REVIEW OF THE SUBCLASSIFICATION OF LYMPH NODE BIOPSIES REPORTED AS REACTIVE LYMPHADENITIS AT KENYATTA NATIONAL HOSPITAL.

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STUDENT'S DECLARATION

This project is my original work under the guidance of my supervisors and has not presented for award of a degree or for any similar purpose in any other institution of learning

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DEDICATION

I dedicate this work to God Almighty, my parents, siblings and my little angel, Carrie.

ACKNOWLEDGEMENT

First, I would like to acknowledge the Almighty God for His abundant grace throughout this journey. I would like to thank my supervisors; Dr. Ndungu J.R and Dr. Nyagol J for their continuous support, encouragement and guidance throughout the study period.

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OPERATIONAL TERMS

WHO-ICD-10: It is a standard tool in medical records archiving for epidemiology, health management and clinical purposes.

REACTIVE LYMPHADENITIS: Lymph node enlargement due to hyperplasia of cellular components reflecting antigenic stimulation

LYMPHOMA: Diverse group of clonal (malignant) lymphoproliferative disorders

INDOLENT LYMPHOMA: Slow-growing lymphomas

LIST OF ABBREVIATIONS

AEC	3-Amino-9-Ethylcarbazole
AIDS	Acquired immunodeficiency syndrome
BSA	Bovine Serum Albumin
$CaCL_2$	Calcium Chloride
CD	Cluster of differentiation
CISH	Chromogenic *in Situ* Hybridization
EDTA	Ethylenediaminetetraacetic acid
EBV	Epstein Barr Virus
FISH	Fluorescence *in Situ* Hybridization
H/E	*Haematoxylin* and *eosin*
IHC	Immunohistochemistry
KNH	Kenyatta National Hospital
NSRH	Non-specific reactive hyperplasia
PBS	Phosphate buffer saline
PI	Principal Investigator
PTGC	Progressive Transformation of Germinal center
SPSS	Statistical Package for the Social Sciences
UON	University of Nairobi

ABSTRACT

Background: Lymphadenopathy is a common clinical finding in both adult and paediatric patients in Kenya. It has a broad differential diagnosis, the most common being nonspecific reactive lymphoid hyperplasia, a benign reversible enlargement of the lymph node which often disguises malignancy. It is known to be of unknown cause. Prevalence and morbidity of malignancies in early stages e.g. micro lymphoma in diagnoses of reactive lymphadenitis have been poorly investigated.

Objective: To evaluate the histopathologic patterns of lymph node biopsies reported as reactive lymphadenitis at Kenyatta National Hospital for the period 2013-2019.

Methods: The study utilized formalin fixed paraffin embedded tissue blocks of lymphadenopathy, previously reported only as reactive lymphadenitis on histopathology, in the department of human pathology at KNH. Consent to conduct the study was obtained from KNH/UoN ERC. The demographic and clinical data were retrieved from the records. *Haematoxylin* and *eosin* was performed to confirm the initial reported diagnosis of non-specific reactive hyperplasia. Further characterization was done through immunohistochemistry evaluation. Data was entered into excel spreadsheet, cleaned and analyzed using SPSS version 20 for statistical correlations.

Results: The study analyzed 86 cases, 64 (73%) which were adults and 22 (27%) paediatric cases. The age range was 1year 3months to 76 years. Out of the 86 cases, males were 49 (57%), while females were 37 (43%). Follicular hyperplasia 63 (73%), sinus expansion 16 (19%) and

progressive transformation of germinal center (PTGC) (1%) were the histopathological patterns of reactive lymphadenitis in the studied cases. Diffuse and mixed patterns were not found among the reviewed cases, and no cases of micro lymphomas were reported in the current study. However, 6(7%) cases were of notable pathology, which were confirmed using immunophenotyping to be DLBCL (1 case), a high grade round blue cell tumor of childhood (1 case), and 4 (3SLL and 1MCL) cases of lymphomas.

Discussion: Follicular hyperplasia was the most predominant sub-classification of cases reported as reactive lymphadenitis in KNH. Further studies to correlate histopathological patterns of reactive lymphadenitis with underlying cause are recommended. IHC is useful when morphologic features are equivocal. Minimum markers that would help improve lymphoma diagnosis in our set-up would include CD20, CD10, CD3, Ki-67, Bcl2, and Bcl6 to differentiate a reactive process from a lymphoma.

Conclusion: This study underscores the need for routine use of the various sub-classifications of reactive lymphadenitis in the diagnosis as they may suggest the underlying pathological condition

CHAPTER ONE: INTRODUCTION

1.1 Background

Lymph nodes are involved by a very wide range of pathologic conditions, and are therefore commonly biopsied for histologic analysis. Lymphadenopathy is the disease process of the lymph nodes that render them abnormal in size and consistency (1). Etiologies are multiple which include neoplastic diseases, infections and effects of drugs. Non-specific reactive hyperplasia is the leading cause of lymphadenopathy (2). It is defined as a benign reversible enlargement of the lymph node that results from the proliferation of part or all of its cellular component.

When lymphoma features in the differential diagnoses, analyses are often very technical requiring second opinion by a specialist haematopathologist. It is not surprising, therefore, that there is paucity of data on prevalence of micro lymphoma in an environment where ancillary testing and specialist's opinion are not readily available or affordable. Indeed, only a few Kenyan studies with the required corroboratory ancillary testing were found after an extensive search (3, 4, 5). There has also been a change in the epidemiology of lymph node diseases over the years. Lymphomas remain a leading cause of cancer mortality, especially with the advent of HIV – AIDS, making studies on it important in our environment (6).

Prevalence and morbidity of malignancies in early stages e.g. micro lymphoma in diagnoses of reactive lymphadenitis have been poorly investigated (7). Limited information is available in developing countries with most of the scientific data obtained from

developed countries. In a developing country such as Kenya, with the incessant problem of late presentation and referral, it is essential for the attending physician to have thorough knowledge of the demographic pattern of reactive lymphadenitis. This will enhance prompt diagnosis and institution of definitive treatment protocols.

It is on the basis of this background information that the present study sought to give an update of the morphologic spectrum of reactive lymphadenitis seen in our facility as well as to determine the prevalence of micro lymphoma.

1.2 Statement of the Problem

Reactive lymphadenopathies are known to be of unknown cause. Prevalence and morbidity of early stage lymphomas and their association with nonspecific reactive lymphadenitis is unknown in our set up. Distinguishing a neoplastic from a reactive lymph node requires the application of histologic criterion that involves the evaluation of cytologic features (10). This includes evaluating for the presence or absence of cytological atypia, as well as cellular polymorphism and monomorphism (11). These criteria, however valid, are not perfect and exceptions exist that may result in diagnostic uncertainty (12). Further, the interpretation of a reactive process in a lymph node is based on a systematic approach and on the dominant histologic architectural pattern. These patterns include whether the nodal architecture is predominantly follicular, sinusoidal, diffuse and mixed interfollicular (13). This assigns subjectivity to the diagnosis of reactive lymphadenitis that needs to be evaluated (14). Difficulties also arise because benign reactive lymphadenopathies may coexist with extra nodal lymphomas and these lymphomas may be focal (15). WHO recommends the use of immunophenotyping

through immunohistochemistry (IHC) and molecular techniques in addition to cytomorphological features to distinguish a reactive from a neoplastic lymph node (16). These techniques, IHC and molecular studies are not routinely done in our set up.

1.3 Justification of the Study

Reactive lymphadenitis presents with increasing diagnostic and therapeutic challenges. It is the most common cause of lymphadenopathy (17), which may disguise malignancy and lead to misdiagnosis. Proportion of lymphomas among cases reported as reactive lymphadenitis in our set up, is not known. In a study done in the US among the paediatric age group with a population size of 75, 55% of lymphadenopathies were diagnosed as reactive lymphadenitis. Out of these cases of reactive lymphadenitis, 17% of were ultimately proved to have a specific pathologic process (18). A case is reported of reactive lymphadenopathy that was subsequently diagnosed as lymphoma nine years later (19) . The paucity of literature on how to effectively diagnose this condition and the impact in our society justifies this study.

1.4 Research Questions

What are the histopathologic patterns of cases of lymph node biopsies reported as reactive lymphadenitis at Kenyatta National Hospital?

What is the prevalence of micro lymphomas in reactive lymphadenitis among the cases reported as reactive lymphadenitis at Kenyatta National Hospital?

Hypothesis

Follicular hyperplasia is the predominant histolopathologic pattern in lymph node biopsies reported as reactive lymphadenitis at Kenyatta National Hospital and there is a low prevalence of micro lymphoma among these cases.

1.5 Objectives

1.5.1 Broad Objective

To evaluate the histopathologic patterns of lymph node biopsies reported as reactive lymphadenitis at Kenyatta National Hospital.

1.5.2 Specific Objectives

1. To sub classify reactive lymphadenitis (according to histomorphology) as reported in the department of Anatomic Pathology, KNH.
2. To perform immunohistochemistry on cases suspicious of lymphomas, and correlate with known molecular features.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction and Background

Lymphadenopathy is a common clinical finding, with a broad differential diagnosis. The most common cause is nonspecific reactive lymphoid hyperplasia, which is defined as a benign reversible enlargement of the lymph node (20). This results from the proliferation of part or all of its cellular components. Non specific reactive hyperplasia is the leading cause of lymphadenopathy Higher incidence is found in the inguinal region as compared to the cervical region ((21, 22). Prevalence and morbidity of malignancies in early stages e.g. micro lymphoma in diagnoses of reactive lymphadenitis have been poorly investigated.

In a study done by Oski *et al* in the US, 55% of lymphadenopathies were diagnosed as reactive lymphadenitis in a population size of 75.Out of this group of 55%, 17% were ultimately proved to have a specific pathologic process (18, 23).

A variety of unusual reactive conditions may be seen in lymph nodes from the pediatric age which are of unknown cause (24).

2.2 Lymph Node Structure and Function

An understanding of the normal lymph node structure and function aids in distinguishing a reactive from a neoplastic lymph node. The lymph node is a small bean-shaped nodular structure made up of lymphoid tissue that filters circulating lymph and participates in

immune reactions as part of the immune system (25, 26). It is a highly organized structure composed of the cortex, paracortex, medulla, and sinuses.

The cortex lies within the periphery of the lymph node beneath the lymph node capsule (27). It is densely populated by B cells and organized into nodular structures called follicles (28).

Depending on the antigen stimulation state, follicles may be designated as either primary or secondary. Primary follicles are composed of antigen-naive, small mature B cells(29). Secondary follicles form when primary follicles react to antigen stimulation (30). They are characterized by a peripheral mantle zone of small mature B cells surrounding a central germinal center made up of centroblasts and centrocytes which segregate into polarized dark and light zones, respectively, with admixed tingible body macrophages and follicular dendritic cells (31).

The paracortex is a T-cell–rich area that lies between B cell follicles extending deep into the cortex (32). The paracortex consists predominantly of small mature T cells and variable numbers of large transformed immunoblasts (T cells or B cells), interdigitating dendritic cells, plasmacytoid dendritic cells, and high endothelial venules (27).

The medulla is the central region of the lymph node, lying adjacent to the lymph node hilum. It is arranged into cords that surround the medullary sinuses containing a mixture of small B and T lymphocytes, plasmacytoid lymphocytes, plasma blasts, and mature plasma cells (33).

The sinuses are endothelium-lined vessels that traverse the lymph node and carry lymph through the node parenchyma. Lymph carried in the afferent lymphatic vessel enters the node through the subcapsular sinus, traversing the node through the intermediary and medullary sinuses finally emptying into the efferent lymphatic vessel. Cells seen in lymphatic sinuses include histiocytes, lymphocytes, plasma cells, and granulocytes (34, 35).

Reactive changes in lymph nodes are routinely classified into histologic patterns based on the aforementioned anatomic compartments prominently affected: follicular/nodular, which affects the cortex; interfollicular/paracortical, which affects the paracortex; sinusoidal, which affects the sinuses throughout the lymph node; and diffuse, which affects 2 or more of the anatomic compartments (13).

2.3 Follicular Hyperplasia

Follicular hyperplasia is the most common reactive change encountered in the lymph node. It is a nonspecific finding of unknown etiology and is seen in the setting of specific etiologies that have unique associated histopathologic findings, including infection (e.g., human immunodeficiency virus, Epstein-Barr virus, toxoplasmosis, syphilis), autoimmune disease (rheumatoid arthritis, systemic lupus erythematosus), Castleman's disease, or immunoglobulin G4 (IgG4)–related disease (36).

Follicular hyperplasia is characterized by an expansion of the cortex due to an increase in both the number and size of secondary follicles. Occasional extension into the paracortex and medulla can be found. Importantly, follicular hyperplasia does not efface normal lymph node architecture, with the other lymph node compartments, although relatively diminished in size, still recognizable.

Reactive follicles may show variability in size and shape but typically exhibit polarized germinal centers that are made up of centroblasts and centrocytes with admixed tingible body macrophages and small mature lymphocytes, which are cuffed by well-formed and defined mantle zone (37).

Immunohistochemical stains show mostly lymphocytes in reactive follicles, including centroblasts, centrocytes, and mantle zone cells which are CD20 positive B cells. Germinal center B cells are positive for CD10 and BCL6 and negative for the antiapoptosis protein BCL2, (38)while mantle zone B cells are positive for IgD and BCL2 and lack expression of CD10 and BCL6. Reactive germinal centers contain scattered small CD3 positive follicular helper T cells that coexpress CD4, CD57, PD1, and BCL2. These T cells are more prominent in the germinal center light zone. CD21 highlights well-formed mesh works of follicular dendritic cells throughout follicles (31).

Follicular lymphoma and mantle cell lymphoma share histologic features with follicular hyperplasia and may be misdiagnosed as such (39, 40, 41).

2.4 Progressive Transformation of Germinal Centers

Progressive Transformation of Germinal Centers is a less frequent but still commonly encountered reactive change as compared to follicular hyperplasia (42). It presents with localized lymphadenopathy and no obvious signs of illness (43). It can rarely, be part of a systemic process with multiple lymph node groups involved. It affects men more often than women and young adults more frequently than children or the elderly.

Histologically, it is seen as a localized change in a background of follicular hyperplasia but can occasionally be seen as a generalized or florid process. Progressive transformation of germinal centers is characterized by expansion of the cortex by large macro nodules of enlarged secondary follicles with expanded mantle zones. These show progressive and multifocal inward migration of mantle zone cells into the germinal center, leading to germinal center disruption and eventual obliteration (37).

In the late stages of progression, the transformed germinal centers consist almost entirely of small mantle zone cells with scattered centroblasts and centrocytes visible. Immunohistochemical stains show the small mantle zone cells are CD20 positive B cells, positive for IgD and BCL2, and negative for CD10 and BCL6 (44). Residual germinal centers B cells are positive for CD10 and BCL6, and negative for BCL2 (45). CD57 PD1follicular helper T cells are also present and are evenly distributed throughout the germinal center, which is in contrast to the polarized distribution seen in the germinal centers of hyperplastic follicles. T-cell rosettes are not typically seen in PTGC. CD21 highlights expanded follicular dendritic cell mesh works that show disruption in the later stages of progression (31).

Progressive transformation of germinal centers has an unknown etiology. It is a benign entity that has a known association with nodular lymphocyte–predominant Hodgkin lymphoma (NLPHL) in a minority of cases, either as disease occurring before or after the discovery of PTGC or as concomitant disease (47).

Thus, when PTGC is identified in a lymph node it is important to go back to the gross pathology laboratory and submit the entire lymph node for comprehensive histologic evaluation to exclude focal involvement by lymphoma. The surgical pathologist should bring the known association of lymphoma with PTGC to the attention of the referring physician and recommend biopsy of any other enlarged lymph nodes and close clinical follow-up if there is a clinical suspicion of malignancy.

Further, lymphocyte-rich classical Hodgkin lymphoma shares overlapping features with PTGC and NLPHL and risks being under diagnosed and inadequately treated without adequate evaluation (48).

Benign monocytoid B-cells are closely related to marginal zone B-lymphocytes. They show cytological diversity and may be recognized in reactive lymph node conditions as large lymphoid cells (49).

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Study Area

This study was conducted at KNH, the largest referral hospital in Kenya. It has a bed capacity of about 1800 and runs 22 outpatient clinics. The areas of interest in this study were the pathology register and the histopathology laboratory at the department of Pathology where retrieval of clinical data and tissue blocks from the archives were done respectively.

3.2 Study Population

All cases reported as reactive lymphadenitis on histopathology at KNH laboratory were obtained from the pathology register, department of Pathology.

3.3 Study design

This was a descriptive cross-sectional study reviewing the sub classification of lymph node biopsies reported as reactive lymphadenitis at Kenyatta National Hospital for the period 2013 to 2019.

3.3.1 Inclusion criteria:

Histopathologic diagnosis of reactive lymphadenitis

Formalin fixed, paraffin embedded and well preserved tissue blocks

3.3.2 Exclusion criteria:

Confirmed histopathologic diagnosis of primary or recurrent lymphomas

Poorly preserved formalin fixed, paraffin embedded blocks

Lost or missing tissue blocks

3.4 Sampling Method

Census sampling was used. The material for this study was obtained from the surgical pathology records and the blocks were retrieved from the archives. All the cases reported as reactive lymphadenitis for the period 2013 to 2019 meeting the inclusion criteria were reviewed.

3.4.1 Sample size determination

The total number of cases reported as reactive lymphadenitis at KNH for the period 2013 to 2019 was 100 (based on a pilot study conducted by the PI). Using census sampling, sample size was 100.

3.5 Materials and Methods

All cases of lymph node biopsies reported as reactive lymphadenitis at the department of Human Pathology, Kenyatta National Hospital, Kenya, from January 2013 to December 2019 was retrospectively reviewed.

3.5.1 *Demographic and clinical data*

These were extracted from the patients' records. This included the patient's age, anatomical site, duration of lymphadenopathy, preliminary and final diagnoses. Previous histopathological diagnoses were obtained from the histology register. Formalin fixed paraffin embedded tissue blocks of cases reported as reactive lymphadenitis were retrieved from the archives once the data was scrutinized and verified by the principal investigator.

3.5.2 *Haematoxylin and eosin*

*Haematoxyli*n and *eosin* was done for each case to corroborate the initial reported diagnosis of reactive lymphadenitis. Two research assistants, trained medical laboratory technologists with experience in histopathology, aided the principal investigator in processing of the tissue blocks to obtain slides as well as in *Haematoxylin* and *eosin* staining.

Deparaffinization of the slides was done by use of xylene. Hydration was achieved by passing the slides through several changes of alcohol to remove xylene. They were then thoroughly rinsed in water and hydrated. *Haematoxylin* nuclear stain was applied. Differentiation was done to improve contrast. *Eosin* counterstain was applied. Following the *eosi*n stain, the slides were passed through several changes of alcohol to remove all traces of water. They were then rinsed in several baths of xylene and mounted.

This was followed by immunohistochemistry which was used to characterize non-specific reactive hyperplasia by immunophenotyping (cell markers e.g. CD 10, CD 20) and where necessary (inconclusive IHC) molecular analyses to further characterize and sub-classify non-specific reactive lymphadenitis. Diagnoses other than non-specific reactive hyperplasia obtained on *Haematoxylin* and *eosin* (verified histology register of non-specific reactive hyperplasia) was subjected to immunohistochemistry by the principal investigator.

The initial readings of nonspecific reactive hyperplasia were done by the surgical pathologists at the department of Anatomic Pathology, KNH. The reviewers included the principal investigator and independent expert pathologists in lymphoma. The principal investigator and two independent expert pathologists in lymphoma on review of the demographic and clinical data, morphologic findings on *Haematoxylin* and *eosin* and IHC findings where applicable, reached concordance.

3.5.3 *Immunophenotyping*

IHC was performed as described by Russo et al (51). Slide preparation was done by deparaffinization and rehydration. The slides were incubated in a 56-60 °C oven for 15minutes and subsequently to a xylene bath and two changes of xylene for 5min each was done.

The slides were rehydrated in two changes of fresh absolute ethanol for 3min followed by 90% and 80% alcohol respectively for 3mins each. Antigen retrieval was done using protease treatment. All the slides were incubated with the respective primary antibodies for 1 hour at room temperature, rinsed with TBST and then incubated with secondary antibody (ABC kit DAKO Cytomation) for another 30 minutes. The sections were rinsed and streptavidin peroxidase was applied, followed by incubation for another 30 minutes. The sections were rinsed with TBST and then stained with DAB for 7 minutes. The sections were then rinsed again, counter-stained with Meyers' *haematoxylin* and mounted. The test was done for CD3, CD5, CD10, CD20, CD23, CD45, CD99, S100, MUM1, BCL2, BCL6, Ki-67, CKAE1, Synaptophysin, Desmin, Cyclin D1. All the dilutions were made at 1:50. The stains were independently assessed by two experienced pathologists as mean percentages of positive cells expressing each parameter. In each case, complete cell counts were evaluated in five different fields at 40X and positive cells were expressed as mean percentages of the total cells in the fields. The inter-observer correlation coefficient was more than 90%.

Table 1: Panel of antibodies used for immunohistochemistry

Antibody	Clone	Source
Ki67	SP6	Roche Diagnostic
CD3	SP162	Roche Diagnostic
CD5	SP19	Roche Diagnostic
CD10	SP177	Roche Diagnostic
CD20	SP32	Roche Diagnostic
CD 23	SP163	Roche Diagnostic
CD45	H130	Roche Diagnostic
CD 99	DN16	Roche Diagnostic
S100	SP127	Roche Diagnostic
MUM1	SP114	Roche Diagnostic
BCL2	SP124	Roche Diagnostic
BCL6	SP155	Roche Diagnostic
CKAE1	RM126	Roche Diagnostic
Synaptophysin	SP15	Roche Diagnostic
Desmin	SP138	Roche Diagnostic
Cyclin D1	SP4	Roche Diagnostic

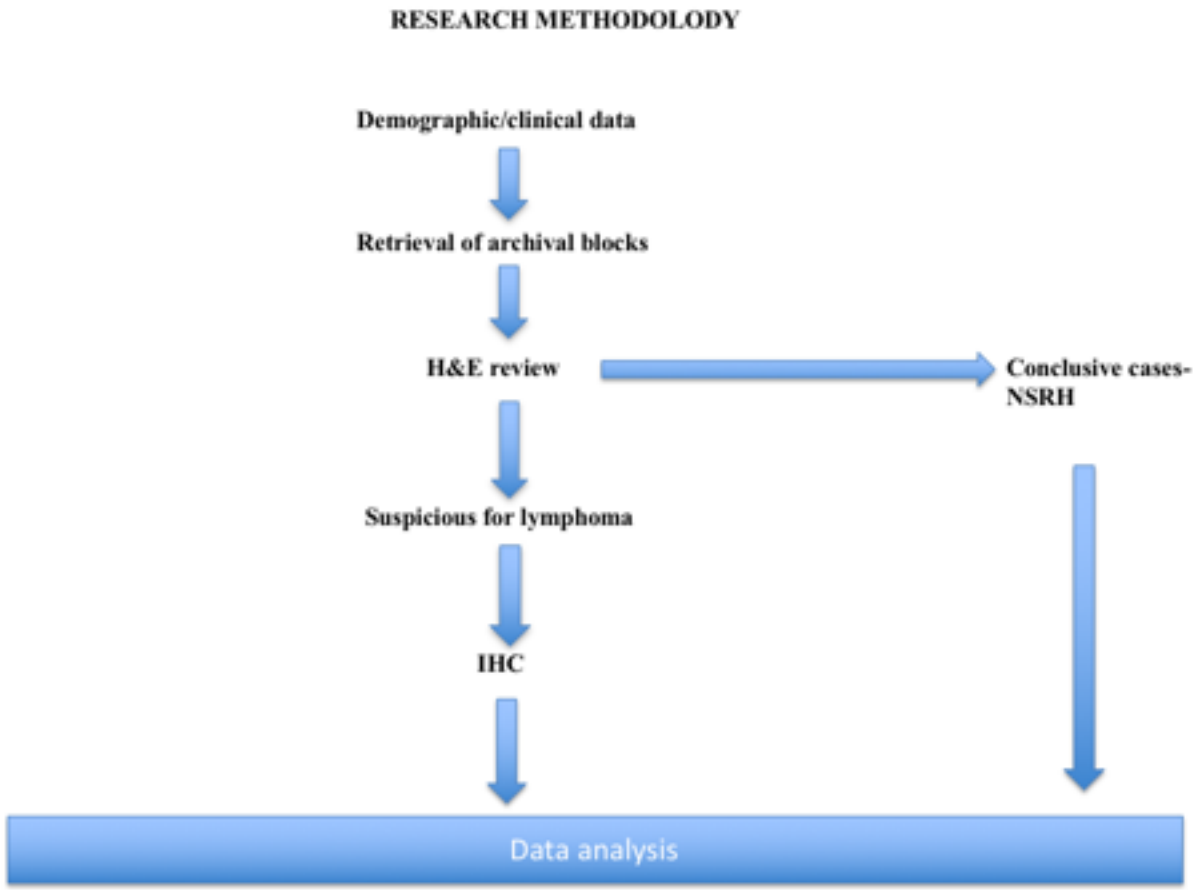

Figure 1 : Flowchart

3.5.1 Flowchart

Demographic and clinical data was obtained from the patients' records. This was followed by retrieval of paraffin embedded tissue blocks from the archives. These were processed to obtain slides that were reviewed on *Haematoxylin* and eosin and immunohistochemistry where applicable. Immunohistochemistry was conclusive in all cases and therefore molecular techniques were not applied.

3.5.2 Quality Assurance

Standard operating procedures were adhered to as well as personal protective equipment worn during sample collection, processing and analysis. This included gloves, lab coats. Rescreening by independent expert pathologist in lymphoma was done.

Epi-info was used for data entry, which was designed to resemble the data collection form. This ensured that the correct data was entered and any mistakes easily identified.

3.6 Ethical Considerations

Ethical approval was obtained from KNH/UON-Ethics Review Committee and permission from KNH.

Participants' and pathologists' details were de-identified during data collection by only assigning a unique number and the records kept confidential.

No data obtained from this study was used for any purpose other than meeting the objectives stated.

3.7 Data Management

Data was entered into the data collection tool. This included age, gender, site as well as duration of lymphadenopathy and diagnosis of nonspecific reactive hyperplasia.

3.7.1 Data processing

Data was processed using Epi Info, which is an access based programme that allows for correction of captured data as well as recheck of data.

3.7.2 Data analysis

The data format in Epi-info was designed in the same format as the data collection tool, simplifying entry. This ensured complete and accurate data for analysis. The data was extracted and converted from an access baseformat into an excel format.

Data in the excel spreadsheet was analyzed using SPSS version 20. Data frequencies were generated and represented in the form of figures and tables. Measures of central tendency were generated for continuous variables of interest such as age. A Shapiro-Wilk normality test was performed on all continuous variables to determine the distribution pattern of the data, which in turn determined the method to be used during analysis, parametric or non-parametric tests. These statistical tests were performed and set at 95% confidence levels.

CHAPTER FOUR: RESULTS

In the period under review, 2013 to 2019, a total of 100 cases were seen at KNH coded as reactive lymphadenitis as per the WHO: ICD-10 coding system. Out of these, 86 cases were included into the study. The cases excluded were; 10 missing/lost blocks and 4 cases that were not actually reactive lymphadenitis.

Out of the 86 cases, males were 49 (57%) and females. 37 (43%). The age range was 1 year 3months to 76 years, while the median age was 30years with an interquartile range of 16-47 years. Table 1 below demonstrates the study population characteristics.

STUDY POPULATION CHARACTERISTICS

GENDER	n %(n=86)
MALE	49(57%)
FEMALE	37(43%)
TOTAL	**86(100%)**

Table 2: Study Population Characteristics

Distribution of Reactive Lymphadenitis based on site.

There was a higher frequency of clinical presentation of reactive lymphadenitis in the cervical region of the neck (52%) than the inguinal region (9%)

DISTRIBUTION OF REACTIVE LYMPHADENITIS BASED ON SITE

Site	n %(n=86)
Cervical(neck)	45(52%)
Mesenteric	10(12%)
Inguinal	8(9%)
Axillary	7(8%)
Others	16(19%)
Total	**86(100%)**

Table 3: Distribution of Reactive Lymphadenitis Based on Site

Follicular hyperplasia was found to be the predominant histopathological pattern 63(73%) followed by sinusoidal 16(19%) and PTGC 1(1%). More females than males presented with the follicular pattern of reactive lymphadenitis (78% vs 65%) p=0.186. Only males presented with the sinusoidal pattern.

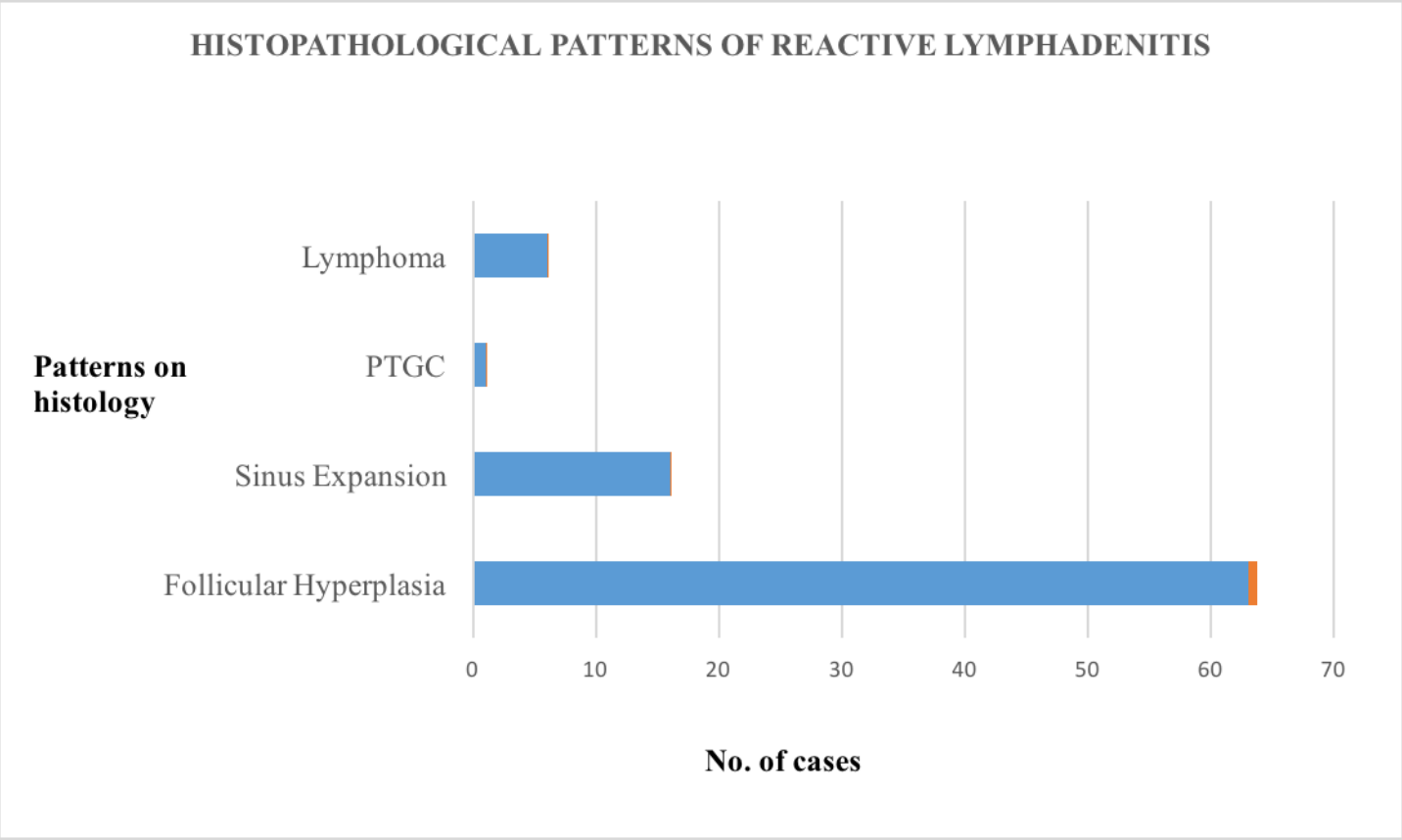

Figure 2: Histopathological patterns of Reactive Lymphadenitis

Social-Demographic features

The study population comprised of 49 males (57%) and 37 females (43%).

The age range was 1year 3months-76years.The median age was 30years with the Interquartile range at 16-47years.

The study analyzed 86 cases, 64 (73%) which were adults and 22 (27%) paediatric cases. The age range was 1year 3months to 76 years. Out of the 86 cases, males were 49 (57%), while females were 37 (43%). Follicular hyperplasia 63 (73%), sinus expansion 16 (19%) and progressive transformation of germinal center (PTGC) (1%) were the histopathological patterns of reactive lymphadenitis in the studied cases. Diffuse and mixed patterns were not found among the reviewed cases, and no cases of micro lymphomas were reported in the current study. However, 6 cases (7%) were of notable pathology, which were confirmed using immunophenotyping to be DLBCL (1 case), a high grade round blue cell tumor of childhood (1 case), and 4 (3SLL and 1MCL) cases of lymphomas.

HISTOPATHOLOGICAL PATTERN BASED ON GENDER

HISTOPATHOLOGICAL PATTERN	MALE	FEMALE	n%(n=86)
FOLLICULAR HYPERPLASIA	32(65%)	29(78%)	61(71%)
SINUS EXPANSION	16	-	16(19%)
PTGC	-	1	1(1%)
LYMPHOMA	1	5	6(7%)
TOTAL	**49**	**37**	**86(100%)**

Table 4: Histopathological Patterns of Reactive Lymphadenitis Based on gender

Reactive lymphadenitis is characterized by proliferation of cellular components of the lymph node and preservation of the general architecture as shown in Figure 3 below:

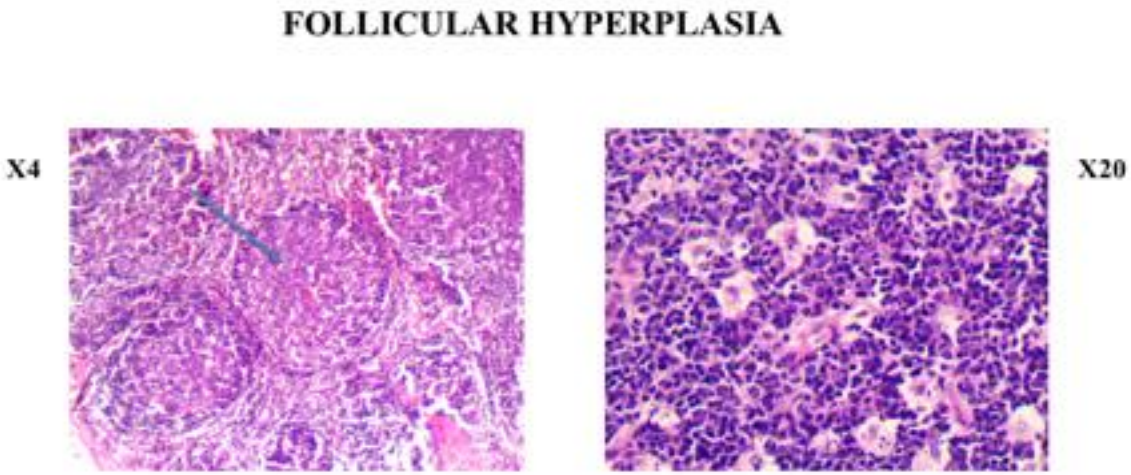

Figure 3: Reactive Lymphadenitis, Follicular Pattern

Arrows indicate follicles. Nodal architecture is preserved with no capsular infiltration. Follicles vary in size and shape and are composed of centrocytes and centroblasts. Tingible body macrophages are common

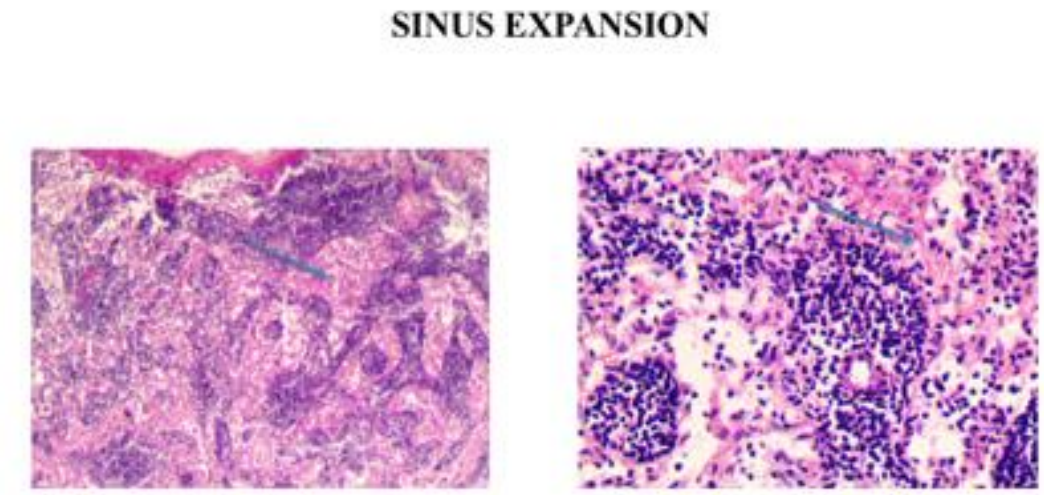

Figure 4: Reactive Lymphadenitis, Sinus Expansion pattern

Arrow indicates sinus expanding to the capsule. Sinuses are prominent and are lined by hyperplastic sinus histiocytes.

One case of Diffuse Large B Cell Lymphoma was diagnosed based on morphological features and IHC as shown in Figure 5 below.

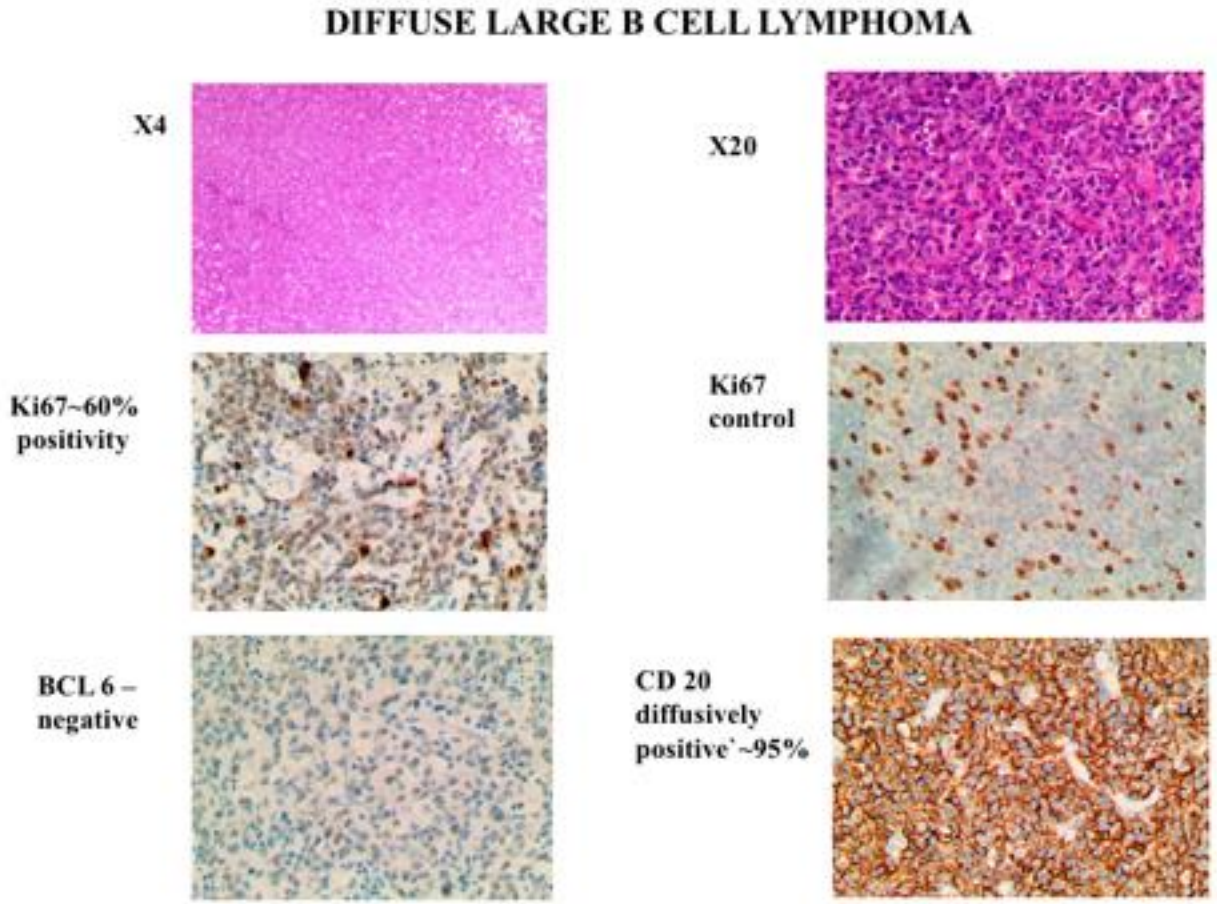

Figure 5: DLBCL exhibiting diffuse growth pattern with large cells. IHC confirms DLBCL of the Activated B cell type, CD 10 positive, MUM1 negative, BCL 6 negative, CD 20 positive, Ki67~80% positivity.

An example of an indolent lymphoma, Small Lymphocytic Lymphoma is shown in Figure 6 and IHC confirmation

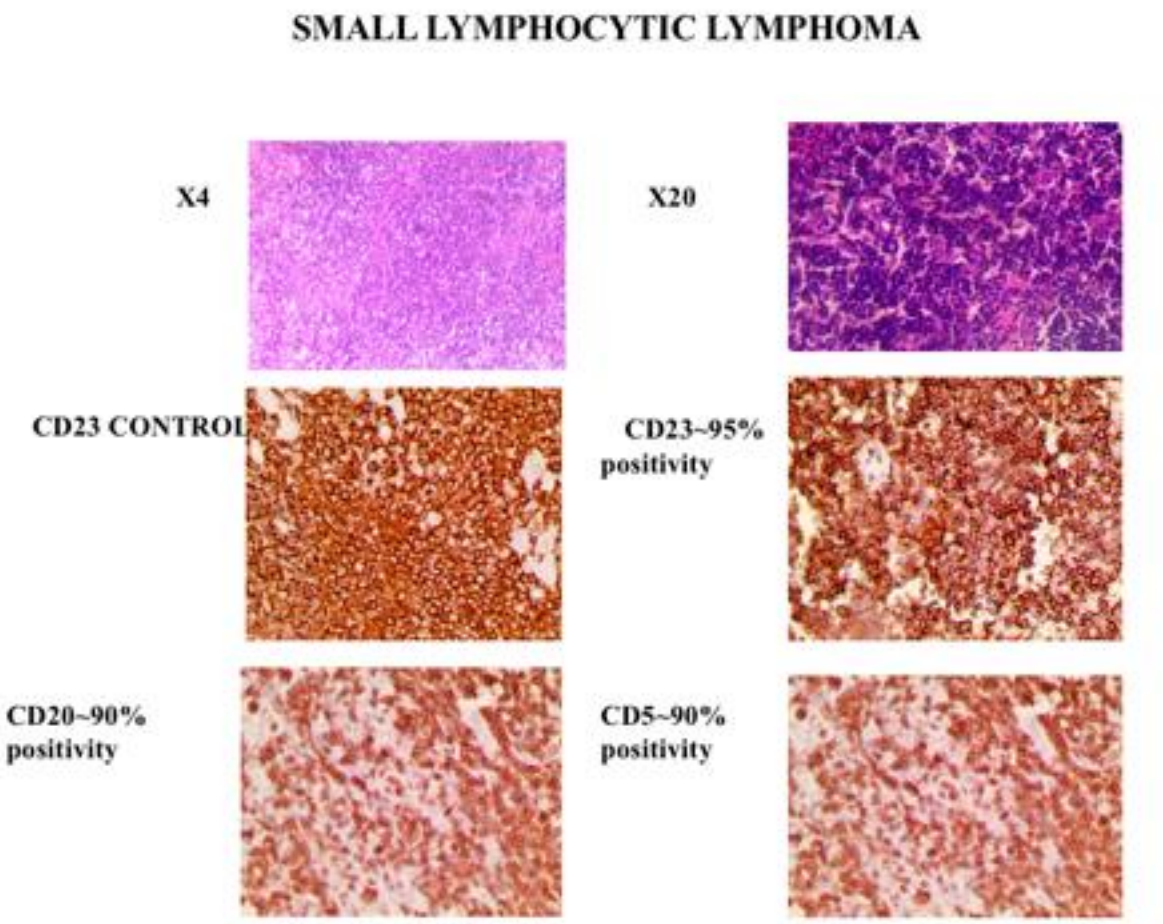

Figure 6: SLL on H/E characterized by diffuse effacement of parenchyma by small mature lymphocytes. IHC confirming SLL-CD 5-positive, CD20-positive, CD23-positive.

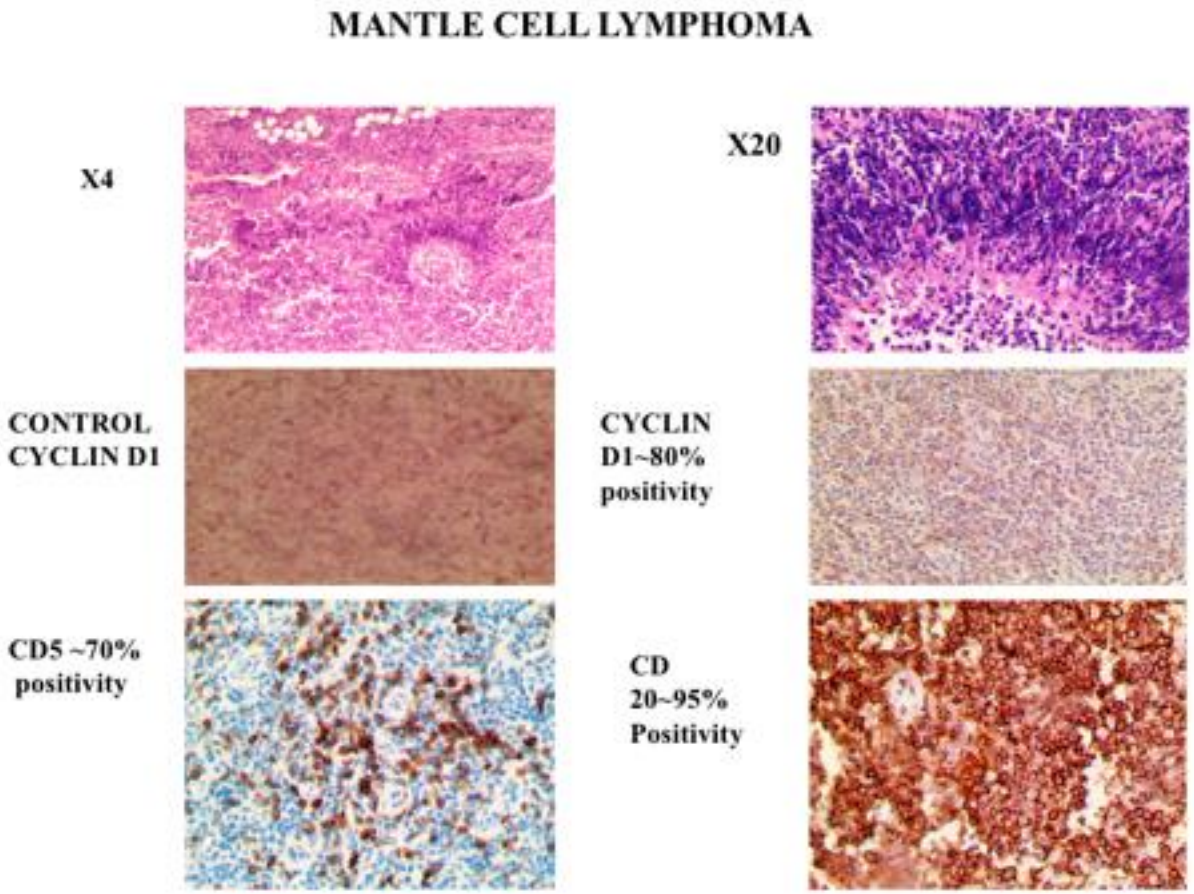

Figure 7: Mantle Cell Lymphoma on H/E at (X4) and at (X20). IHC positive for MCL-Cyclin D1, CD5, CD10, CD20

CHAPTER FIVE: DISCUSSION.

This study reviewed the morphologic spectrum of cases reported as reactive lymphadenitis at KNH.A total of 86 cases were reviewed in a 7-year period (2013 to 2019) with 14 missing files.

The study population comprised of 49 males (57%) and 37 females (43%). Of the 86 cases analyzed, 64 cases were of the adult population (73%) while 22 cases were of the paediatric population (27%). This age distribution is similar to studies done in Nigeria (21, 22). This can be attributed to the generally young population in our set-up and in Africa.

There was a higher frequency of clinical presentation of reactive lymphadenitis in the cervical region of the neck 45(52%). This is consistent with studies done in South Sudan where cervical nodal presentation was at 69.4% in a study population of 80 (1). Studies done in Nigeria had similar findings at 46% in a population of 275 (4). This may be related to its location near a common primary site of infection and the fact that these nodes drain the upper aero-digestive tract through which foreign antigens enter the body via ingestion or inhalation.

The most frequent encountered pattern of reactive lymphadenitis was follicular hyperplasia 63(73%). This finding is similar to a study done in Saudi Arabia that found follicular hyperplasia as the predominant pattern of reactive lymphadenitis at 60% in a study population of 289 (17). This finding is also consistent with studies done in South Africa, where it was found to be at 68% in a population of 560 (36). Similar studies done in Nigeria found follicular hyperplasia to predominate at 66% in a study of 275 (4). The findings in this study though are much higher than in most other places. This is not surprising as a wide variety of conditions such as infection, drugs, chemicals, environmental pollutants, and even malignancy are associated with this pattern. Infections and

autoimmune conditions associated with this pattern are common in our set up and could explain these findings.

Sinus hyperplasia was found to be the second most common pattern of reactive lymphadenitis, similar to studies done in SA and Nigeria (36, 21). This is seen in relation with monocytoid B-cell hyperplasia, hemophagocytic syndromes, Whipple's disease, or lymph nodes draining sites of prostheses or malignancy.

PTGC was found to be the least common histopathological pattern of reactive lymphadenitis. This is similar to studies done in the US where it was found to be at 2% in a population of 75 (3). This is also consistent with studies done in Nigeria where it was found to be at 1% in a study population 191 (20). The findings in this study are comparable to those in other places. This could be because it is part of the spectrum of reactive follicular hyperplasia and is possibly, the ultimate fate of a follicular center in response to antigen.

There were no cases of micro lymphoma found in cases reported as reactive lymphadenitis at KNH. This can be attributed to health seeking behavior in our set-up which is commonly late presentation in disease.

Lymphoma was found in 6 cases (7%) reported as reactive lymphadenitis. Studies in the US and SA found lymphoma in 17% of cases reported as reactive lymphadenitis while in Nigeria it was found at 6% in a population of 235 (18, 22, 36). This can be attributed to advanced healthcare systems and early health seeking behavior patterns in the West as compared to that in our set-up and also to earlier diagnosis of malignancies before onset of nodal metastases.

CONCLUSION

From the study findings, Follicular hyperplasia, Sinus expansion and PTGC were the histopathological patterns of reactive lymphadenitis at KNH. In addition, diffuse and mixed patterns were not found in cases reported as reactive lymphadenitis at KNH. There were no cases of micro lymphoma in cases reported as reactive lymphadenitis at KNH. There was however, lymphomas in 7% of cases reviewed.

RECOMMENDATIONS

There is need for routine use of the various sub-classifications of reactive lymphadenitis in diagnosis, as they suggest the underlying cause. However, further studies to correlate histopathological patterns of reactive lymphadenitis with underlying cause is recommended. IHC is useful when morphologic features are equivocal. Minimum markers that would help improve lymphoma diagnosis in our set-up would include CD20, CD10, CD3, Ki-67, Bcl2, and Bcl6. This will aid in differentiating a reactive process from a lymphoma and avoid the pitfalls of misdiagnosis.

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CHAPTER SIX: APPENDICES

6.1 DATA COLLECTION TOOL

Case No.	Gender	Age	Clinical data(site and duration lymphadenopathy)	Presumed Diagnosis	New Diagnosis	Disparity

6.2 HAEMATOXYLIN AND EOSIN STAINING PROCEDURE

The H&E stain provides a comprehensive picture of the histology of organs and tissues.

Hematoxylin precisely stains nuclear components, including heterochromatin and nucleoli, while eosin stains cytoplasmic components including collagen and elastic fibers, muscle fibers and red blood cells. In a high quality H&E, subtle differences in the shades of color produced by the stains, particularly eosin, preside which aids in the detection and interpretation of morphological changes associated with disease.

Steps in performing an H&E stain are as follows:

1. Wax removal

Following a paraffin section preparation, all the elements are infiltrated with and surrounded by paraffin wax, which is a hydrophobic agent impervious to aqueous reagents. The majority of cell and tissue components having no natural color, are not visible. The first step in performing an H&E stain is to dissolve all the wax away with xylene (a hydrocarbon solvent).

2. Hydration

After thorough de-waxing, the slide is passed through several changes of alcohol to remove the Xylene. It is then thoroughly rinsed in water and hydrated so that aqueous reagents will readily penetrate the cells and tissue elements.

3. Hematoxylin Nuclear Stain application

The slide is stained with a nuclear stain such as Harris hematoxylin, which consists of a dye (oxidized hematoxylin or hematein) and a mordant or binding agent (an aluminium salt) in solution. This initially stains the nuclei reddish-purple color.

4. Nuclear Stain by completion

After rinsing in tap water, the section is “blued” by treatment with a weakly alkaline solution.

This step converts the hematoxylin to a dark blue color. The section is rinsed and checked to see if the nuclei are properly stained, showing adequate contrast and to assess the level of background stain.

5. Differentiation

When Harris hematoxylin is employed, a differentiation (de-staining) step is required to remove non-specific background staining as well as to improve contrast. A weak acid alcohol is used. After this, blueing and thorough rinsing is required.

6. Eosin Counterstain

The section is stained with an aqueous or alcoholic solution of eosin. This colors the different non-nuclear elements in different shades of pink.

7. Rinse, Dehydrate, Clear and Mount (Apply Cover Glass)

Following the eosin stain, the slide is passed through several changes of alcohol to remove all traces of water. It is then rinsed in several baths of xylene which “clears” the tissue and renders it

completely transparent. A thin layer of polystyrene mountant is applied, followed by a glass

cover slip. With proper performance of these steps, the slide will reveal all the important microscopic components. It will also be stable for many years.

6.3 IMMUNOHISTOCHEMISTRY PROCEDURE

Immunohistochemical staining is a valuable tool for detecting specific antigens in tissues. To perform the standard staining procedure, the tissue section has to be deparaffinized and rehydrated before applying the primary antibody. Enzyme-conjugated secondary antibodies are then applied and the specific staining can be visualized after adding the enzyme-specific substrate. Occasionally, antigen "unmasking" by enzyme digestion may be used when weak or no staining is observed.

The procedure below describes the application of peroxidase or alkaline phosphatase conjugates in the immunohistochemical labeling of formalin-fixed, paraffin-embedded tissue sections.

Reagents and Equipment

1. Formalin-fixed, paraffin-embedded tissue sections
2. 0.01 M phosphate buffered saline, pH 7.4 (PBS)
3. Bovine serum albumin (BSA)
4. Diluent: 1% BSA in PBS
5. Xylene
6. Ethanol absolute
7. 0.1% Trypsin in PBS or Trypsin tablet in 4mM $CaCl_2$, 200 mm Tris, pH 7.7, or 0.1% Protease in PBS.
8. Microwave antigen retrieval solution: 10 mM sodium citrate buffer pH 6.0, 1 mM EDTA pH 8.0
9. Primary antibody
10. **Option 1:** Biotinylated secondary antibody and ExtrAvidin-Peroxidase or ExtrAvidin-Alkaline Phosphatase or **Option 2:** Secondary antibody conjugated to peroxidase or alkaline phosphatase
11. When using peroxidase-conjugated secondary antibodies or ExtrAvidin-Peroxidase: 3% hydrogen peroxide.
12. Enzyme substrate: depends on the enzyme and color needed .

13. For peroxidase: AEC Staining Kit
14. For alkaline phosphatase: Fast Red /Naphtol
15. Mayer's hematoxylin
16. Coplin staining jars
17. Microwave
18. Microscope

Procedure

Slide preparation

Primary Antibody Reaction

Secondary Antibody Reaction

Substrate preparation

Development

Counterstaining

1. **Slide Preparation**

Deparaffinization and Rehydration

1. Place the slides in a 56-60 °C oven for 15 min. (Oven temperature not to exceed 60 °C).
2. Transfer to a xylene bath and perform two changes of xylene for 5 min. each.
3. Shake off excess liquid and rehydrate slides in two changes of fresh absolute ethanol for 3 min. each.
4. Shake off excess liquid and place slides in fresh 90% ethanol for 3 min.
5. Shake off excess liquid and place slides in fresh 80% ethanol for 3 min.
6. Rinse the slides in gently running tap water for 30 seconds (avoid direct jets which may wash off or loosen the section).

7. Place in PBS wash bath for further rehydration (30 min. at room temperature)

Antigen Retrieval - Unmasking of Antigen

This step is performed in cases where weak or no staining occurs, or for antigens requiring

"unmasking" according to the primary antibody specifications

Enzyme retrieval:

1. Apply 0.1% trypsin in PBS or 0.1% protease in PBS for 2-30 min. at 37 °C. Extending

   incubation time may enhance specific staining. Rinse in PBS for 10 min.

Microwave retrieval:

1. Wash the slides with deionized H_2O and place them in a microwave-resistant plastic staining jar containing antigen retrieval solution. Slides are fully covered with solution.
2. Operate the microwave oven for 5 min. on high power (~700 watts).
3. This process can be repeated 2-3 times.
4. Cool slowly at room temperature for at least 20 min.

Inactivation of Endogenous Peroxidase

This step is performed when using peroxidase-conjugated secondary antibodies or ExtrAvidin-Peroxidase.

1. Place the slides on a flat level surface. Slides should not ouch each other. Sections should not dry out at any time.
2. Add enough drops of 3% hydrogen peroxide.
3. Incubate 5 min. at room temperature.

4. Rinse with PBS from a wash bottle.
5. Place the slide in PBS wash bath for 2 min.

Primary Antibody Reaction

a. Pre-incubation of the sample with 5% BSA for 10 min. prior to the primary antibody reaction may decrease background staining. For best results with animal tissues, use 5 to 10% normal serum from the same species as the host of the secondary antibody.
b. Optimal dilution and incubation times should be determined for each primary antibody prior to use.

1. Allow the slides to drain, shake off excess fluid with a brisk motion and carefully wipe each slide around the sections.
2. Dilute the primary antibody or negative control reagent to its optimal dilution in diluent. The diluent alone may be used as a negative control. A positive control slide (a tissue known to contain the antigen under study) should also be run.
3. Apply 100 µl primary antibody solution to the appropriate slides, covering the tissue sections.
4. Tilt each slide in two different directions, so the liquid is spread evenly over the slide.
5. Incubate for at least 60 min. at 37 °C in humidified chamber. Longer incubations are advised for low density antigens.
6. Rinse gently with PBS from a wash bottle. Place the slide in a PBS wash bath for 5 min.

Secondary Antibody Reaction

Option 1 - Biotin/ExtrAvidin Detection

1. Allow the slides to drain, shake off excess fluid and carefully wipe the slide as before.
2. Dilute the biotinylated secondary antibody in diluent to its optimal concentration.
3. Apply 100 µl to each slide, covering the tissue sections.
4. Tilt each slide in two different directions.
5. Incubate in a humidity chamber for at least 30 min. at room temperature.

6. Rinse gently with PBS from a wash bottle.
7. Place the slide in a PBS wash bath for 5 min.

ExtrAvidin Reaction

8. Allow the slides to drain. Shake off excess fluid.
9. Dilute ExtrAvidin peroxidase or ExtrAvidin alkaline phosphatase, in diluent to its optimal concentration.
10. Apply 100 µl to all slides; cover the section.
11. Tilt each slide in two different directions.
12. Incubate in humidified chamber for at least 20 min. at room temperature.
13. Rinse gently with PBS from a wash bottle.
14. Place the slide in PBS wash bath for 5 min.

Option 2 - Enzyme-labeled Secondary Antibody

1. Allow the slide to drain. Shake off excess fluid with a brisk motion
2. Dilute the peroxidase or alkaline phosphatase conjugated secondary antibody in the diluent to its optimal dilution.
3. Apply 100 µl to all slides, covering the tissue sections.
4. Tilt each slide in two different directions.
5. Incubate 30 min. at room temperature or at 37 °C in humidified chamber.
6. Rinse gently with PBS from a wash bottle.
7. Place the slides in a PBS wash bath for 5 min.

Substrate Preparation

Substrate mixture preparation is done during the final wash step.

Addition of 1 mm levamisole

Development

1. Allow each slides to drain. Shake off excess fluid .
2. Apply enough drops of freshly prepared substrate mixture to cover the tissue section.
3. Incubate 5-10 min. or until desired color reaction is observed when monitored with the microscope. Terminate the reaction before background staining appears in the negative controls by rinsing gently with distilled water from a wash bottle.

Counterstaining

When using AEC substrate, do not use alcohol-containing solutions for counter-staining (e.g., Harris' hematoxylin, acid alcohol), since the AEC stain formed by this method is soluble in organic solvents. The slide must not be dehydrated, brought back to toluene (or xylene), or mounted in toluene-containing mountants.

1. Apply enough Mayer's hematoxylin to cover the section or place the slide in a bath of Mayer's hematoxylin.
2. Incubate for 0.5-5 min., depending on strength of the hematoxylin used.
3. Rinse the slide gently with distilled water from a wash bottle.
4. Rinse the slide under gently running tap water for 5 min. (avoid direct jets which may wash off or loosen the section).
5. Mount the sections using aqueous mounting medium such as glycerol gelatin. Coverslip may be sealed with clear nail polish.

6.4 PLAGIARISM REPORT

6.5 KNH-UON ERC APPROVAL LETTER