olsson H, Akerman M: Mitotic activity and survival in advanced non-Hodgkin's lymphoma of unfavourable histology. Eur J Cancer 1990, 26:227-230
180. Macartney JC, Camplejohn RS, Morris R, Hollowood K, Clarke D, Timothy A: DNA flow cytometry of follicular non-Hodgkin's lymphoma. J Clin Pathol 1991, 44:215- 218
181. Knecht H., Odermatt B.F., Maurer R., and Rüttner J.R.: Diagnostic and prognostic value of monoclonal antibodies in immunophenotyping of angioimmunoblastic lymphadenopathy/lymphogranulomatosis X. Br J Haematol 1987; 67: pp. 19-24

182. Patsouris E., Stocker U., Kallmeyer V., Keiditsch E., Mehraein P., and Stavrou D.: Relationship between Ki-67 positive cells, growth rate and histological type of human intracranial tumors. Anticancer Res 1988; 8: pp. 537-544.
183. Ueda T., Aozasa K., Tsujimoto M., Ohsawa M., Uchida A., Aoki Y., et al: Prognostic significance of Ki-67 reactivity in soft tissue sarcomas. Cancer 1989; 15: pp. 1607-1611\
184.Scholzen T, Gerdes J (March 2000). "The Ki-67 protein: from the known and the unknown". *J. Cell. Physiol.* **182** (3): 311–22. doi:10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9. PMID 10653597.
185. Role and prognostic significance of the Ki-67 index in non-Hodgkin's lymphoma Adi Broyde,1 Olga Boycov,2 Yulia Strenov,2 Elimelech Okon,2 Ofer Shpilberg,1 and Osnat Bairey1* Am. J. Hematol .84: 338-343,2009.
186. Soumia Zeggai1, Noria Harir1, Abdelnacer Tou2, Feriel Sellam1, Meriem N. Mrabent1, Rachida Salah1Immunohistochemistry and scoring of Ki-67 proliferative index and p53 expression in gastric B cell lymphoma from Northern African population: a pilot study: Journal of Gastrointestinal Oncology Vol 7, No 3 June 2016 :462-469
187. Scholzen T, Gerdes J. The ki-67 protein: From the known and the unknown. J Cell Physiol 2000;182:311–322.
188. Olga Boycov, Yulia Strenov, Elimelech Okon, Ofer Shpilberg, and Osnat Bairey . Role and prognostic significance of the Ki-67 index in non-Hodgkin's Lymphoma: Am. J. Hematol .84: 338-343,2009.
189. Lorenzo Leoncini, Maria Teresa Del Vecchio,Tiziana Megha, Paolo Barbini, Piero Galieni,t Stefano Pileri,t Elena Sabattini,t Filippo Gherlinzoni, Piero Tosi, Rainer Kraft, and Hans Cottier: Correlations Between Apoptotic and Proliferative Indices in Malignant Non-Hodgkin's Lymphomas- American Journal of Pathology, Vol. 142, No. 3, March 1993 755-762.
190. Y. Soini, P. Pa¨a¨kko¨ , and V-P. Lehto: Histopathological Evaluation of Apoptosis in Cancer: *American Journal of Pathology, Vol. 153, No. 4, October 1998;1041-1053*
191. Roser F, Saini M, Meliss R, Ostertag H, Samii M and Bellinzona M: Apoptosis, vascularity, and proliferation in primary central nervous system lymphomas (PCNSL): a histopathological study. Surg Neurol *62(5)*: 393-399, 2004
192. Gerdes J: Ki-67 and other proliferation markers useful for immunohistologocal diagnostic and prognostic evaluations in human malignancies. Semin Cancer Biol *1*: 199-206, 1990.
193.Hall PA, Richards MA, Gregory WM, d'Ardenne AJ, Lister TA and Stansfeld AG: The prognostic value of Ki-67 immunostaining in non-Hodgkin's lymphoma. J Pathol *154*: 223-235, 1998.
194.Velders GA, Kluin-Nelemans JC, De Boer CJ, Hermans J, Noodijk EM, Schuuring E, Kramer Mh, Van Deijk WA, Rahder JB, Kluin PM and Van Krieken JH: Mantle-cell lymphoma: a populationbased clinical study. J Clin Oncol *14(4)*: 1269-1274, 1996.

195. Tiemann M, Schrader C, Klapper W, Dreyling MH, Campo E, Norton A, Berger F, Kluin P, Ott G, Pileri S, Pedrinis E, Feller AC, Merz H, Janssen D, Hansmann ML, Krieken H, Moller P, Stein H, Unterhalt M, Hiddemann W and Parwaresch R: Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL Network. Br J Haematol *131*: 29-38, 2005.
196. Martin AR, Weisenburger DD, Chan WC, Ruby EI, Anderson JR, Vose JM, Bierman PJ, Bast MA, Daley DT and Armitage JO: Prognostic value of cellular proliferation and histologic grade in follicular lymphoma. Blood *85*: 3671-3678, 1995
197.Smith A,Roman E,Howell D et al. The haematological malignancy research work(HRMN): British journal of haematology.2010;148(5):739-753.
198.Ahmad M,Khan AH,Mansoor A et al.NHL-clinicopathological pattern.Journal-pakistan medical association 1992;42:205-207.
199.Chakrabarti S,Sarkar S,Goswami et al. HL and NHLin Indian Rural Medical Association :comparative clinicopathologic analysis. Asian pacific J cancer prevention.2010;11;1605-1608.

MASTER CHART

Sl.no	Block number	Age	Sex	Site	classification by working formulation	Proliferative indices	Apoptotic indices
1	491/13	31YRS	MALE	Cervical node	LOW GRADE -SMALL LYMPHOCYTIC	60%	1.60%
2	441A2/15	5YRS	MALE	Cervical node	LOW GRADE -SMALL LYMPHOCYTIC	10%	2.40%
3	797A1/13	11YRS	MALE	Axillary node	LOW GRADE -SMALL LYMPHOCYTIC	20%	1.30%
4	58 A2/13	50YRS	MALE	Cervical node	LOW GRADE -SMALL LYMPHOCYTIC	10%	1.10%
5	1726R2/16	80YRS	FEMALE	Cervical node	LOW GRADE-SMALL LYMPHOCYTIC	10%	0.70%
6	1567A11	64YRS	FEMALE	Inguinal node	LOW GRADE-SMALL LYMPHOCYTIC	20%	1.50%
7	491/13	31YRS	MALE	Cervical node	LOW GRADE-SMALL LYMPHOCYTIC	60%	1.60%
8	2316R4/13	57YRS	FEMALE	Inguinal node	LOW GRADE-SMALL LYMPHOCYTIC	25%	1.40%
9	1427/10	40YRS	MALE	Cervical node	LOW GRADE-SMALL LYMPHOCYTIC	25%	2.40%
10	3790B1/15	60YRS	FEMALE	Cervical node	LOW GRADE-SMALL LYMPHOCYTIC	10%	0.70%
11	1813/11	65YRS	FEMALE	Cervical node	LOW GRADE-SMALL LYMPHOCYTIC	45%	0.90%
12	2426/11	78YRS	MALE	Inguinal node	LOW GRADE-SMALL LYMPHOCYTIC	8%	2.30%
13	1593N3/11	62YRS	FEMALE	Axillary node	LOW GRADE-SMALL LYMPHOCYTIC	40%	2.70%
14	394/12	65YRS	MALE	Inguinal node	LOW GRADE-SMALL LYMPHOCYTIC	10%	0.20%
15	2444A/13	62YRS	MALE	Inguinal node	LOW GRADE-SMALL LYMPHOCYTIC	10%	0.30%
16	2857R3/11	15YRS	MALE	submandibular node	LOW GRADE-FOLLICULAR PREDOMINANT SMALL CLEAVED	10%	1.40%
17	3130B/15	57YRS	MALE	Cervical node	LOW GRADE-FOLLICULAR PREDOMINANT SMALL CLEAVED	15%	1.50%
18	2607/13	45YRS	FEMALE	Cervical node	LOW GRADE-FOLLICULAR PREDOMINANT SMALL CLEAVED	15%	1.60%

19	1217/13	60YRS	MALE	tonsillar growth	LOW GRADE-FOLLICULAR PREDOMINANT SMALL CLEAVED	10%	1.80%
20	1304A2/12	39YRS	MALE	Inguinal node	LOW GRADE-FOLLICULAR DIFFUSE AREAS	7%	2.50%
21	2072B/15	48YRS	MALE	Cervical node	LOW GRADE-FOLLICULAR DIFFUSE AREAS	72%	2.90%
22	189A3/15	71YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	50%	2%
23	190/13	4YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	60%	2.50%
24	1117A3/12	9YRS	FEMALE	Inguinal node	INTERMEDIATE GRADE -DIFFUSE SMALL CLEAVED	60%	3.60%
25	273A2/14	7YRS	MALE	Inguinal node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	30%	3.10%
26	1146A1/14	5YRS	MALE	Axillary node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	70%	2.90%
27	614A2/15	8YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	50%	1.90%
28	5938/15	35YRS	MALE	Cervical node	INTERMEDIATE GRADE -DIFFUSE SMALL CLEAVED	62%	1.80%
29	607F/15	45YRS	FEMALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	50%	4.60%
30	2218/10	47YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	50%	1.70%
31	1158/10	70YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	45%	1.20%
32	211/11	8YRS	MALE	Axillary node	INTERMEDIATE GRADE-DIFFUSE SMALL CLEAVED	70%	4.10%

33	2551B/12	60YRS	FEMALE	Inguinal node	INTERMEDIATE GRADE-DIFFUSE LARGE CLEAVED	84%	2.50%
34	768A/14	60YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE LARGE CLEAVED	70%	2.50%
35	539/14	5YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE LARGE CLEAVED	15%	2.70%
36	615A2/14	5YRS	FEMALE	Inguinal node	INTERMEDIATE GRADE-DIFFUSE LARGE CLEAVED	95%	1.70%
37	59r10/15	5YRS	MALE	Mesentric node	INTERMEDIATE GRADE-LARGE NON CLEAVED	10%	1.20%
38	680A2/15	10 YRS	FEMALE	Mesentric node	INTERMEDIATE GRADE-LARGE NON CLEAVED	90%	2.10%
39	3413/15	50YRS	FEMALE	submandibular node	INTERMEDIATE GRADE-DIFFUSE MIXED SMALL AND LARGE CELL	30%	4.50%
40	3550A/15	11YRS	MALE	submandibular node	INTERMEDIATE GRADE-DIFFUSE MIXED SMALL AND LARGE CELL	85%	3.80%
41	1341/10	57YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE MIXED SMALL AND LARGE CELL	35%	1.20%
42	681A2/14	10YRS	FEMALE	Mesentric node	INTERMEDIATE GRADE-DIFFUSE MIXED SMALL AND LARGE CELL	65%	4%
43	576/10	14YRS	MALE	supraclavicular node	INTERMEDIATE GRADE-DIFFUSE MIXED SMALL AND LARGE CELL	83%	0.40%
44	2408A/16	13YRS	MALE	Cervical node	INTERMEDIATE GRADE-DIFFUSE MIXED SMALL AND LARGE CELL	50%	2%
45	771C/14	10YRS	FEMALE	Cervical node	HIGH GRADE-BURKITT'S LIKE	85%	5.60%
46	2139/16	20YRS	MALE	supraclavicular node	HIGH GRADE -BURKITTS LIKE	90%	6.50%

47	1998/14	65YRS	MALE	Inguinal node	HIGH GRADE-BURKITT'S LIKE	95%	5.50%
48	1761/14	32YRS	FEMALE	Axillary node	HIGH GRADE -BURKITTS LIKE	90%	4.90%
49	1104A/14	65YRS	MALE	Inguinal node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	85%	1.70%
50	1567/14	47YRS	MALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	90%	5.10%
51	1148/14	4YRS	MALE	Cervical node	HIGH GRADE- LARGE CELL IMMUNOBLASTIC	95%	3.40%
52	71A2/15	12YRS	FEMALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	85%	4.10%
53	2111B/16	55YRS	FEMALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	90%	3.30%
54	478/16	63YRS	MALE	Cervical node	HIGH GRADE IMMUNOBLASTIC LARGE CELL	82%	4.60%
55	2928B/13	60YRS	FEMALE	Cervical node	HIGH GRADE LYMPHOBLASTIC LARGE CELL	84%	0.80%
56	119B/16	52YRS	MALE	Cervical node	HIGH GRADE LYMPHOBLASTIC LARGE CELL	85%	2.50%
57	980/14	53YRS	FEMALE	Cutaneous lymphoma	HIGH GRADE-CUTANEOUS T CELL LYMPHOMA	90%	0.60%