IMMUNOPHENOTYPIC CHARACTERIZATION OF NON-HODGKIN LYMPHOMAS

BY

JOSEPH ADOMAKO

ID: 10313073

DEPARTMENT OF HAEMATOLOGY

SCHOOL OF MEDICINE AND DENTISTRY

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA (UG)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL HAEMATOLOGY DEGREE

OCTOBER 2020

DECLARATION

I, Joseph Adomako, hereby declare that except for reference to other people's work which I have duly cited, this thesis is the result of an original research work carried out by me under the supervision of supervisors whose signatures are below.

Signed: _____ _____

Joseph Adomako Date

(Student)

Signed: _____ _____

Yvonne A. Dei-Adomakoh, MBBS, FWACP Date

Department of Haematology, University of Ghana

(Supervisor)

Signed: _____ _____

Afua O. D. Abrahams, FWACP, FGCP Date

Department of Pathology, University of Ghana.

(Supervisor)

DEDICATION

This project work is dedicated to the Holy Spirit of God who is and has been my allos parakletos throughout this research. Also to my lovely mother, Diana Adomako for her support, inspiration and courage.

ACKNOWLEDGEMENTS

To God be the Glory. A very special appreciation to the Almighty God for His grace and love throughout this work.

I owe this unique opportunity to place on record my deep sense of gratitude and indebtedness to my supervisors, Dr. Yvonne A. Dei-Adomakoh (Haematology Department, University of Ghana) and Dr. Afua O. D. Abrahams (Pathology Department, University of Ghana) for their scholastic and expert guidance, prudent suggestions, helpful criticism, persistent endeavor and counselling throughout this work.

I wish to express my profound appreciation to Roche Pharmaceuticals for the educational grant support that made it possible for me to conduct this study.

I would also like to thank other lecturers of the Haematology Department including Prof. J. K. Acquaye, Dr. Amma Anima Benneh and Prof. Edeghonghon Olayemi for their investment, advice and encouragement. Again, my appreciation goes to all lecturers and staffs of Pathology Department for their support and encouragement.

I wish to express my sincere gratitude to Mr. Emmanuel M. K. Ametefe, Mr. Andrew Richard Hooper and Ms. Bernice Anane Mawuli of the Pathology Department for their patience, time and assistance in the immunohistochemical analysis. Again, my appreciation goes to Dr. David Nana Adjei for assisting me in the statistical analysis.

My great sense of gratitude also goes to all administrative and technical staff of the Haematology Department, especially Mr. Enock Alabi Mensah and Mr. Francisco Torto, for their help and support.

TABLE OF CONTENTS

DECLARATION i

DEDICATION ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF TABLES ix

LIST OF FIGURES x

ABBREVIATIONS xi

ABSTRACT xiv

CHAPTER ONE 1

1.0 INTRODUCTION 1

1.1 Background 1

1.2 Problem statement 3

1.3 Justification 4

1.4 Aim 5

1.5 Specific objectives 5

CHAPTER TWO 6

2.0 LITERATURE REVIEW 6

2.1 Historical perspective 6

2.2 Clinical manifestations of NHLs 7

2.3 Pathogenesis of non-Hodgkin lymphomas 8

2.4 Aetiology/risk factors of non-Hodgkin lymphomas 10

2.5 Classification of NHL 11

2.6 Diagnosis and staging of NHLs 12

2.7 Immunophenotyping 14

2.8 Common phenotypic subtypes of non-Hodgkin lymphoma 16

2.8.1 Small lymphocytic lymphoma 16

2.8.2 Follicular lymphoma (FL) 17

2.8.3 Marginal Zone Lymphoma 18

2.8.4 Diffuse Large B-cell Lymphoma (DLBCL) 19

2.8.5 Burkitt lymphoma 20

2.8.6 Lymphoplasmacytic lymphoma 21

2.8.7 Mantle cell lymphoma 22

2.8.8 T/NK cell lymphomas 23

2.9 Antigenic markers 24

2.9.1 B cell markers 24

2.9.2 T/NK cell markers 29

2.10 Treatment of NHLS 30

2.10.1 Chemotherapy 31

2.10.2 Immunotherapy 31
2.10.3 Target therapy 32
2.10.5 Radiotherapy 32
2.11 Treatment outcomes of NHLs 33
CHAPTER THREE 34
3.0 METHODOLOGY 34
3.1 Study design 34
3.2 Study site 34
3.3 Study period and population 35
3.4 Inclusion criteria 35
3.5 Exclusion criteria 35
3.6 Sample size determination 35
3.7 Sample and data collection 37
3.8 Materials and methods 37
3.9 Procedure 38
3.9.1 Microtomy 38
3.9.2 Immuno-staining 38
3.9.3 Mounting 41
3.9.4 Examination of slides 41
3.10 Data handling 42

3.11 Statistical analysis 42

3.12 Ethical issues 42

CHAPTER FOUR 43

4.0 RESULTS 43

4.1 Demographic Characteristics 43

4.2 Antigen expression in various NHLs 45

4.3 Characterization of NHLs according to cell type 46

4.4 Cell types and sex 47

4.5 Phenotypic distribution of NHLs 48

4.6 Selected images of slides of non-Hodgkin lymphomas 49

4.7 Clinical characteristics and cell types 53

4.8 Morphology diagnosis and immunophenotype 54

4.9 Cell types and outcomes 56

CHAPTER FIVE 57

5.0 DISCUSSION AND CONCLUSION 57

5.1 Discussion 57

5.1.1 Demographic characteristics and cell types distribution by sex 57

5.1.2 Antigen expressions and NHLs distribution by cell type 58

5.1.3 Phenotypic subtypes of non-Hodgkin lymphomas 60

5.1.4 Clinical features and cell types 61

5.1.5 Morphology diagnosis and immunophenotype 61

5.1.6 Cell types and outcomes of NHLs 62

5.2 Limitation of the study 62

5.3 Conclusion 63

5.4 Recommendation 63

REFERENCES 64

APPENDIX A - DATA ABSTRACTION FORM 76

APPENDIX B 76

APPENDIX C 77

APPENDIX D 78

APPENDIX E 79

APPENDIX F 79

LIST OF TABLES

Table 1: Ann Arbor Staging of NHLs..... 13

Table 2: Immunophenotypic behaviour of some B-cell NHLs..... 28

Table 3: Immunophenotypic behaviour of some NK/T-cell NHLs..... 30

Table 4: Demographic characteristics of participants..... 44

Table 5: Immunophenotypic pattern of non-Hodgkin lymphomas..... 45

Table 6: Distribution of subtypes of NHLs..... 48

Table 7: Association between clinical presentations and cell types..... 53

Table 8: Association between pathological cell types and diagnosis made by morphology..... 55

Table 9: Association between cell types of NHLs and clinical outcomes..... 56

LIST OF FIGURES

Figure 1: Diagrammatic presentation of the ‘optiview’ detection technique 40

Figure 2: Distribution of NHLs by cell type 46

Figure 3: Frequency of cell types according to sex 47

Figure 4: Photomicrograph of diffuse large cell Lymphoma 49

Figure 5: Photomicrograph of B-cell Lymphoma 49

Figure 6: Photomicrograph of T-cell Lymphoma 50

Figure 7: Photomicrograph of H&E and IHC staining of small lymphocytic lymphoma 51

Figure 8: Photomicrograph of H&E and IHC staining of mantle cell lymphoma 52

ABBREVIATIONS

AIDS -	Acquired immunodeficiency syndrome
ABC -	Activated B-cell like
ALCL -	Anaplastic large cell lymphoma
ALK+ -	Anaplastic lymphoma kinase positive
BL -	Burkitt lymphoma
BMA -	Bone marrow aspirate
BCL2	B cell lymphoma 2
BCL6	B cell lymphoma 6
BCR	B-cell receptor
CCDN1 -	Cyclin D1 gene
CCDN2 -	Cyclin D2 gene
CCDN3 -	Cyclin D3 gene
CD -	Cluster of differentiation
CNS -	Central nervous system
CALLA	Common acute lymphoblastic leukaemia antigen
CSR	Class- switch recombination
CLL -	Chronic lymphocytic lymphoma
CHOP -	Cyclophosphamide, doxorubicin, vincristine, prednisolone
DNA -	Deoxyribonucleic acid
DLBCL -	Diffuse large B cell lymphoma
DAB -	3,3-Diaminobenzidine tetrahydrochloride
EBV -	Epstein barr virus
FL -	Follicular lymphoma
FBC -	Full blood count
GC -	Germinal center
GCB -	Germinal center B-cell like
H&E	Hematoxylin and eosin
HRP	Horseradish peroxidase

HLs - Hodgkin lymphomas

HIV - Human immunodeficiency virus

HHV - Human herpes virus

HTLV - Human T lymphotropic virus

HCV - Hepatitis C virus

IPI - International prognostic index

Ig - Immunoglobulin

IHC - Immunohistochemistry

ISH - *In situ* hybridization

KBTH - Korle Bu Teaching Hospital

LPL - Lymphoplasmacytic lymphoma

LDH - Lactate dehydrogenase

MALT - Mucosa associated lymphoid tissues

MCL - Mantle cell lymphoma

MZL - Marginal zone lymphoma

MUM1 - Multiple myeloma oncogene 1

MF - Mycosis fungoides

NHLs - Non-Hodgkin lymphomas

NK - Natural killer

REAL - Revised European-American classification of lymphoid neoplasm

RS - Reed-Sternberg

SLL - Small lymphocytic lymphoma

SLE - Systemic lupus erythematosus

SHM Somatic hypermutation

SPSS - Statistical package for social science

TFH Follicular T-helper cells

T-NHL - T cell non-Hodgkin lymphoma

UG University of Ghana

V(D)J Variable, diversity, joining

WM - Waldenstrom’s macroglobulinemia

WF - Working formulation

WHO - World Health Organization

ABSTRACT

Background: Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of clonal lymphoid tumours originating from lymphocytes. They constitute about 90% of an estimated 3-4% worldwide distribution of malignant lymphomas among various cancers. It is the commonest haematological malignancy and fifth most common cancer worldwide. Despite the continuous rise and associated deaths, research on non-Hodgkin lymphomas, and in particular the area of immunophenotypic spectrum is limited in Ghana. The situation is not different from other African countries. Identification of NHL-associated immunophenotype plays an essential role towards patient management.

Aim: To determine the antigen expressions in non-Hodgkin lymphomas by immunohistochemistry and the identification of the distribution of cell and subtypes.

Methods: This is a retrospective, descriptive study in which archived formalin-fixed paraffin-embedded tissue blocks of morphologically diagnosed NHLs were used. Clinical and laboratory information of participants were retrieved from the medical records of the Haematology Department, Korle Bu Teaching Hospital. Antigenic phenotypes were determined by immunohistochemistry.

Results: A total of 66 cases of NHLs were selected for the study. Mean age of the study was 50.2±16.1 with a range of 16 to 78 years. Males outnumbered females with ratio of 1.54:1. Among the targeted markers, CD20 was the most commonly expressed in 89.4% (59) cases. Immunohistochemistry studies revealed greater proportion of B cell lymphomas of 89.4%. Five subtypes were successfully identified of which diffuse large B cell lymphoma constitutes the predominant group (40.9%). There was 100% agreement between diagnosis made by morphology

and immunophenotype by cell type (P=0.001). Of the clinical characteristics, B-symptoms were the most common feature presented in the study with 87.9% (58) whereas hepatomegaly was the least expressed, 7.6% (5). However, there was no significant association between obvious clinical manifestations and pathological cell types. A substantial amount of B cell 37.3% (22) and T cell 14.7% (1) lymphomas attained clinical remission. A significant association was observed between phenotypic cell types and outcomes of NHLs (p=0.011).

Conclusion: Adult Non-Hodgkin lymphomas were mostly due to the malignant transformation of B cell lineage with diffuse large B cell lymphoma being the commonest subtype. Majority of B and T cell lymphomas occurred in males. A significant association was observed between phenotypic cell types and morphology (P=0.001) as well as outcomes of NHLs. The present study therefore serves as a preliminary data for further research towards the adoption of an improved treatment regimen and management of non-Hodgkin lymphomas in Ghana.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of clonal lymphoid tumours originating from B cell, T cell, or natural killer (NK) lymphocytes. B cell lymphomas represent about 85% of cases with the rest being mainly T cell and occasionally NK lymphomas worldwide (Zelenetz et al., 2011). NHLs occur in individuals at virtually all ages, with relatively reduced frequency in children. Their presentation significantly increases with age, with a peak incidence age of 55-60 years (Howard & Hamilton, 2013). NHLs generally develop in the lymph nodes or in lymphatic tissue found in organs such as the stomach, intestines or skin. There may be bone marrow involvement. Lymphoma cells may develop in one or many sites in the body (Leukaemia and Lymphoma Society, 2013). They vary from highly proliferative and potentially rapidly fatal diseases (aggressive lymphomas) to well-tolerated malignancies (indolent lymphomas). The clinical groupings of NHLs into aggressive, moderately aggressive, highly aggressive and indolent lymphomas showed respectively 68%, 3%, 3% and 26% (Jairajpuri, Ghai, Saluja, Kapur, & Bhowmik, 2017).

The 2018 global cancer report estimates five hundred and nine thousand, five hundred and ninety (509,590) new cases of NHLs and about two hundred forty eight thousand, seven hundred and twenty four (248,724) deaths due to NHLs. Haematological malignancies represent about 7% of all cancers worldwide (Miranda-Filho et al., 2019). Malignant lymphomas which comprise NHLs and Hodgkin Lymphomas (HLs) make up an estimated 3–4% of the worldwide distribution of malignancies (Hjalgrim & Engels, 2008). NHLs make up 80-90%, and HLs account for the

remaining of all malignant lymphomas (Batra, Kaur, & Jindal, 2014). They are the most common haematological malignancy and are currently the fifth most common cancer diagnoses among patients in some developed countries (Howard & Hamilton, 2013). Haematological malignancies including non-Hodgkin lymphoma, leukemia, Hodgkin lymphoma, and multiple myeloma have emerged as a major cause of morbidity and mortality in sub-Saharan Africa and account for nearly 10% of the overall cancer burden in the region (Gopal et al., 2012). A national strategy for control of cancer document developed by the Ministry of Health, Ghana, shows that out of eight hundred and thirty (830) adult haematological malignancies recorded over a decade at the Haematology/Oncology clinic, Korle Bu, NHLs recorded the highest frequency of 218 (26.3%) (Ministry of Health-Ghana, 2011). Retrospective review of already diagnosed NHLs of patients aged 13 and above at the same unit, in a period of 4 years and 10 months showed a dramatic figure of 279 (Dei-Adomakoh et al., 2017). They consist of many subtypes with diverse epidemiology, aetiology, morphological features, growth pattern, antigenic phenotype and biological behaviour (Jaffe et al., 2017). It is therefore imperative that a multidisciplinary approach be used in diagnosing Non-Hodgkin lymphomas. These include routine morphological evaluation, immunophenotyping and genetic studies (Bakshi & Maghfoor, 2012). Immunohistochemistry of the lymph node or involved tissue is essential in detecting antigenic markers located in or on the surfaces of lymphocytes. This allows the detection of specific types of NHL (Hoffbrand & Moss, 2016) and serves as a profound milestone in bridging the gap in the classification. Medical laboratory units in Ghana and other West African countries are more presently placed to diagnose common infectious diseases due to their massive impact on individuals and the economy. Lack of resources makes it difficult to develop and apply most current technologies including molecular

and cytogenetic techniques and immunohistochemistry or flow cytometry for routine clinical use (Charwudzi et al., 2014).

1.2 Problem statement

NHLs are a diverse group of haematological cancers of the lymphoid system affecting the functional cells of the adaptive immune system, lymphocytes. NHLs continue to rise and remains the leading malignancy diagnosed among patients at the Haematology Department of KBTH, Ghana. The occurrence of NHLs have increased markedly across the global world (Howard & Hamilton, 2013) with an incidence of approximately 17 in 100,000 (Hoffbrand & Moss, 2016). It is therefore estimated that more than five thousand Ghanaians may suffer from NHLs. The disease accounts for 2.7% of deaths due to cancer (Sharma, Mannan, Madhukar, & Navani, 2014). Disparities exist in the incidence rates and distribution at various geographic regions of the world and NHLs are apparently frequent in countries with limited resources (Disanto et al., 2016). This may be explained by different genetic, antigenic and environmental influences that affect disease risk (Segbefia, Renner, Dei-Adomakoh, & Welbeck, 2013) and increased population density.

Moreover, NHLs are a wide group of cancers that have unique responses to treatment. Diversity of NHLs requires the determination of particular cell type and disease sub-type in order to maximize treatment through the selection of the most appropriate therapy. Treatment of particular NHLs may not produce the intended result without identifying the specific immunophenotypic subtype. However, diagnosis and treatment are largely based on morphology (histopathology examination) due to limited resources and financial constraints. Dei-Adomakoh et al (2017) reported 17 out of 279 (only 6.1%) non-Hodgkin lymphoma cases with phenotypic studies. Thus, inability to follow the current bench mark for classification of lymphoid tumors by World Health

Organization (WHO); which classifies lymphoid malignancies as distinct biological entities based on morphology, immunophenotype, genetics, and clinical features (Jaffe et al., 2017).

Research on the NHLs and the immunophenotypic distribution is limited in Africa. As at now, not much systematic scientific assessment has been carried out on NHLs in the area of immunophenotypic spectrum in Ghana. There is therefore no clear cut data on the distribution of phenotypic cell and sub-types necessary for policy directions on local treatment protocols. Immunophenotyping is necessary for early diagnosis and treatment. The era of antibody targeted therapy demands standardized reporting on potential targets (markers) in tumour cells (Andrade, 2007). Identification of NHL-associated immunophenotype is used to monitor minimal residual disease during follow-up.

1.3 Justification

Immunophenotyping is a fundamental step and key component for the precise diagnosis and classification of haematological malignancies, including Non-Hodgkin lymphomas. In recent times, cancer treatment response and disease progression increasingly rely on immunohistochemistry to monitor changes in targeted antigens. The study will form the basis to categorize NHLs and identify the various disease behaviours that bears specific impact on prognosis (monitoring) and patient management. Classification remains the gold standard for defining heterogeneities of related diseases, hence the study will facilitate the understanding in the treatment of NHLs at the local setting. This will in turn reduce morbidity and mortality associated with NHLs in Ghana as well as the overall cancer burden.

1.4 Aim

This study aims to determine the antigen expressions in non-Hodgkin lymphomas by immunohistochemistry and the identification of the distribution of cell and subtypes.

1.5 Specific objectives

1. To determine the immunophenotypic patterns of non-Hodgkin Lymphomas by immunohistochemistry.
2. To characterize the non-Hodgkin lymphomas according to pathological cell and subtypes by immunophenotypic findings.
3. To determine association between clinical characteristics of non-Hodgkin lymphomas (B symptoms, lymphadenopathy, splenomegaly, Hepatomegaly, extranodal involvement) and cell types.
4. To compare diagnosis made by morphology and immunophenotype (pathological cell type)
5. To determine association between cell types and outcomes of non-Hodgkin lymphomas.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical perspective

Lymphomas are malignancies of solid tumours involving lymphoid tissues. The implicated cells in lymphomas are lymphocytes. They are divided into Hodgkin and non-Hodgkin lymphomas. Non-Hodgkin lymphomas are varied entities of lymphatic tumours that have distinct histological, immunophenotypic, genetic, clinical features (Stathis & Owens, 2016) and epidemiological characteristics (Hjalgrim & Engels, 2008). Virchow and Cohnheim observed a disease characterized by enlargement of lymph nodes in 1864 and 1865 which was named as lymphosarcoma and pseudoleukaemia respectively. Subsequently, Bilroth (1871), for the first time introduced the name 'malignant lymphoma'. There were efforts made in identifying NHLs between the end of the 19th and the middle of the 20th century by various scientists (Lakhtakia & Burney, 2015). The identification and differentiation of lymphomas became clear after the remarkable discovery of the Reed-Sternberg (RS) cell early in the 20th century. Cases with characteristic RS cell were described as Hodgkin disease and those without (other lymphomas) were grouped together as NHLs (Andrade, 2007). Giant follicular lymphoma was introduced by Brill and Symmers in 1925 and reticulum cell sarcoma by Oberling in 1928 (Lakhtakia & Burney, 2015). These led to further recognition, description and classification of NHLs. Rappaport first classified NHLs based on cytology and the presence or absence of follicular structure. Burkitt described endemic (African) Burkitt's lymphoma in 1858. Scientists took advantage of the evolution of immunologic tools in the 1960s and tried to compare malignant cells to their normal

counterparts. (Lakhtakia & Burney, 2015). Recently, over 80 NHLs are known and classified (Jaffe et al., 2017).

2.2 Clinical manifestations of NHLs

NHLs have diverse clinical features. The commonest clinical features of NHLs are: B symptoms such as fever, night sweats, weight loss and anorexia; and painless lymphadenopathy. Lymphadenopathy can be seen in one or several peripheral lymph node regions and usually lead to pressure effect when significantly enlarged. The most common lymph node site affected by lymphoma are the cervical, axillary and groin areas (Dehghani, Haddadi, & Vojdani, 2015). Retroperitoneal or mesenteric nodes are also frequently involved (Hoffbrand & Moss, 2016). Significant number of NHLs are situated in tissues outside the lymph nodes and also from sites with no native lymphoid tissue. These sites of involvement are termed extranodal. Extranodal NHLs can occur in any anatomic site of the body such as gastrointestinal tract, head and neck (such as Waldeyer's ring, nasopharynx, salivary glands), skin, central nervous system (CNS), bone marrow, testis, thyroid, breast, orbit, pancreas, rarely adrenal and the genitourinary tract (Bangash, Hussain, Zakaria, & Piracha, 2014). Patients may present with acute gastrointestinal symptoms, splenomegaly, and hepatomegaly. Waldeyer's ring (oropharyngeal lymphoid structures) is affected by NHLs in 5–10% of patients and often associated with inflammation of the throat, noisy or obstructed external respiration. Diffuse bone marrow involvement in advanced cases leads to anaemia and associated symptoms, infections due to neutropenia or petechial hemorrhages due to thrombocytopenia. The commonest extranodal site of NHL involvement is the gastrointestinal tract. This is followed by the bone marrow. The skin is also usually affected by closely linked lymphomas of T cell origin namely, mycosis fungoides and Sézary syndrome (Hoffbrand & Moss, 2016). However some NHLs appear asymptomatic and may be detected incidentally during routine

medical examination for another reason (Dehghani et al., 2015). These include low grade follicular lymphomas (Armitage & Longo, 2016) and mucosa associated lymphoid tissue (MALT) lymphoma with no threatened vital organ function (Shimizu et al., 2010).

2.3 Pathogenesis of non-Hodgkin lymphomas

B-cell lymphomas form throughout different stages of B-cell development and are therefore linked to their normal counterpart (Nogai, Dorken, & Lenz, 2011). Immunoglobulin (Ig) gene rearrangement by V(D)J recombination in the bone marrow has an essential role in the production of normal B cells with functional surface receptor. Also somatic hypermutation (SHM) and class switching (CSR) of Ig genes are vital procedures during B cell differentiation in germinal centers after an encounter with an antigen. The profound deoxyribonucleic acid (DNA) rearrangements by V(D)J recombination, SHM, and CSR are key processes that might prone B cells to be malignant (Seifert, Scholtysik, & Küppers, 2013). Majority of Follicular lymphomas have characteristic t(14;18) and t(11;14) translocation which arise from V(D)J recombination and juxtaposes BCL2 and IgH gene. Some cases of DLBCL also express BCL2 translocation.

A myriad of lymphomas originate from cells in the germinal centers or post germinal center reactions(Kuppers & Dalla-favera, 2001). A fraction of DLBCL exhibits mutated BCL6 as the result of errors in SHM. Abnormality in the CSR results in chromosomal switch translocations often seen in Burkitt's lymphoma, multiple myeloma, and other lymphoid malignancies (Nogai et al., 2011).

The expression of particular B-cell receptor (BCR) and emergence of patterns of differentiation protein markers indicates the distinct stages of B-cell development and differentiation. Hence,

investigation of these features is used to identify the origin of the various B-cell lymphomas (Küppers, 2005).

The germinal center (GC) is enclosed by a mantle zone of naive B cells, most of which express the CD5 marker (Sagaert, Tousseyn, & Yantiss, 2012). An outer B-cell-rich zone, marginal zone, surrounds the entire follicle. The cells of marginal zone serves as the origin for lymphomas including; extranodal mucosa-associated lymphoid tissue (MALT) lymphomas, nodal and splenic marginal-zone lymphomas. (Küppers, 2005). Also, chronic lymphocytic leukaemia/small lymphocytic lymphoma is believed to derive from CD5 B cells, memory B cells or marginal-zone B cells (Seifert et al., 2013).

In addition, it is now known that the genome of certain lymphoma subtypes can be altered by the introduction of exogenous genes by oncogenic viruses (Stathis & Owens, 2016).

Majority of T-cell lymphomas manifest the immunophenotypic features of post-thymic T lymphocytes (Elaine S. Jaffe, 2006). T-cell lymphomas are the end result of accumulated genetic alterations leading to abnormal signaling pathways and increased proliferation and survival advantage (Arnam, Lim, & Elenitoba-johnson, 2018).

A lot of T-cell and NK-cell lymphomas that often occur in children and young adults are due to malignant transformation of cells of the innate immune system. Examples include aggressive NK-cell leukemia, fulminant EBV-positive T-cell lymphoproliferative disease and hepatosplenic T-cell lymphoma. A subset of T-cells, follicular T-helper cells (TFH), are derived from germinal center (Elaine S. Jaffe, 2006) and implicated in angioimmunoblastic T-cell lymphoma (Arnam et al., 2018). Germinal center–associated markers, BCL6 and CD10 are therefore expressed (Elaine S. Jaffe, 2006).

2.4 Aetiology/risk factors of non-Hodgkin lymphomas

The aetiology of the majority of cases of NHLs are not well known. However, several predisposing factors have been established. These include immunodeficiency disorders (such as HIV/AIDS, organ transplantation), autoimmune disorders, some infectious agents and genetic factors (Karin, 2006). There are some inherited genetic syndromes associated with a higher risk of developing NHLs. They include autoimmune lymphoproliferative syndrome, severe combined immunodeficiency, ataxia-telangiectasia, Wiskott-Aldrich syndrome, common variable immunodeficiency, hyper-IgM syndrome and Nijmegen breakage syndrome (Stathis & Owens, 2016). Infectious agents associated with lymphomas include the Epstein-Bar virus (EBV) and human herpesvirus-8 (HHV-8), human T-cell lymphotrophic virus 1 (HTLV-1)], hepatitis C virus (HCV) and bacteria such as *Helicobacter pylori* (*H. pylori*). Some particular occupations such as pesticide applicators, farmers, wood and forestry workers, grain millers and workers in the, rubber, plastic, petroleum and synthetics industries are at higher risk of developing NHLs. This is due to continuous contact with chemicals including benzene and pesticides containing organophosphates, phenoxy acids, and organochlorines. Acute and chronic psychological stress have the ability to lower the activities of the immune system which may affect the daily immune regulation of suspected developing tumors (Karin, 2006).

2.5 Classification of NHL

Classification is a critical step in determining diagnosis and disease outcomes of various subtypes of NHLs. Various known classification schemes include: the Lukes-Collins, Rappaport, Working Formulation (WF), Kiel, Revised European-American Classification of Lymphoid Neoplasm (REAL) and World Health Organization (WHO) (Dei-Adomakoh et al., 2017).

The oldest and morphologically oriented Rappaport system was initially published in 1956 (Jaffe, Harris, Stein, & Isaacson, 2008). This system divided NHLs into two categories with clinical significance: nodular lymphomas and diffuse lymphomas. The latter is associated with poor diagnosis (Skarin & Dorfman, 1997). The Kiel and the Lukes-Collins classification, popular in Europe and the United States respectively, were both introduced in 1974. Kiel system is primarily based on cell lineage and differentiation whereas Lukes-Collins system was the first to identify lymphomas according to B-cell and T-cell types using immunologic techniques. The WF stratified lymphomas according to clinical outcome in 1970s (Jaffe et al., 2008). It puts NHLs into low, intermediate, and high-grade types, based upon prognosis and therapeutic response. Relatively simple and reproducible morphologic categories that may cover several individual diseases were solely used with no consideration for cell origin.

In 1994, the International Lymphoma Study Group proposed the REAL system. The REAL classifies NHLs by origin as B-cell, T/natural killer (NK)-cell types. T-cell and B-cell lymphomas are further divided into precursor and peripheral cell types. The REAL system also proposed clinically useful categories; indolent, aggressive and highly aggressive lymphoma types. The indolent ones were further put into disseminated, extranodal, or nodal (Skarin & Dorfman, 1997). It used an approach where each disease entity was defined by morphology, immunophenotype, genetic features and clinical features. WHO adopted the REAL classification system and has

expanded the principles to the classification of myeloid, histiocytic and mast cell neoplasms (Sharma et al., 2014). This multi-parameter approach adopted by WHO has opened up a new and common understanding, as well as effective cooperation and communication between oncologists and haematopathologists in different parts of the world (Harris, Jaffe, Diebold, Flandrin, & Vardiman, 2000).

2.6 Diagnosis and staging of NHLs

Diagnosing NHLs commences with careful investigation of the medical history and clinical presentation, assessment of results of full blood count, examination of the blood film and other laboratory tests including lactate dehydrogenase (LDH) and liver function test. Excisional biopsy of lymph node or other involved tissue for histopathological examination remains the gold standard for primary diagnosis and classification of NHLs. It involves careful observation of altered architecture and cytologic features with any lymphomatous infiltrates. The abnormal cytomorphologic features can be small cleaved or non-cleaved, intermediate, or large cell, and can have a follicular or diffuse pattern (Andrade, 2007)**.** Morphological examination of the biopsy is confirmed by immunophenotyping and, in some cases, genetic analysis (Hoffbrand & Moss, 2016). Bone marrow aspirate (BMA) is usually done for cytological assessment and also for immunophenotypic, genetic studies. A trephine biopsy (TB) is also useful for the diagnosis of NHLs, especially low-grade lymphoma types which often presents with marrow infiltration. BMA is used in staging and assessing the response to treatment (Andrade, 2007)**.** Staging and monitoring treatment responses of NHL requires laboratory tests such as lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), C-reactive protein, full blood count (FBC), liver function test, BMA, and trephine biopsy and radiological investigations such as computed tomography or

positron emission tomography scan. The international prognostic index (IPI) based on age, stage, bulk of disease, performance status and serum LDH levels is used (Howard & Hamilton, 2013). NHLs can be staged using the Ann Arbor staging system. The principle of the staging is based on the site(s) of occurrence of the disease. The disease may occur at a single or multiple lymph node/extranodal site. Localized extranodal sites of involvement are denoted by a subscript E (Armitage, 2005). The table below shows the criteria for clinical staging of various NHLs.

Table 1: Ann Arbor Staging of NHLs

Stage	**Features**
I	Involvement of a single lymph node region or lymphoid structure
II	Involvement of two or more lymph node regions on the same side of the diaphragm
III	Involvement of lymph node regions or structures on both sides of the diaphragm
III_1	Involvement of splenic, coeliac or portal nodes
III_2	Involvement of para-aortic, iliac or mesenteric nodes
IV	Extensive disease in liver, bone marrow or other extranodal sites
Sub-stage **E**	Localized extranodal disease/ Symptom Status **A** - $Fever^0$, $Sweats^0$ or $weight\ loss^0$ Symptom Status **B** - Unexplained fever >38^oC Drenching Night Sweats Weight loss of > 10% in preceding 6/12

2.7 Immunophenotyping

Immunophenotyping is an immunological technique that is used to identify cells in relation to the types of antigens (protein markers) located in the nucleus, cytoplasm or surface membrane of cells (McLaughlin, 2000). It therefore determines the degree of maturation and differentiation of malignant cells, as well as the cell of origin (Howard & Hamilton, 2013) and specific sub-types (Townsend & Marcus, 2016).

Immunophenotyping seeks to establish clonality and determine the pattern of expression of cell markers for specific subtyping. The availability of polyclonal lymphocytes indicates an ongoing immune response whereas monoclonal proliferation of lymphocytes demonstrates malignancy. This is exhibited by same set of cell markers on all lymphoid cells (Pallister & Watson, 2011).

Malignant cells may show same protein markers as their normal counterparts but some NHLs also acquire deviant antigens not expressed in normal mates or even lose normally expressed antigens. For example CD7 may be lost in T cell neoplasms (Australian Cancer Network, 2005).

B cell disorders are distinguished from T cell disorders by the possession of specific antigens. Neoplastic B cells may be differentiated from their normal mates by the expression of surface immunoglobulin light chain restriction. Surface immunoglobulin light chain restrictions demonstrates mono-clonality but do not occur in all B-cell neoplasms (Bain, Bates, Laffan, & Lewis, 2011).

Immunophenotyping has diagnostic, prognostic and clinical significance. In evaluating a patient suspected of having NHL, marker studies may be essential to confirm and make correct diagnosis. It is important to note that immunophenotyping is not interpreted alone, but done in relation to morphology, clinical and other laboratory information (Bain et al., 2011). Clinically, identification of various antigens mostly specific to neoplastic cells is crucial as some drugs targets those

antigens and stimulate destruction of the malignant cells. Diagnosis and distribution of NHLs into specific subtype has prognostic significance as subtypes have varying treatment outcomes (Bain et al., 2011).

Histology presents with considerable morphologic overlap with reactive lymphoid proliferations (McLaughlin, 2000). Immunophenotyping helps distinguish reactive from neoplastic lymphoid infiltrates, lymphoid from non-lymphoid malignancies, and specific lymphoid neoplasms. The specific phenotypic markers have been identified so far to assist in correct histopathological diagnosis and therefore providing the patient the chance of early treatment (Sathiya & Muthuchelian, 2009). Immunophenotyping may be carried out by immunohistochemistry, immunofluorescence (Howard & Hamilton, 2013), immunocytochemistry (Bain et al., 2011) and flow cytometry. Immunohistochemistry determines cellular antigens on solid tissues such as bone marrow and lymphoid biopsies. Immunocytochemistry evaluates cells from body fluids spun or smeared onto slides as well as blood and bone marrow smears. Flow cytometry is useful in determining both viable and fixed cells in suspension. B cell antigenic markers include CD19, CD20 and CD22 and T cell markers include CD5, CD3, CD2 and CD7. Markers used to define B cell subsets are, but not limited to; BCL-2, CD10, CD5, cyclin D1, CD23, BCL-6 and MUM1. Markers for distinguishing T cell subsets include CD 4, 8, 56, 57, beta F1 (Hoffbrand & Moss, 2016;Townsend & Marcus, 2016). Hence, a complete panel of antibodies to various surface and intra-cytoplasmic markers need to be applied to help in detecting specific types of NHL.

Immunophenotypic findings of NHLs done in India, by Sharma et al showed higher B-cell origin of 89.3% and the rest being T-cell types. No NK- NHL was recorded. The distribution of sub-types had majority being diffuse large B-cell lymphoma (46.8%), followed by B-cell small lymphocytic lymphoma and then Mantle-Cell Lymphoma (MCL) representing 17% and 12.8% respectively .

T-cell lymphoblastic lymphoma was the most common T-cell type showing 6.4% of all NHL cases (Sharma et al., 2014). In the review by Dei-Adomakoh *et al*, the commonest subtype recorded was diffuse large B cell lymphoma (DLBCL) with 53%, followed by small lymphocytic lymphoma, also covering 22.2% (Dei-Adomakoh et al., 2017). Sharma *et al* conducted the study in a relatively shorter period, using a comparatively smaller sample size. A study conducted in Nigeria presented relatively high B cell lymphoma type of 98.8%, with only 1.2% for T cell types. The subtype distribution of 66 NHL cases obtained include DLBCL (59%), marginal zone lymphoma (MZL) [22.2%], Burkitt lymphoma (BL) [10.6%], small lymphocytic lymphoma (SLL) [9.1%] and anaplastic large cell lymphoma (ALCL) [1.5%] (Onwubuya, Adelusola, Durosinmi, Sabageh, & Ezike, 2015). Another study done in South Africa with a relatively larger sample size of 487 had B and T cell lymphoma distribution of 85.8% and 14.2% respectively. The subtypes reported included DLBCL (38.2%), follicular lymphoma (FL) [18.1%], SLL (8.4%), BL (8.2%), peripheral T cell lymphoma (9.7%) and T lymphoblastic lymphoma [3.7%] (Perry et al., 2016). Additionally, much higher B cell lymphoma of 87.3% against 12.7% T cell lymphomas were identified in Central and South America (Laurini et al., 2012).

2.8 Common phenotypic subtypes of non-Hodgkin lymphoma

2.8.1 Small lymphocytic lymphoma

Chronic lymphocytic leukaemia (CLL) and Small lymphocytic lymphoma (SLL) are the same disease with different presentations. The different names are due to the predominant location of small and mature looking lymphocytes. CLL has significant number of lymphocytes that accumulate in the bone marrow and peripheral blood. The bone marrow and lymph node is populated with significant lymphocytes in SLL. The same morphologic presentation and

immunophenotype are exhibited in CLL and SLL hence, the same treatment regimen is employed (Zelenetz et al., 2015). SLL is diagnosed based on the presence of $< 5 \times 10^9$/L peripheral B lymphocytes (Hoffbrand & Moss, 2016) and clinical presentation (lymphadenopathy and or splenomegaly). CLL may be characterized by the absence of lymphadenopathy and $\geq 5 \times 10^9$/L peripheral B lymphocytes (Zelenetz et al., 2015). Cell surface markers are determined by flow cytometry in CLL using peripheral blood. Immunohistochemical analysis is best done on lymph node biopsy in SLL. CLL/SLL shows positivity for CD5, CD19, CD20 (dim intensity), and CD23 and surface immunoglobulin (kappa/lambda). CLL/SLL does not exhibit CD10, cyclin D1. Majority of people are affected around the age of 65 with male to female ratio of 2:1 (Yin et al., 2005).

2.8.2 Follicular lymphoma (FL)

Follicular lymphoma (FL) affects cells of the germinal centres and normally exhibits follicular growth pattern with different proportions of small centrocytes and large centroblasts (Townsend & Marcus, 2016). It emanates from the malignant transformation of mature B lymphocytes of follicular center (Fauzi et al., 2015).

FL exhibits markers of B cell such as CD19, CD20, CD22 and CD79a and germinal center makers, BCL 2, BCL 6 and CD10. They are also characterized by the expression of single type surface immunoglobulin (Goteri et al., 2011). About 90% of FL cases exhibit t (14; 18) (q32; q21) chromosomal translocation. This t (14; 18) translocation leads to the expression of altered BCL 2 gene with significantly reduced apoptotic rate of cells (Hoffbrand & Moss, 2016). The development of FL is not associated with this alone but is believed to be linked to other genomic processes (Ning et al., 2013). It is an indolent NHL type and tends to widely spread in most cases

at the time of diagnosis, affecting several lymph nodes, liver and spleen (Tageja, Padheye, Dandawate, Al-katib, & Mohammad, 2009). The frequent spread and bone marrow involvement is due to the fact that some FL patients (15%) have circulating lymphoma cells (Townsend & Marcus, 2016). The current treatment is unable to cure the disease and most patients tend to relapse after treatment with shorter intervals of remission in between. Approximately 3 out of 10 patients experience disease transformation to Diffuse Large B-Cell Lymphoma (DLBCL). FL is the second most common form of NHL occurring in the United States (Tageja et al., 2009).

2.8.3 Marginal Zone Lymphoma

Marginal zone lymphoma (MZL) is a neoplasm that originates from mature B-cells. It is an indolent form and is put into three groups as nodal, splenic and extra-nodal type of mucosa tissue. Although these three types vary in the clinical presentation and prognosis, they appear similar in phenotype (Olszewski & Castillo, 2013). The cells that constitute marginal zone has distinct morphology of abundant dim cytoplasm that surrounds an oval or reniform nucleus. There is the presence of an irregular and moderate-sized central nucleolus and relatively smaller bar-formed chromatin. The unique morphology of the cells accounts for pale perifollicular corona appearance of the marginal region. The surface markers, CD20, CD79a, BCL2 and surface immunoglobulins (IgM, IgG or IgA), are expressed in MZL. The marginal zone cells are negative for CD5, CD10, CD11c and CD23 (Diebold, Tourneau, Comperat, & Molina, 2005).

2.8.4 Diffuse Large B-cell Lymphoma (DLBCL)

The World Health Organization defines DLBCL as a clinically, morphologically and genetically heterogeneous group of malignant proliferations of large lymphoid B cells (Paepe & Wolf-Peetrs, 2007). DLBCL is aggressive and the most common subtype of NHL. The disease presents as an advanced form in most patients at the time of diagnosis (Horvat, Zadnik, Šetina, & Boltežar, 2018). It originates from large B cells. The majority of cases of DLBCL (about 80%) constitute cells resembling germinal center (GC) centroblasts (Friedberg, 2008). These are termed germinal center B-cell-like (GCB) DLBCL. The second sub-group, activated B-cell-like (ABC) DLBCL, has antigenic pattern that is similar to post-germinal center cells (Colomo et al., 2003). The third sub-group (type 3) presents genes which are different from either sub-groups above. GCB-like type has better prognosis compared to that of ABC-like and type 3. The three groups of DLBCL above are determined by microarray analysis. However, the two prognostic groups (GCB and ABC DLBCL) can now be shown by immunohistochemistry. Phenotypically, GCB-like type are identified by CD10 and bcl-6. MUM1or interferon regulatory factor 4 are used as ABC-like or non-GCB-like markers (Paepe & Wolf-Peetrs, 2007). Thus, GCB are positive for either CD10 or BCL6 without MUM1 whiles non-GCB are either negative for both CD10 and BCL6 or are CD10 negative, but express both BCL6 and MUM1 (Okosun & Cwynarski, 2016). The immunohistochemical (IHC) panel of DLBCL include BCL2, BCL6, CD3, CD5, CD10, CD20, CD45, Ki-67 AND MYC. The IHC is often positive for CD20 and CD45 and negative for CD3 (Colomo et al., 2003).

Morphological variants of DLBCL include centroblastic type, immunoblastic type, anaplastic type, T cell-rich/Histiocyte-rich type. The centroblastic type is identified by germinal center centroblast and occurs in about 80% of DLBCL cases. Immunoblastic type has almost entirely

immunoblasts and constitute about 10% cases of DLBCL. It belongs to the ABC type. A background of reactive T cells and histiocytes characterizes T cell-rich/Histiocyte-rich type (Friedberg, 2008). Several sub-types of DLBCL are associated with specific anatomic sites including; primary CNS DLBCL, primary cutaneous DLBCL (leg type) and intravascular large B-cell lymphoma. Primary CNS DLBCL is restricted and affects the central nervous system. Patients with intraocular disease may have simultaneous or sequential CNS involvement. Intravascular large B-cell lymphoma occurs within the lumina of small or intermediate vessels making it difficult to be seen.

EBV positive DLBCL is clonal proliferation of B cells linked to positive EBV disease. This subtype is common in the elderly (Xie, Pittaluga, & Jaffe, 2015). DLBCL can arise de novo or as the result of transformation of already existing lymphoma (Ondrejka & Hsi, 2015).

An improved treatment outcome is seen in DLBCL patients with combination of chemotherapy [cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP)] and rituximab (R-CHOP) (Horvat et al., 2018).

2.8.5 Burkitt lymphoma

Burkitt lymphoma (BL) is the commonest cancer occurring in children in sub-Saharan Africa. It often occurs in young adults and is frequently linked to HIV (Gopal & Gross, 2018). It is a highly aggressive B cell NHL type and serves as the most rapidly proliferating tumour with almost 100% rate of proliferation. However, BL experiences high rates of apoptosis (Molyneux et al., 2012). It is divided into three clinical subcategories as endemic, sporadic and immunodeficiency-associated BL according to the World Health Organization (Bellan et al., 2003).

The endemic BL coincides with malaria distribution in Africa. It ranges from 2 to 16 years but dominates in individuals from 4 to 7 years. The disease is relatively common in male children with a 2:1 male to female ratio. It has a strong association with Epstein Barr Virus (EBV) infection. Endemic BL affects the bones of the jaw and face, gastrointestinal tract, ovaries, breast, renal system and other extranodal sites (Olaniyi, 2012). Sporadic BL occurs in all geographical locations. It however occurs among adults and the common sites of involvement are the terminal ileum and lymph nodes (Bellan et al., 2003). Immunodeficiency-related BL is highly observed among patients with HIV infection. There is a risk of developing BL after 4 to 5 years of organ transplantation. BL shows morphological 'starry sky' appearance that emanates from dispersed macrophages that contain dead tumour cells. The malignant cells originate from B cells with positivity for CD20 and CD79a. Also, CD10 and Bcl-6 are often expressed (Molyneux et al., 2012).

BL arises from lack of genetic conformity by the c-MYC proto-oncogene as a result of chromosomal translocations. The commonest translocation, occurring in about 80% of cases involves chromosome 8 and 14, t (8; 14) (q24.1; q32.3). This leads to subsequent translocation between the c-MYC proto-oncogene and IgH gene. About 15% of BL shows translocations between c-MYC and the gene for kappa light chain t (2; 8). The translocation between lambda light chain and c-MYC t(8;22) is exhibited in the rest of cases (Olaniyi, 2012).

2.8.6 Lymphoplasmacytic lymphoma

Lymphoplasmacytic lymphoma (LPL) is a B cell neoplasm characterized by the accumulation of lymphoplasmacytic cells, mostly within the bone marrow, and in extramedullary sites (including the lymph nodes and spleen). Majority of cases of LPL are termed as Waldenström's

macroglobulinemia (WM) when LPL is associated with the clonal production and accumulation of IgM paraprotein. There are rare cases of LPL which are not WM including; LPL cases with lymphadenopathy and clonal IgA or IgG production in bone marrow (Kristinsson, Goldin, & Turesson, 2012). The condition is commonly found in men beyond 50 years of age. It is known to originate from post-germinal B cells with the characteristics of an IgM-bearing memory B cell. Majority of cases (over 90%) are due to mutation of the gene *MYD88* (Hoffbrand & Moss, 2016). Immunohistochemical studies show the expression of CD19, CD20, CD22, FMC7, BCL2, CD38, and CD79a with monotypic surface light chain. About less than 20% of cases may be positive for CD5, CD10, or CD23 (Naderi & Yang, 2013).

2.8.7 Mantle cell lymphoma

Mantle cell lymphoma (MCL) is an aggressive NHL that originates from mature B lymphocytes (Schieber, Gordon, & Karmali, 2018). The malignant cells come from the pre-germinal center cells in the primary follicles or the mantle region of the secondary follicles (Hoffbrand & Moss, 2016). The disease is underlined mostly by t (11; 14) (q13; q32) translocation involving the CCDN1 gene encoding cyclin D1, and the immunoglobulin heavy chain gene. The translocation subsequently leads to the overexpression of cyclin D1. Other few cases of MCL which do not show t(11;14)(q13;q32) translocation may exhibit modifications in *CCND2* and *CCND3* genes which encode respectively cyclin D 2 and D3 (Schieber et al., 2018). It is frequent in males with 3:1 male to female ratio. It expresses CD5, CD19 and CD20 markers (Dreyling et al., 2017).

2.8.8 T/NK cell lymphomas

T cell non-Hodgkin lymphomas (T-NHLs) are relatively uncommon and constitute diverse neoplasms that affect the lymphoid system. T-NHLs are generally aggressive, resistant to treatment and present with poor prognosis. This is an exception to few indolent types (Poggio, Duyster, & Illert, 2018). T-NHLs include angioimmunoblastic T cell lymphoma, anaplastic large cell lymphoma, extranodal NK/T cell lymphoma, mycosis fungoides and sezary syndrome. Angioimmunoblastic T cell lymphoma (AITL) is one of the commonest T cell lymphomas (Rizvi, Evens, Tallman, Nelson, & Rosen, 2006). AITL presents with the common clinical feature of generalized lymphadenopathy. It is commonly associated with immune-mediated haemolytic anaemia, hypergammaglobulinaemia and eosinophilia (Mosalpuria, Bociek, & Vose, 2014) The cells involved are small and medium-sized types together with plasma cells and B-immunoblasts. A bunch of eosinophils and histiocytes may be seen. AITL shows CD4 and CD8 markers (Rizvi et al., 2006).

Anaplastic large-cell lymphoma (ALCL) is clinically put into two, systemic and primary cutaneous types. Anaplastic lymphoma kinase positive *(ALK*+) ALCL affects most often the lymph nodes with high frequency in children. The common extranodal sites of involvement include skin, bones, soft tissue, lung and liver. Majority of cases of ALCL present as end stage disease (Mundada, Ahmed, & Santa, 2017).

2.9 Antigenic markers

2.9.1 B cell markers

CD20

CD20 is a protein embedded in the membrane of B cells with exception of early pro-B cells, plasmablasts and plasma cells. It is one of the best targets for detection of B-cell lineage development in B-cell lymphomas (Chu, Loera, Huang, & Weiss, 2006). It is a 297 amino acid molecule. It presents as a four-part polypeptide that traverses B cell membranes. It is involved in the differentiation, maturation and activation of B cells. CD20 activates intracellular proteins such as Lyn and Fyn through a phosphorylation cascade by first making contact with Src family tyrosine kinases. The exhibition of CD20 varies in different lymphoma subtypes (Katchi & Liu, 2017). Determination of CD20 is useful for treatment of B cell lymphomas. A monoclonal antibody Rituximab is given to CD20 positive B-cell NHL patients. Other B cell markers such as CD19, PAX-5 and CD79a become useful in determining B cell lineage after treatment with Rituximab as CD20 turns negative (Rizzo & Nassiri, 2012). These markers are also useful in determining rare CD20 negative B cell-NHL cases. Less than 2% cases of NHLs that originate from B cells do not express usual CD20 antigens. This occurs in plasmablastic lymphoma, primary effusion lymphoma, large B-cell lymphoma arising from HHV8-associated multicentric Castleman's disease, and ALK+ large B cell lymphoma (Katchi & Liu, 2017).

CD5

CD5 is a 67-kDa transmembrane glycoprotein. It is not lineage specific and therefore exhibited by almost all T cells and some subset of B cells (B1a) in the follicular mantle zones (Pramoda et al., 2014). It plays a role in T and B cell receptor signaling (Rizzo & Nassiri, 2012) and is involved

in activation of T cells. CD5 reduces the effect of activated B-lymphocytes. CD5 also induces the generation of some cytokines such as IL 10 which preserves B cells from autoimmunity (Pramoda et al., 2014). It is not expressed in NK cells (Higgins, Blankenship, & Kinney, 2008). The levels of CD5 on normal B cells are high in the foetus and the elderly and low in children and young adults. Normal CD5 B cells are characterised by the presence of CD23 immunoglobulins M and D. The variable region of the immunoglobulin has no mutations. Conversely, the malignant CD5 B-cells often have hypermutations on their variable region of immunoglobulin heavy chain and are antigen experienced (Pramoda et al., 2014). Anti-CD5 becomes useful in classifying T cell lymphomas and some B cell neoplasms including mantle cell lymphoma and small lymphocytic lymphoma (Rizzo & Nassiri, 2012).

CD23

CD23 serves as a receptor located on resting cells and some activated B cells and binds weakly to immunoglobulin (Ig) E (Diraimondo et al., 2002). It belongs to the C-type lectin family(Liu, Richard, Melvin, Zhu, & Conrad, 2016). Co-expression of CD23 and CD5 allows the distinction of SLL/CLL from mantle cell lymphoma. CD23 protein is exhibited by virtually all cases of SLL/CLL. The reverse occurs in other small B cell neoplasms such as mantle cell lymphoma and marginal zone lymphomas. There is therefore the tendency of missing some rare subsets of mantle cell lymphoma (Schlette, Fu, & Medeiros, 2003). The expression of CD23 markers is known to have prognostic significance (Diraimondo et al., 2002).

CD10

CD10 is a cell surface enzyme involved in proteolysis. CD10 is also called common acute lymphoblastic leukaemia antigen (CALLA) as it was initially observed to be frequently expressed in acute lymphoblastic leukaemic cells (Mishra, Singh, & Narayan, 2016). It is commonly found on surfaces of GCB cells and other cells including epithelial cells and lymphoid precursor cells. It is a follicular center cell maker and therefore serves as a signal for GCB-like DLBCL. It occurs in about 30 to 40% of DLBCL cases. So far CD10 has no identified function but manifests in follicular, Burkitt's and lymphoblastic cancers (Paepe & Wolf-Peetrs, 2007).

BCL2

B cell lymphoma 2 (BCL2) belongs to BCL2 family of proteins which have both stimulatory and inhibitory roles on apoptosis. The anti-apoptotic is strongly associated with many neoplasms, including NHLs (Adams, Mitra, Gong, & Eischen, 2017). BCL2 is highly expressed in follicular lymphoma and some DLBCL (Aaron, Engelman, & Faber, 2016). The follicular hyperplasia is differentiated from malignant follicular morphology by the exhibition of BCL2 in neoplastic cells together with CD10 protein (Chen, Jensen, & Li, 2003). It is localized at the membrane and/or the cytoplasm (Portier & Taglialatela, 2006).

BCL6

B cell lymphoma 6 (BCL6) serves as a transcription factor that is involved in vital antibody responses in both normal B cells and T cells (Wagner, Ahearne, & Ferrigno, 2010). BCL6 is associated with lymphomas that originate from the germinal centers (Jia et al., 2019). It is linked with cells of the germinal centers. It is associated with chromosomal translocations in DLBCL and nodular lymphocyte predominant Hodgkin lymphoma. It is frequently exhibited in follicular lymphoma, Burkitt's lymphoma and T-cells of angioimmunoblastic T-cell lymphoma. Thus it allows the classification of DLBCL into germinal center (GC) subtype. GC-DLBCL is associated with good prognosis(Wagner et al., 2010).

Tables 2 below gives a summary of the characteristics of some specific B cell NHLs in immunohistochemical studies.

Table 2: Immunophenotypic behaviour of some B-cell NHLs

B-cell Non-Hodgkin lymphomas		CD20	CD5	CD10	CD23	BCL2	BCL6	MUM1	SIg
Diffuse large B-cell lymphoma	GCB	+	-	+	+/-	+/-	+	-	+/-
	ABC	+	-	-	+/-	+/-	-/+	+	+/-
Small lymphocytic lymphoma		+	+	-	+	+	-	-	Wk
Follicular lymphoma		+	-	+	+/-	+	+	-	+
MALT lymphoma		+	-	-	+/-	+	-		-
Mantle cell lymphoma		+	+	-	-	-	-	-	+
Burkitt lymphoma		+	-	+	-	-	+	-	+
Marginal Zone lymphoma (Nodal)		+	-	-	-	+	+/-	+	+/-
Marginal Zone lymphoma (Splenic)		+	-	-	-	+	-	+/-	+
Hairy cell lymphoma			-	+/-	+/-	+	-		-
Lymphoplasmacytic lymphoma		+	-	-	-		-	-	+

wk, weak; **GCB**, germinal center B cell type; **ABC**, activated B cell type; **CD**, cluster of differentiation; **MALT**, mucosa associated lymphoid tissue; **SIg**, surface immunoglobulin; **+**, means more than 50%; **-**, means less than 5%; **+/-**, means 5-25%; **MUM1**, multiple myeloma oncogene 1; **BCL2**, B cell lymphoma 2 protein; **BCL6**, B cell lymphoma 6 protein.

-------- (Barroca & Marques, 2016; Townsend & Marcus, 2016; Okosun & Cwynarski, 2016; Higgins et al., 2008)

2.9.2 T/NK cell markers

CD3 forms part of receptor T cell marker, hence, known as T cell lineage specific. It is expressed in an early stage during T lymphocytes, natural killer (NK) cells, and T cell and NK cell neoplasms (Rizzo & Nassiri, 2012). Some few lymphoid malignancies arising from T cells may not express CD3 markers. Anaplastic large cell lymphoma is known for this situation. CD3 marker is also expressed by some NK cells (Higgins et al., 2008). CD3 marker is localized at the membrane of T cells or cytoplasm of NK cells.

A greater proportion of T cell lymphomas express **CD4** protein. These include; adult T cell lymphoma, angioimmunoblastic T-cell lymphoma, anaplastic large cell lymphoma (ALCL) and mycosis fungoides (MF). Both T cells and NK cells that have undergone malignant transformation may have **CD8** marker. This usually involves extra-nodal sites. There may be expression of both CD4 and CD8 in some few cases of lymphomas such as ALCL and MF (Higgins et al., 2008).

The tables 3 below gives a summary of the characteristics of some specific T cell NHLs in immunohistochemical studies.

Table 3: Immunophenotypic behaviour of some NK/T-cell NHLs

T-cell Non-Hodgkin lymphomas	CD3	CD5	CD4	CD7	CD8	CD30	CD56
Angioimmunoblastic T-cell	+	+	+/-	+	+/-	-	
Anaplastic large cell lymphoma	+/-	+/-	+/-		+/-	+	
Mycosis fungoides	+	+	+	+/-	-	-	
Extranodal NK/T-cell lymphoma, nasal type **	+/-						+
Sezary syndrome **	+		+	-			

CD, cluster of differentiation; **+**, more than 50%; **-**, less than 5%; **+/-**, 5 to 25%; Extranodal NK/T-cell lymphoma, nasal type has additional markers, CD2+, EBV+ and exhibit sCD3–, cCD3+; Sezary syndrome shows erythroderma and circulating cells >1000/mm3.

---------- (Hoffbrand & Moss, 2016; Turner et al., 2004)

2.10 Treatment of NHLS

The determination of treatment regimens for patients suffering from NHL depends on the person's age, general health and ability to tolerate side effects, the disease sub-type and stage (Armitage, 1993) and the tumor grade (Macdonald, 2009). Treatment options of NHLs include one or combination of; chemotherapy, immunotherapy, target therapy, high dose chemotherapy and stem cell transplant, surgery and radiotherapy. Supportive care (palliative treatment) may be necessary to alleviate the symptoms due to the disease including infections and low blood cell counts (American Cancer Society, 2018).

2.10.1 Chemotherapy

Chemotherapy employs the use of anticancer drugs. It serves as an effective treatment regimen for advanced and aggressive NHLs (Cai, Hao, Wang, & Xu, 2017). Chemotherapy drugs disrupts the life cycle of cancer cells by causing harm to the DNA and cellular parts needed for the production of new cells. The commonly used drugs include; corticosteroids such as prednisone and dexamethasone, alkylating agents such as cyclophosphamide and chlorambucil, purine analogs, anti-metabolites and platinum drugs (American Cancer Society, 2018). It is usually administered by combining several drugs. The first combination therapy introduced comprises cyclophosphamide, doxorubicin (hydroxydaunorubicin), vincristine (oncovin) and prednisone [CHOP]. Several second or third-generation regimens have been developed. New drugs needed to increase fractional dose are incorporated in subsequent generations. Some new CHOP therapies are combined with immunotherapy (Cai et al., 2017). However, chemotherapy drugs lack the ability to distinguish between malignant and normal cells. They kill rapidly dividing cells and generate side effects including bone marrow suppression, gastrointestinal problems and hair loss (Macdonald, 2009)

2.10.2 Immunotherapy

Immunotherapy is used currently for treating haematological malignancies, including NHLs. The localization of certain proteins in tumors serve as potential targets for treatment of NHLs (Poggio et al., 2018). Immunotherapy treatment technique seeks to improve the body's natural defense mechanism. Rituximab, an anti-CD 20 immunotherapy drug attaches to CD20 proteins on cancer cells and marks them for destruction.

2.10.3 Target therapy

The aim of any cancer therapy (including targeted therapy option) involves targeting specific surface and intracellular proteins, thereby marking cancerous cells for destruction and avoiding damage to normal cells. In this regard, several monoclonal antibodies are designed against specific antigens on or in lymphocytes. The monoclonal antibodies are designed against cells at different maturation stage and target antigens including CD20, CD22, CD19, CD37, CD80, CD52, CD4 and CD3 (Khubchandani & Czuczman, 2009). The expression of CD20 is highly limited to benign and neoplastic B cells and remain one of the best targets. Rituximab and ofatumumab monoclonal antibody therapy are first and second generations respectively against CD20 (Anas Younes, 2011).

2.10.5 Radiotherapy

Radiotherapy is useful for a localized and early stage curative/primary treatment of some NHLs including indolent lymphomas. It is given to consolidate systemic chemotherapy in a combination therapy approach for localized and aggressive NHLs types. Radiotherapy on the other hand may not be effective in well advanced cases (Zimmermann, Oehler, Mey, Ghadjar, & Zwahlen, 2016).

2.11 Treatment outcomes of NHLs

NHLs are characterized by various age-related differences in clinical presentation, biology, and outcome. Treatment outcomes of NHLs differ from various age groups and this may be attributed to sociologic, psychosocial, general medical condition and biology of NHL type. The spectrum of NHL subtypes varies with age. High-grade tumors including Burkitt lymphoma, diffuse large B-cell lymphoma, lymphoblastic lymphoma, and anaplastic large cell lymphoma, are seen very often in children. Low-grade forms such as follicular lymphoma and marginal zone B-cell lymphomas on the other hand are frequent in young adults and the elderly. The differences in disease biology of various subtypes between age groups have major effects on treatment approach and outcome (Sandlund & Martin, 2016).

The disease outcome is therefore influenced by the ability to classify lymphoma sub-type and the selection of effective therapy (Klener & Klanova, 2020). There has been the development of improved and novel therapies over the past years (Hazarika et al., 2015). More than 50% of patients confirmed for NHLs tend to do well on the conventional chemotherapy. However, a significant percentage experience relapse after some time and others are refractory to treatment (Chao, 2013; Hafez, Hussein, & Ismail, 2018).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

This was a retrospective, descriptive study. Archived formalin-fixed paraffin-embedded tissue blocks of previously diagnosed NHL cases (by morphology) were used for the study. Retrospective descriptive analysis is an accepted approach to conducting research. This was adopted to allow the attainment of a required sample (archived) for the study with limited population. Archived tissue blocks remain a viable sample for immunohistochemistry studies.

3.2 Study site

The study was carried out at the Haematology Department, Korle-Bu Teaching Hospital (KBTH). KBTH is currently the third largest hospital in Africa and serves as the leading national referral center in Ghana. The Haematology Department receives about three hundred and eighty two (382) new cases in a year with majority being referred from the Out Patients Department (OPD). The average number of new NHLs received in a year is 55 with about 40 being adults (aged ≥ 13). However, about 4,538 OPD attendants are being followed up in a year.

Immunohistochemical analysis of the archived tissues (mostly lymphoid) was carried out at the Department of Pathology, UG/KBTH. The Department's research unit is fully equipped and has the technical expertise to undertake immunohistochemical studies.

3.3 Study period and population

The study used archived tissue blocks from patients histologically diagnosed with NHL at the Department of Haematology, KBTH, between 2015 and 2019. The actual study was however conducted between October 2019 and April 2020.

3.4 Inclusion criteria

- All patients aged fifteen (15) and above.
- Cases histologically (morphology) diagnosed as NHL.

3.5 Exclusion criteria

- Patients whose archived tissue blocks were exhausted or could not be found.

3.6 Sample size determination

The sample size was estimated using Cochran's sample size formula below:

$$\mathbf{n} = \frac{Z^2_{\alpha/2}\{P(1-P)\}}{e^2}$$ ---------- (Pourhoseingholi, Vahedi, & Rahimzadeh, 2013)

Where **n** is the minimum sample size; $Z_{\alpha/2}$ (Z score) is the standard score for 95% confidence level (1.96); **P** is the sample proportion (0.9); **e** is the allowable margin of error (5%).

Non-Hodgkin lymphomas constitute about 90% of cases of malignant lymphoma (Batra et al., 2014). This proportion covers B cell, T cell and NK cell antigenic markers which determine the origin and distribution of NHL.

Hence, $\mathbf{n} = \frac{\mathbf{(1.96)^2 \{ 0.9 (1\text{-}0.9)\}}}{\mathbf{(0.05)2}} = 138.29 \approx 138$

However, modification for the Cochran's formula for sample size calculation was opted for due to the research being a retrospective study involving a finite population. Thus, the research is intended to investigate past cases of NHL within a limited period (2015 to 2019). Therefore, the formula below was used to determine a new minimum sample size (adjusted sample size).

$$N = \frac{n_0 * \mathbf{n}}{n_0 + (\mathbf{n} - 1)}$$ …… (Naing, Winn, & Rusli, 2006)

Where N is the new, adjusted sample size; $\mathbf{n}$ =138 (calculated above), n_0 is Cochran's sample size recommendation, which is the estimated limited population size (120).

$$N = \frac{120 * 138}{120 + (138 - 1)} = 64.43 \approx 64$$

The minimum sample size that was targeted for the study is sixty four (64).

3.7 Sample and data collection

The folders of all histopathologically confirmed NHLs at the Haematology Department within the study period were retrieved from the medical records. A unique study number was assigned to each corresponding folder. A data abstraction form (Appendix A) was used to retrieve personal, laboratory and clinical information from the folders. Archived tissue blocks and hematoxylin and eosin (H&E) stained slides of these patients were retrieved at the Pathology Department, KBTH. The histopathology diagnosis of all cases were reviewed (by pathologist) using H&E slides and appropriate tissue blocks were selected for immunohistochemistry.

3.8 Materials and methods

Immunohistochemical staining of tissue sections was carried out using antibodies, anti-CD20, anti-CD23, anti-CD5, anti-CD3 and an 'optiview' DAB detection kit. This allowed the distribution of NHL into T and B cell types as well as detection of some specific subtypes.

Below are the major equipment and apparatus used for the studies;

a. Rotary microtome (LEICA RM2125 RTS)
b. Water bath (Boekel Scientific)
c. Thermometer
d. VENTANA BenchMark GX IHC/ISH automated instrument
e. Light microscope (Olympus)
f. Barcode label equipment
g. Thermostat oven (DHG-9053A)

3.9 Procedure

3.9.1 Microtomy

Formalin-fixed paraffin-embedded tissues were sectioned at 4 microns and mounted on positively charged slides. Positive control samples were placed on the same slide as the patient tissue. Slides were labelled and dewaxed in a hot air oven for 2 hours at 60°C.

3.9.2 Immuno-staining

Immunohistochemistry studies were performed using BenchMark GX automated IHC/ISH staining instrument (Tucson, VENTANA-Roche, USA). Monoclonal (mAb) antibodies, **anti-CD20** (L26, VENTANA-Roche, Tucson, USA), **anti-CD3** (2GV6, VENTANA-Roche, Tucson, USA), **anti-CD5** (SP19, VENTANA-Roche, Tucson, USA) and **anti-CD23** (SP23, VENTANA-Roche, Tucson, USA) were used with **OptiView DAB IHC detection kit**.

A standard operating procedure for BenchMark GX automated IHC/ISH staining instrument was followed. The reagents were registered on the BenchMark GX instrument. The primary antibody, counterstain and detection kit dispensers were loaded on the reagent tray and placed on the automated slide stainer. Bar code labels were generated and applied on each slide.

Slides were loaded on to the automated slide stainer and the 'run' button was engaged to start analysis. The IHC protocol shown in appendix C was used to programme staining processes by the BenchMark GX instrument. Slides were removed from the stainer after completion of a run. They were washed in mild dish washing detergent to get rid of cover slip solution. They were then rinsed in enough distilled water before permanent mounting was done.

The IHC principle of the main procedures used by the VENTANA BenchMark GX instrument are explained below:

Deparaffinization

Deparaffinization involves the removal of paraffin from sections. This is achieved on the BenchMark GX instrument using EZ prep concentrate solution.

Cell conditioning (antigen unmasking)

Tissues are subjected to heat-induced epitope retrieval technique to allow renaturation of proteins damaged by tissue fixation and hence regeneration of surface antigenic epitopes recognized by antibodies. BenchMark GX instrument uses cell conditioning solution (CC1) to attain this purpose. CC1 contains tris-based ethylenediamine tetraacetic acid (EDTA) with pH of 7.8.

Pre-primary peroxidase inhibition

Peroxidase inhibition tackles nonspecific antibody binding due to tissue peroxidase. The automated stainer undergoes tissue incubation with 3% H_2O_2.

Primary antibody incubation

Monoclonal antibodies directed against particular markers of interest are incubated with the tissues.

Detection technique

The localization of an antigen by primary antibody was observed by an Optiview detection technique. This technique employs a secondary antibody, HQ universal linker, which is linked to non-endogenous HQ haptens and directed against the monoclonal antibodies. There is a tertiary antibody linked to several horseradish peroxidase (HRP) multimer and also directed to the HQ haptens. 3,3-Diaminobenzidine tetrahydrochloride (DAB) serves as the substrate for HRP. It therefore reacts with HRP and hydrogen peroxide to produce a brown coloured signal.

The diagram below gives a snap shot of immunohistochemistry using the Optiview detection technique.

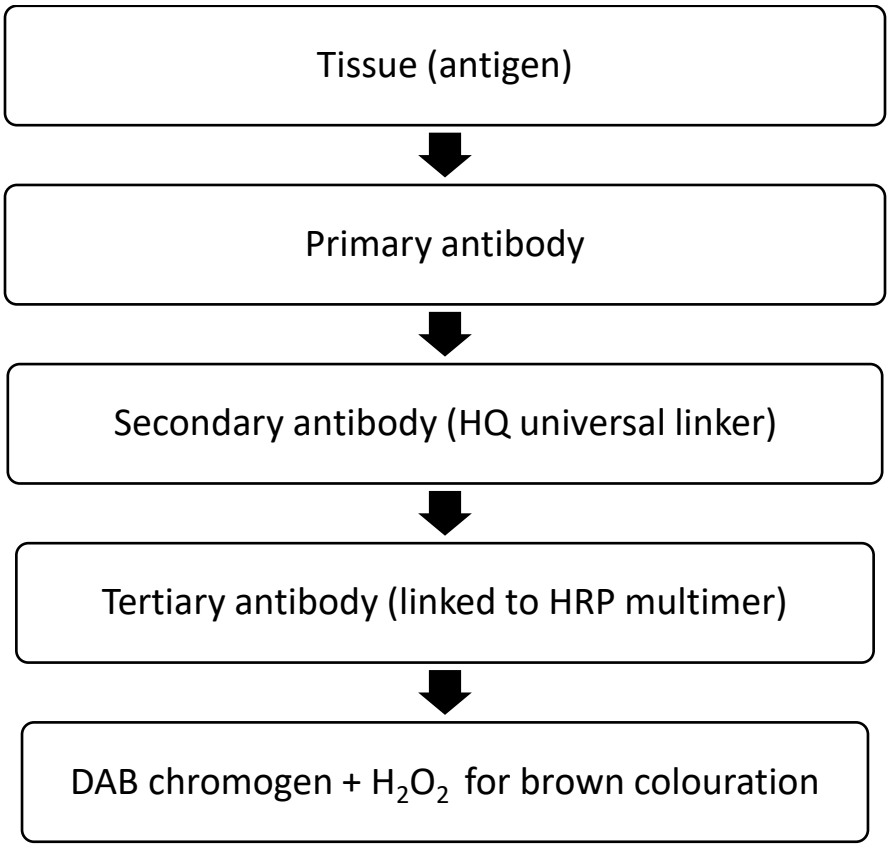


Figure 1: Diagrammatic presentation of the 'optiview' detection technique

Quality control and optimization

Quality control samples (positive and negative) were first analyzed. Lymphoid tissue known to express various antigenic markers (tonsil) was used for the positive control. Conversely, lymphoid tissues known not to express the target markers were used for negative control. Control samples were obtained from the Pathology Department, UG/KBTH. Unstained study samples were

observed under the microscope to monitor internal background staining. Reagent control was also carried out by tissue incubation with diluent of primary antibody alone. This was followed by incubation with secondary antibodies and detection reagents.

The antibodies were then optimized and validated for specific incubation and antigen retrieval times. Incubation times for the detection system were also varied and validated.

3.9.3 Mounting

The stained tissues prior to mounting were dehydrated and cleared according to the protocol in appendix B. Seven drops of organic mountant were added to the tissue on the slide and allowed to spread. The cover glass was flipped, placed down and centered on prepared slides with the mountant. The slides were placed on a leveled bench to dry for 24 hours.

3.9.4 Examination of slides

Immunohistochemical stained slides were examined under transmitted light illumination. The slides were scored based on the colour intensity and the percentage of involved tumour cells. Cellular staining patterns were determined. Scoring was done independently by two pathologists. Final scores were then determined by averaging independent scores. Discordant scores were re-examined by a third, independent individual and final score made from the two closest. A score of 0, 1+, 2+ and 3+ were defined as negative, weak, moderate and intense brown stained tissues respectively. The proportion of stained tumor cells were put to 3 categories: 0 for <25%, 1 for 25% to 75%, 2 for >75% of stained cells. Final conclusions (interpretation) were made with the help of the Pathologist, taking into consideration the morphological diagnosis, relevant clinical history and control results.

3.10 Data handling

All data obtained for the study was handled anonymously and confidentially. Data was doubly entered and checked for entry and range errors. During data entry and validation, database files were accessible by study investigator and supervisors only. Data was protected by password.

3.11 Statistical analysis

Data was entered into 2016 Microsoft excel and analyzed using Statistical Package for Social Science (SPSS) version 22. A summary was presented using the descriptive statistics of mean, median, standard deviation and frequency of variables. Graphical displays such as pie chart, box plots, frequency distributions or scattergrams, were created where appropriate. Pearson's chi-square test was used to determine associations between phenotypic cell types (based on immunological markers) and treatment outcome, as well as clinical features. In cases of sparse data (cells with less than 5) the Fisher's exact test was used. The z-test for proportions was used to compare diagnosis made by morphology and immunophenotypic patterns of adult NHL patients. All tests were two-sided and a p-value less than 0.05 was interpreted as significant.

3.12 Ethical issues

Ethical approval was sought and received from the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana.

CHAPTER FOUR

4.0 RESULTS

This chapter shows findings of the study. The findings are presented in accordance with the objectives of the study. Also, findings are presented as per the data collection tool used. A total of 66 cases of NHL were successfully selected for immunohistochemistry studies and hence used for analysis.

4.1 Demographic Characteristics

The youngest age recorded for the study was 16 years while the oldest was 78 years. The average age of participants in this study was 50.2±16.1 years. Majority 45.5% (n=30) of the participants fall within 41 to 60 year group. More than half 60.6% (n=40) of the participants were males. These are shown in Table 4.

Table 4: Demographic characteristics of participants

Characteristics	Frequency	Percent
Age group (years)		
≤ 40	18	27.3
41-60	30	45.5
> 60	18	27.3
Total	66	100
Sex		
Male	40	60.6
Female	26	39.4
Total	66	100

4.2 Antigen expression in various NHLs

In this study immunological markers (antigens) in various NHLs were identified using antibodies. Markers used included CD3, CD5, CD20 and CD23. Only 10.6% (n=7) of CD3 antigens were observed in this study. Also, 31.8% (n=21) were positive for CD5 antigens. Majority 89.4% (n=59) of the cases were positive for CD20. Additionally, 28.8% (n=19) of the cases were positive for CD23 antibodies. These are shown in table 5 below.

Table 5: Immunophenotypic pattern of non-Hodgkin lymphomas

Markers (antigens)	Frequency	Percent
CD3	7	10.6
CD5	21	31.8
CD20	59	89.4
CD23	19	28.8

4.3 Characterization of NHLs according to cell type

Figure 2 shows the distribution of NHLs according to pathological cell type. Majority 89.4% (n=59) were due to malignant transformation of B cells.

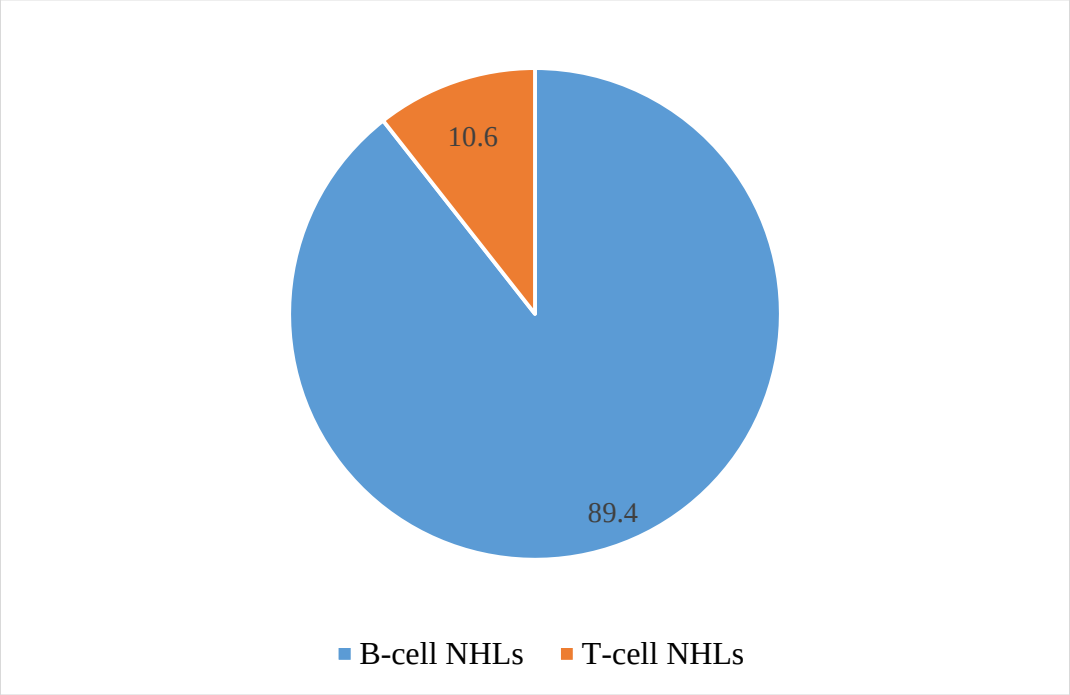


Figure 2: Distribution of NHLs by cell type

4.4 Cell types and sex

Distribution of cell types by sex is shown by figure 3.

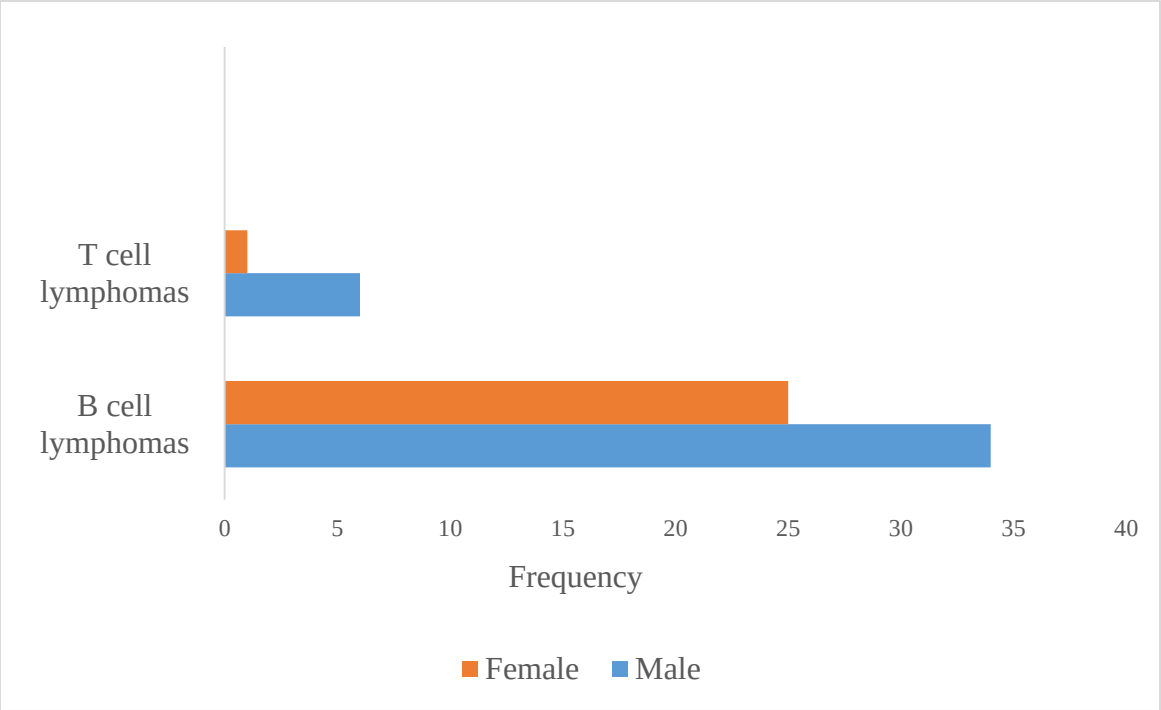


Figure 3: Frequency of cell types according to sex

4.5 Phenotypic distribution of NHLs

The commonest subtype 40.9% (n=27) observed in the study was diffuse large B cell lymphoma. Small lymphocytic lymphoma was identified in 12.1% (n=8) cases. Also, significant proportion 7.6% (n=5) of cases were mantle cell lymphoma. T cell lymphomas, mostly diffuse large forms 7.6% (n=5). However, substantial percentage of B cell lymphomas 25.8% (n=17) were not classified into specific subtypes. These are shown in table 6 below.

Table 6: Distribution of subtypes of NHLs

Phenotypic subtype	Frequency	Percent
B Cell NHLs		
Diffuse large B cell lymphoma	27	40.9
Small lymphocytic lymphoma	8	12.1
Mantle cell lymphoma	5	7.6
Extranodal-marginal zone lymphoma	1	1.5
Lymphoplasmacytic lymphoma	1	1.5
Other B cell types (non-classified)	17	25.8
T Cell NHLs		
Diffuse large T cell lymphoma	5	7.6
Other T cell types (non-classified)	2	3.0
Total	66	100

4.6 Selected images of slides of non-Hodgkin lymphomas

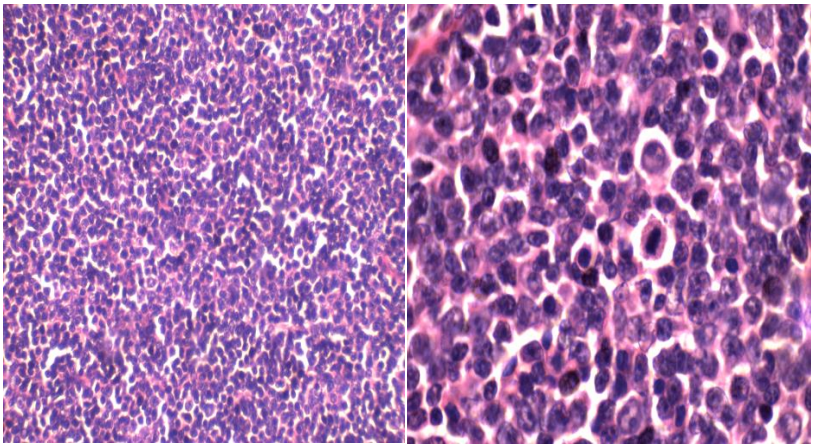


Figure 4: Photomicrograph of diffuse large cell Lymphoma

In figure 4, H & E (x10, *Left*; x40, *right*) showing a diffuse proliferation of large lymphoid cells. The cytoplasm is moderate to abundant, nuclei are irregular and nucleoli conspicuous. Mitotic figures are frequent.

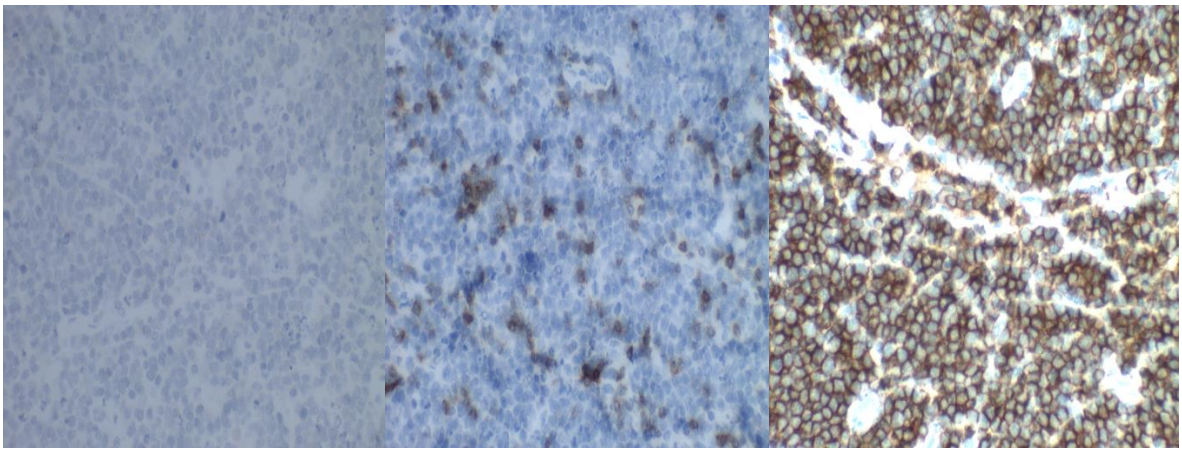


Figure 5: Photomicrograph of B-cell Lymphoma

Immuno-histochemical staining in the figure above shows strong membranous positivity for CD20

(x40, *right*). CD 3 staining (x10, *left*) is negative and CD 5 staining (x40, *middle*) highlights the reactive lymphocytes present.

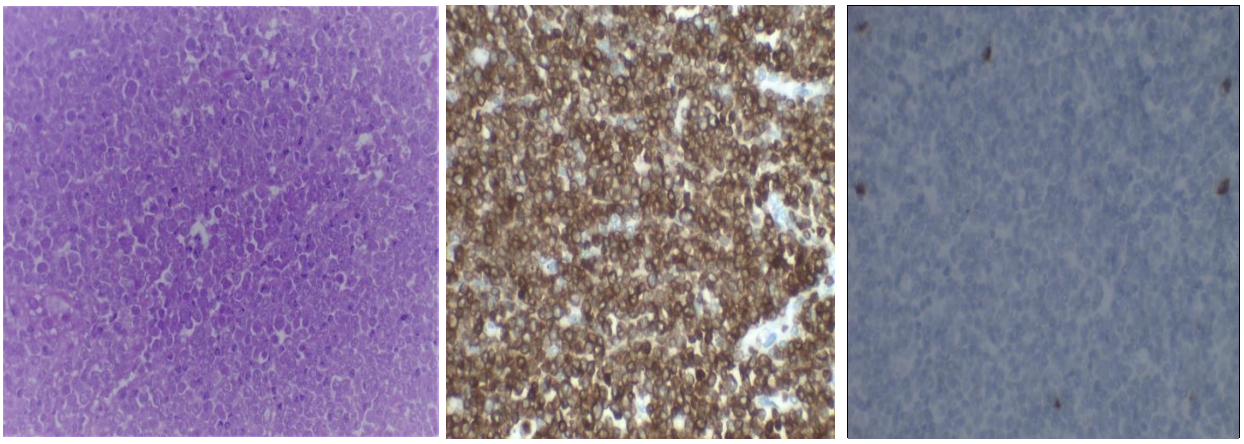
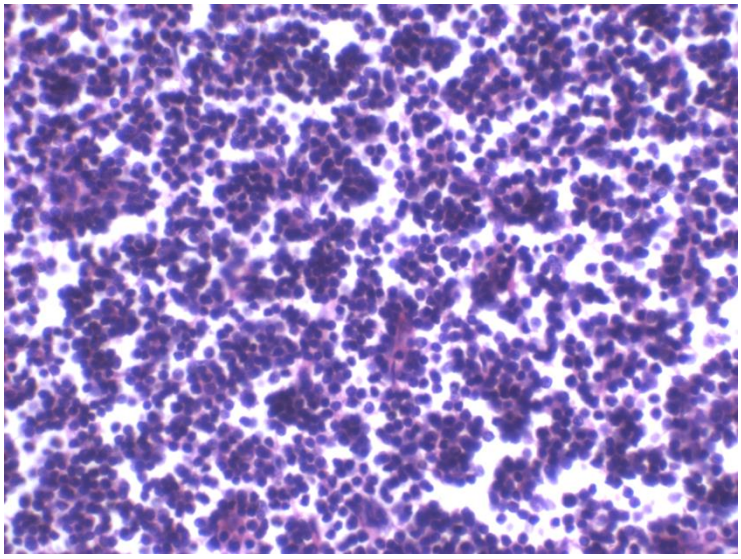
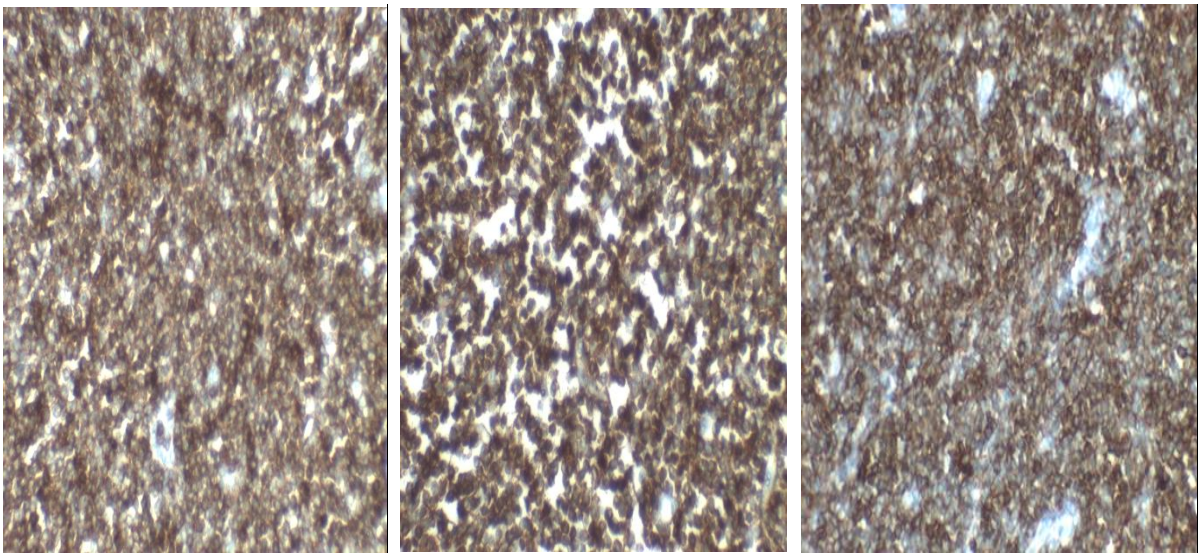


Figure 6: Photomicrograph of T-cell Lymphoma

In figure 6, H&E (x40, *Left*) showing a diffuse proliferation of variably sized lymphocytes with irregular nuclei; the neoplastic cells show strong cell membrane positivity to CD 3 (x40, *middle*); and do not express CD 20 (x40, *right*)

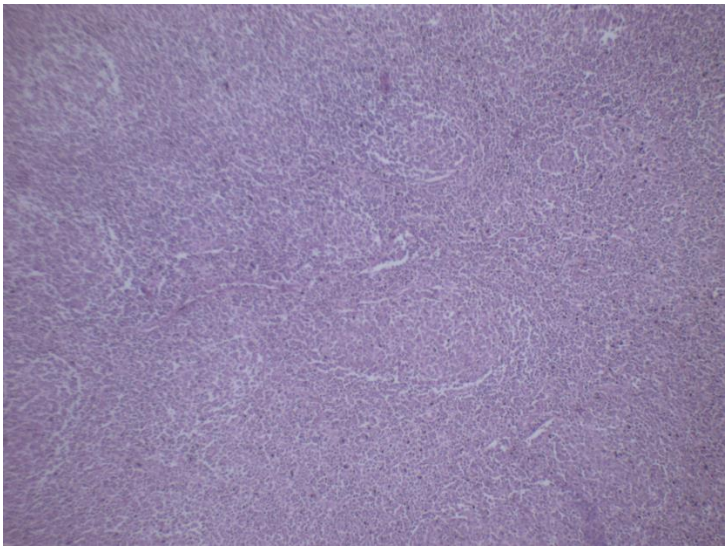


H & E staining (*x40)***:** Diffuse proliferation of small dark-staining, mature-looking lymphocytes. Neoplastic cells have scanty cytoplasm, small nuclei, condensed chromatin and conspicuous nucleoli. Mitotic count is low.

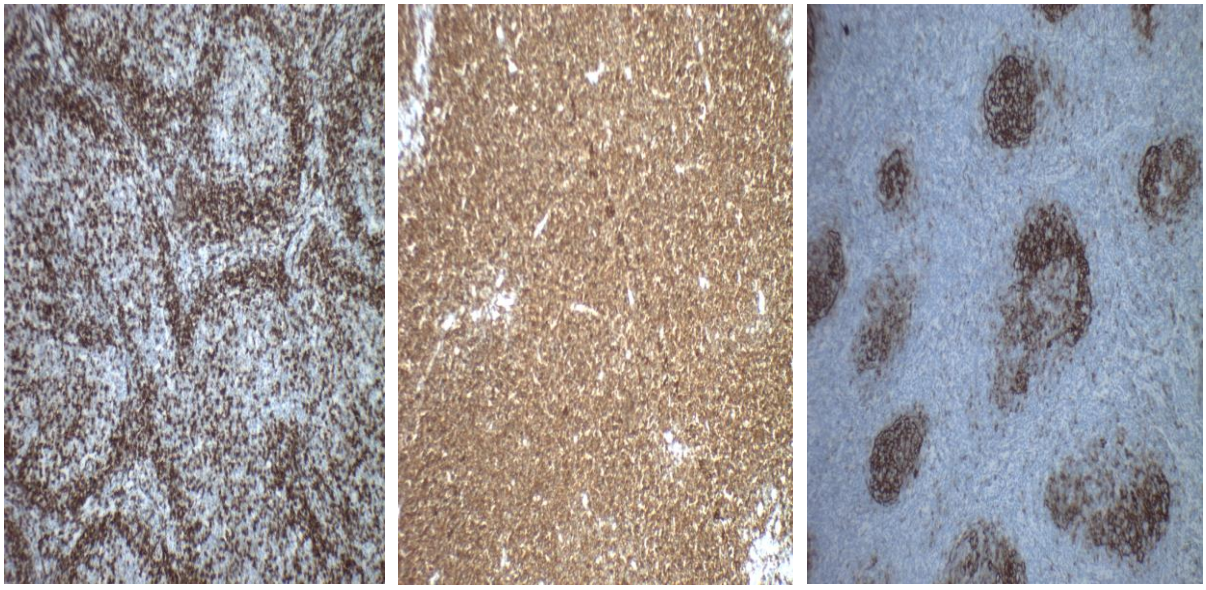


IHC staining: The neoplastic cells show membrane positivity for CD5 (*x40*, *left*), CD20 (*x40*, *middle*) and CD 23 (*x40*, *right*)

Figure 7: Photomicrograph of H&E and IHC staining of small lymphocytic lymphoma



H & E staining (*x10)***:** There is a vaguely nodular proliferation of small mature-looking lymphocytes. Scattered hyalinized venules are present.



A (CD 5) B (CD 20) C (CD 23)

IHC staining (*x40)***: B** shows membranous CD20 positive staining of the vaguely nodular neoplastic cells. In **A**, the neoplastic mantle B cells co-express CD5. In **C**, the neoplastic cells are CD23 negative, with highlighting of the non-neoplastic follicular B cells.

Figure 8: Photomicrograph of H&E and IHC staining of mantle cell lymphoma

4.7 Clinical characteristics and cell types

Table 7 shows clinical features exhibited by participants and their association with pathological cell types. Majority 87.9% (n=58) of the participants showed B-symptoms. Lymphadenopathy was observed among 66.7% (n=44) of the participants. However, only 9.1% (n=6) of the participants had splenomegaly and 7.6% (n=5) had hepatomegaly. Extra-nodal involvement was observed among 56.1% (n=37) of the participants. There was no significant association between clinical presentations (B-symptoms, lymphadenopathy, splenomegaly, hepatomegaly and extra-nodal presentation) and cell types.

Table 7: Association between clinical presentations and cell types

Clinical characteristics		B cell type	T cell type	Total	χ^2	p-value
B-symptoms	Yes	51 (86.4)	7 (100.0)	58 (87.9)	1.081	**0.299**
	No	8 (13.6)	0 (0.0)	8 (12.1)		
Lymphadenopathy	Yes	40 (67.8)	4 (57.1)	44 (66.7)	0.319	**0.572**
	No	19 (32.2)	3 (42.9)	22 (33.3)		
Splenomegaly	Yes	5 (8.5)	1 (14.3)	6(9.1)	0.256	**0.613**
	No	54 (91.5)	6 (85.7)	60(90.9)		
Hepatomegaly	Yes	4 (6.8)	1 (14.3)	5 (7.6)	0.504	**0.478**
	No	55 (93.2)	6 (85.7)	61 (92.4)		
Extranodal involvement	Yes	33 (55.9)	4 (57.1)	37 (56.1)	0.004	**0.951**
	No	26 (44.1)	3 (42.9)	29 (43.9)		

4.8 Morphology diagnosis and immunophenotype

Table 8 shows morphology diagnosis and corresponding pathological cell types. There was significant association between cell types and diagnosis made by morphology (p=0.001). In this study, all those diagnosed with diffuse large cell lymphoma 22 (100%), Non-Hodgkin lymphoma (small cell type) 12 (100%); Non-Hodgkin lymphoma (follicular type) 7 (100%); diffuse large and small cell lymphoma 4 (100%) and MALT lymphoma had B-cell types. However, all those with NHL with CNS involvement 2 (100%); NHL-Anaplastic large cell type 1 (100%) and NHL large cell type had T-cell types.

Table 8: Association between pathological cell types and diagnosis made by morphology

	CELL TYPES ON IHC				
HISTOLOGICAL DIAGNOSIS	**B-cells**	**T-cells**	**Total**	χ^2	**p-value**
? Burkitt Lymphoma	1 (100.0)	0 (0.0)	1 (100.0)	43.85	0.001*
Diffuse large and small cell lymphoma	4 (100.0)	0 (0.0)	4 (100.0)		
Diffuse large cell lymphoma	22 (100.0)	0 (0.0)	22 (100.0)		
Diffuse large cell lymphoma with CNS involvement	1 (100.0)	0 (0.0)	1 (100.0)		
MALT lymphoma	3 (100.0)	0 (0.0)	3 (100.0)		
NHL -Anaplastic large cell type	0 (0.0)	1 (100.0)	1 (100.0)		
NHL with CNS involvement	0 (0.0)	2 (100.0)	2 (100.0)		
NHL, lymphoplasmacytic type	1 (100.0)	0 (0.0)	1 (100.0)		
Non-Hodgkin lymphoma (NHL)	7 (70.0)	3 (30.0)	10 (100.0)		
Non-Hodgkin lymphoma (follicular type)	7 (100.0)	0 (0.0)	7 (100.0)		
Non-Hodgkin lymphoma (large cell type)	0 (0.0)	1 (100.0)	1 (100.0)		
Non-Hodgkin lymphoma (small cell type)	12 (100.0)	0 (0.0)	12 (100.0)		
Small and large cell lymphoma (follicular type)	1 (100.0)	0 (0.0)	1 (100.0)		

***Significant at 5%**

4.9 Cell types and outcomes

In this study, 37.3% (n=22) of those with B cell lymphomas had clinical remission while 14.7% (n=1) of those with T cell had clinical remission. Also, only 1.7% (n=1) of those with B cell lymphoma had a relapse while 28.6% (n=2) of those with T cell had a relapse. There was a significant association between cell types and clinical outcome (p=0.011). These are shown in table 9.

Table 9: Association between cell types of NHLs and clinical outcomes

Cell types	Clinical remission	No remission status	Refractory	Relapse	Total	χ^2	p-value
B cell	22 (37.3)	34 (57.6)	2 (3.4)	1 (1.7)	59 (100.0)	11.13	0.011*
T cell	1 (14.3)	4 (57.1)	0 (0.0)	2 (28.6)	7 (100.0)		
Total	23 (34.9)	38 (57.6)	2 (3.1)	3 (4.6)	66 (100.0)		

***Significant at 5%**

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

Immunohistochemistry studies throw more light and confirm already morphologically diagnosed non-Hodgkin lymphomas. This study was able to determine the phenotype of abnormal forms revealed by morphology and further characterized these neoplastic populations according to specific lymphoid lineage.

5.1.1 Demographic characteristics and cell types distribution by sex

Age and sex are known predisposing factors associated with non-Hodgkin lymphomas according to Alyahya *et al.* (2019). For the purpose of this study, the age of participants were put into adolescent and young adults (15-40) and older adults groups based on risk and guidance from National Cancer Institute, USA. Older adults were further put into two groups (41-60 and >60). The mean age was 50.2±16.1 years and comparable to a previous report done in the same unit by Dei-Adomakoh et al. Again, age distribution shows that a greater proportion (45.5%) of participants were within 41 to 60 older adult year group. Together, these indicate a relatively early onset of disease contrary to reports from Saudi Arabia and United states by Alyahya et al and Teras et al (2016) respectively which follow the pattern that majority of NHLs occur after 60 years. The early occurrence of NHL may be attributed to younger age distribution among the population and personal risk factors. Majority of Ghana's population is between 15 and 59 years according to the Ghana Statistical Service (Kpessa-Whyte, 2018).

Meanwhile, the study observed majority (72.8%) of cases in the older adult group as a whole and therefore suggest that NHL generally increases with age. Additionally, this agrees with the data

generated by the National Cancer Institute's Surveillance, Epidemiology, and End Results Program, USA (SEER) which shows a higher age-standardized rate of 10.5-116.4 per 100000 in the older adult groups as compared to 1.8-7.2 per 100000 for adolescent and young adult (AYA) group (Sandlund & Martin, 2016).

In the study, males outnumbered females with 1.54:1 male to female ratio. This observation is in agreement with other authors (Onwubuya et al., 2015; Yakubu et al., 2015; Alyahya et al. (2019). Also, male predominance was evident in both B and T cell types, although B cell lymphomas especially subtypes such as DLBCL and Burkitts lymphoma occur more in males according to SEER (Horesh & Horowitz, 2014).

The greater occurrence of NHLs in males may be attributed to socioeconomic factors such as occupation. Thus, the issue of sex-segregation continues to linger in labour especially in the traditional (informal) occupations in Ghana according to Abukari & Odai (2018) such that certain male dominated jobs including pesticide applicators, grain millers, wood and forestry workers, and farmers may be associated with higher NHLs in men in the study. Moreover, Horesh and Horowitz linked increasing number of pregnancies and live births as well as sex hormones such as estrogen to the reduction in the risk of developing NHLs in females.

5.1.2 Antigen expressions and NHLs distribution by cell type

This work mainly utilized the pattern of expression of markers of differentiation. A panel of differentiation markers, CD20, CD23 and CD5 were used to confirm neoplasms of B cells while T cell neoplasms were identified with CD3 and CD5. CD20, a pan-B cell maker, was observed in 59 (89.4%) of tumours while 27 of these co-expressed CD23. There was simultaneous co-

expression of CD5 with some CD3 and largely with some CD20 proteins. CD3, a pan T cell marker, was least expressed (10.6%). This is similar to findings in a previous study where at least CD20, CD5, CD23 and CD3 markers were used. CD20 was the commonest expressed marker (81.3%) followed by CD5 (50%). However, CD23 was the least expressed in that study (Atri, Singhal, Kataria, & Mohini, 2020).

The immunophenotypic findings show that 89.4% of the NHLs were due to malignant transformation of B cells whereas relatively few cases (10.6%) were derived from T cells. Research work done by Onwubuya et al, Perry et al, Sharma et al, Jairajpuri et al and Laurini et al in Nigeria, South Africa, India, Iran and Central and South America respectively indicates overall greater proportions of B cell lymphomas and hence, consistent with the findings of this study. Documented B cell lymphomas for these studies ranged from 66% - 98.8%. Despite this similarity, differences are observed in the incidence rates of B and T cell lymphomas. T cell lymphomas reported by Jairajpuri *et al* were about 3 times higher (34%) than that reported in my study while Onwubuya et al had only 1.2%. The disparities may be attributed to different geographical locations with varied but related underlying risk factors. For example, some infectious agents (such as *H. pylori*, HTLV-1 and HIV/AIDS) are known to be strongly associated with particular NHLs such that higher prevalence of those infections in various locations may correspond to the development of greater proportion of specific cell and sub-types of NHLs. Karin, (2006) associates much higher adult T cell lymphoma in Japan and Caribbean to the endemic nature of HTLV-1 in these locations. Karin also reports frequent occurrence of some rare T-cell neoplasms in Asia than other continents.

5.1.3 Phenotypic subtypes of non-Hodgkin lymphomas

Moreover, in this study NHLs were put into broad subtypes based on immunophenotype, and knowledge on the relevant clinical features and morphology. CD20 positive diffuse large forms were broadly categorized as diffuse large B cell lymphomas without specific division into GCB and ABC-like types. CD20 positive Small B cell neoplasms with simultaneous co-expression of CD5 and CD23 were identified as small lymphocytic lymphoma. Mantle cell lymphomas were confirmed by the expression of CD20 and CD5 without CD23. About a quarter of B cell neoplasms (25.8%) with morphology and features other than the ones explained above could not be further categorized beyond the cell types. Also, CD3 positive diffuse large cell types and others identified as T cell lymphomas in the study could not be classified into specific subtypes. The vast majority of subtypes recorded were diffuse large B cell lymphoma (40.9%). Although other subtypes, small lymphocytic lymphoma (12.1%), mantle B cell lymphoma (7.6%), extra-nodal B cell marginal zone lymphoma (1.5%) and lymphoplasmacytic lymphoma (1.5%), were recovered in the study, ranking of rate of occurrences cannot be appropriately done as significant proportion (25.8%) remain unclassified. The findings of the study is consistent with previous work in Nigeria by Onwubuya et al (2015) and in India by Atri et al (2020). Onwubuya et al reported 47% for DLBCL whiles Atri et al had 66.66% for DLBCL and 3.7% for marginal B cell lymphoma. Conversely, more mantle cell lymphoma (7.4%) than small lymphocytic lymphoma (3.7%) were obtained by Atri et al and therefore indicates geographical locations with increased possible triggers of particular lymphoma types.

5.1.4 Clinical features and cell types

In this study, the most common clinical manifestation was B-symptoms (87.9%) followed by lymphadenopathy (66.7%). Hepatomegaly was the least (7.6%) observed. The finding is different from previous works in India by Atri et al who recorded 81.3% for lymphadenopathy followed by B symptoms (53.1%) and Devi et al who reported 76% and 48% for lymphadenopathy and B symptoms respectively (Devi, Sharma, Singh, & Sonia, 2017).The higher proportion of B symptoms in the present study may be associated with general delay in seeking medical attention and therefore presenting with advanced disease. Extra-nodal involvement in the study (56.1%) was comparatively higher than that documented by Devi et al (43%). Meanwhile, the findings of this study suggest no significant association between clinical presentations (B-symptoms, lymphadenopathy, splenomegaly, hepatomegaly and extra-nodal presentation) and the observed cell types of NHLs.

5.1.5 Morphology diagnosis and immunophenotype

The study observed significant association between histopathological diagnosis and cell types based on immunohistochemistry (P=0.001). There was 100% agreement in each case of NHL determined by morphology and the phenotypic distribution by particular cell lineage. This may have been influenced by a broad manner of characterization (B and T cell types) by the study hence, this finding may change upon identification of specific subtypes.

5.1.6 Cell types and outcomes of NHLs

Treatment outcomes were categorized as clinical remission, refractory and relapse. Remission status for greater proportion of both B cell (57.6) and T cell (57.1) lymphomas could not be determined as patients were lost to follow up before completion of a required treatment cycle. A distribution of 37.3% and 14.3% for B and T cell NHLs respectively was recorded for clinical remission. This is consistent with the second year outcome of a three-year follow up on the outcomes of NHLs by Dei-Adomako et al (2017), which recorded an impressive outcome (78.9% for clinical remission) at the first year with a sharp decline in clinical remission (31.5% for second year and 11.8% for third year) in the following years due to loss to follow up. Furthermore, a significant association between cell types and clinical outcome was observed for the study ($p=0.011$), which indicates a varied treatment response and behaviour of NHLs as a result of heterogeneous cell types.

5.2 Limitation of the study

Additional immunological markers including CD10, BCL 2, BCL6, MUM1, CD79a, CD2, CD30 and TdT of B or T/NK cell lineage would have been useful to determine the distribution according to specific subtypes. Clinical outcome of some selected cases could not be determined due to failure of clinical attendance and treatment discontinuation. In spite of the limitations, the reliability and validity of the study was not compromised.

5.3 Conclusion

Non-Hodgkin lymphomas were mostly due to the malignant transformation of B cell lineage with phenotypic distribution of 89.4% for B cells and 10.6% for T cells. Majority of B and T cell lymphomas occurred in males. The predominant subtype observed in the study was diffuse large B cell lymphoma. A significant association was observed between phenotypic cell types and morphology (P=0.001) as well as outcomes of NHLs (p=0.011). However, there was no statistically significant association between obvious clinical manifestations and pathological cell types. The present study therefore serves as a preliminary data for further research towards the adoption of an improved treatment regimen and management of non-Hodgkin lymphomas in Ghana.

5.4 Recommendation

Upon the findings of this work, it is recommended that more detailed research be conducted in the area of immunophenotyping (by employing markers of maturation, proliferation and clonality) as well as genetic studies. This will enhance effective characterization and the understanding of the biology and expression of the disease in patients at the local setting, leading to the development of improved treatment protocols of NHLs in Ghana. It is also recommended that a minimum panel of markers capable of determining particular cell lineage and/or broad subtypes be considered in making diagnosis in a resource constrained setting like Ghana as that directly affects the outcomes of NHLs.

REFERENCES

Aaron, N. H., Engelman, A. J., & Faber, C. A. (2016). The BCL-2 family: key mediators of the apoptotic response to targeted anti-cancer therapeutics. *Cancer Discov*, *5*(5), 475–487. https://doi.org/10.1158/2159-8290.CD-15-0011

Abukari, R., & Odai, R. O. (2018). Gender and the Labour Market in Ghana : The Relationship in Terms of the Family , the Market and the State. *Advances in Applied Sociology*, *8*, 285–294. https://doi.org/10.4236/aasoci.2018.84015

Adams, C. M., Mitra, R., Gong, J. Z., & Eischen, C. M. (2017). Non-Hodgkin and Hodgkin Lymphomas Select for Overexpression of BCLW. *Clin Cancer Res*, *23*(22), 7119–7129. https://doi.org/10.1158/1078-0432.CCR-17-1144

Alyahya, N., Adiga, B., Alwadei, A., Alshahrani, G., & Alyahya, F. (2019). The clinico-pathological profile of non - Hodgkin ' s lymphoma in Aseer region of Saudi Arabia. *BMC Res Notes*, *12*(418), 1–7. https://doi.org/10.1186/s13104-019-4447-1

American Cancer Society. (2018). Non-Hodgkin Lymphoma Treatment. Retrieved January 10, 2020, from https://www.cancer.org/content/dam/CRC/PDF/Public/8719.00.pdf

Andrade, J. A. (2007). Diagnosing Non-Hodgkin's Lymphoma. *LABMEDICINE*, *38*(4), 244–247. https://doi.org/10.1309/5N0T9HRK89MKQTH1

Armitage, J. O. (1993). Treatment of non-Hodgkin's lymphoma. *The New England Journal of Medicine*, *328*(14), 1023–1030.

Armitage, J. O. (2005). Staging non-Hodgkin lymphoma. *CA Cancer J Clin*, *55*(6), 368–376.

Armitage, J. O., & Longo, D. L. (2016). Perspectives Is watch and wait still acceptable for patients with low-grade follicular lymphoma? *BLOOD*, *127*(23), 2804–2809. https://doi.org/10.1182/blood-2015-11- 632745.

Arnam, J. S. Van, Lim, M. S., & Elenitoba-johnson, K. S. J. (2018). Novel insights into the pathogenesis of T-cell lymphomas. *BLOOD*, *131*(21), 2320–2330. https://doi.org/10.1182/blood-2017-11-764357

Atri, S. K., Singhal, A., Kataria, S. P., & Mohini. (2020). Correlation between clinical features and immunohistochemistry profile of lymphoma patients. *International Journal of Biomedical and Advance Research*, *11*(01), 1–6. https://doi.org/10.7439/ijbar.v11i1.5086

Australian Cancer Network. (2005). Clinical Practice Guidelines for the Diagnosis and Management of Lymphoma. Retrieved June 4, 2020, from www.cancer.org.au/guidelines

Bain, B. J., Bates, I., Laffan, M. A., & Lewis, S. M. (2011). *Dacie and Lewis Practical Haematology* (11th Edit.). Elsevier Churchill Livingstone.

Bakshi, N., & Maghfoor, I. (2012). The Current Lymphoma Classification: New Concepts and Practical Applications—Triumphs and Woes. *Ann Saudi Med*, *32*(3), 296–305. https://doi.org/10.5144/0256-4947.2012.296

Bangash, M. H., Hussain, I., Zakaria, M., & Piracha, M. N. (2014). Pattern of extranodal involvement in non-Hodgkin's lymphoma. *Pak Armed Forces Med J*, *64*(4), 605–608.

Barroca, H., & Marques, C. (2016). A Basic Approach to Lymph Node and Flow Cytometry Fine-Needle Cytology. *Acta Cytologica*, *60*, 284–301. https://doi.org/10.1159/000448679

Batra, R., Kaur, H., & Jindal, S. (2014). Extranodal large B-cell type aggressive non-Hodgkin's lymphoma. *Indian J Dent*, *5*(4), 225–228. https://doi.org/10.4103/0975-962X.144742

Bellan, C., Lazzi, S., Falco, G. De, Nyongo, A., Giordano, A., & Leoncini, L. (2003). Burkitt's lymphoma: new insights into molecular pathogenesis. *J Clin Pathol*, *56*, 188–192.

Cai, P., Hao, J., Wang, D., & Xu, J. (2017). Comparative efficacy of different chemotherapies for non- Hodgkin lymphoma: a network-meta analysis. *Oncotarget*, *8*(53), 91238–91247.

Chao, M. P. (2013). Treatment challenges in the management of relapsed or refractory non-Hodgkin's lymphoma – novel and emerging therapies. *Cancer Management and Research*, *5*, 251–269. https://doi.org/10.2147/CMAR.S34273

Charwudzi, A., Olayemi, E. E., Ekem, I., Olopade, O., Coyle, M., Benneh, A. A., & Allotey, E. A. (2014). A preliminary study of the suitability of archival bone marrow and peripheral blood smears for diagnosis of CML using fish. *Advances in Hematology*, *2014*, 1–5. https://doi.org/10.1155/2014/604165

Chen, X., Jensen, P. E., & Li, S. (2003). A Useful Marker to Distinguish Florid Follicular Hyperplasia From Follicular Lymphoma by Flow Cytometry. *Am J Clin Pathol*, *119*, 842–851. https://doi.org/10.1309/WP6H6UD4DXMRQ86T

Chu, P. G., Loera, S., Huang, Q., & Weiss, L. M. (2006). Lineage Determination of CD20 – B-Cell Neoplasms. An Immunohistochemical Study. *Am J Clin Pathol*, *126*, 534–544. https://doi.org/10.1309/3WG32YRAMQ7RB9D4

Colomo, L., Lopez-Guillermo, A., Perales, M., Rives, S., Martınez, A., Bosch, F., … Campo, E. (2003). Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. *BLOOD*, *101*(1), 78–84.

Dehghani, M., Haddadi, S., & Vojdani, R. (2015). Signs , Symptoms and Complications of Non-Hodgkin's Lymphoma According to Grade and Stage in South Iran. *Asian Pac J Cancer Prev*, *16*(8), 3551–3557. https://doi.org/10.7314/APJCP.2015.16.8.3551

Dei-Adomakoh, Y. A., Asare, E. V., Amedonu, E. S. A., Abraham, E. D., Segbefia, C. I., & Acquaye, J. K. (2017). Sub-Types and Treatment Outcomes of Adolescent and Adult non-Hodgkin Lymphomas in a Resource Poor Setting. *Journal of Hematology and Oncology Research*, *2*(3), 15–23. https://doi.org/10.14302/issn.2372-6601.jhor-17-1423

Devi, A. A., Sharma, D. T., Singh, I. Y., & Sonia, H. (2017). Clinicopathological Profile of Patients with Non-Hodgkin's Lymphoma at a Regional Cancer Center in Northeast India. *J Sci Soc*, *44*, 140–144. https://doi.org/10.4103/jss.JSS

Diebold, J., Tourneau, L., Comperat, E., & Molina, T. (2005). Primary Splenic and Nodal Marginal Zone Lymphoma : *J. Clin. Exp. Hematopathol*, *45*(1), 1–14.

Diraimondo, F., Albitar, M., Huh, Y., Brien, S. O., Montillo, M., Tedeschi, A., … Keating, M. (2002). The Clinical and Diagnostic Relevance of CD23 Expression in the Chronic Lymphoproliferative Disease. *Cancer*, *94*(6), 1721–1730.

Disanto, M. G., Ambrosio, M. R., Rocca, B. J., Ibrahim, H. A. H., Leoncini, L., & Naresh, K. N. (2016). Optimal Minimal Panels of Immunohistochemistry for Diagnosis of B-Cell Lymphoma for Application in Countries With Limited Resources and for Triaging Cases Before Referral to Specialist Centers. *Am J Clin Pathol*, *145*, 687–695.

https://doi.org/10.1093/AJCP/AQW060

Dreyling, M., Campo, E., Hermine, O., Jerkeman, M., Le Gouill, S., Rule, S., … Ladetto, M. (2017). Newly diagnosed and relapsed mantle cell lymphoma : ESMO Clinical Practice Guidelines for diagnosis , treatment and follow-up. *Annals of Oncology*, *28*(Supplement 4), 62–71. https://doi.org/10.1093/annonc/mdx223

Fauzi, M. F. A., Pennell, M., Sahiner, B., Chen, W., Shana'Ah, A., Hemminger, J., … Gurcan, M. N. (2015). Classification of follicular lymphoma: The effect of computer aid on pathologists grading Clinical decision-making, knowledge support systems, and theory. *BMC Medical Informatics and Decision Making*, *15*(115), 1–10. https://doi.org/10.1186/s12911-015-0235-6

Friedberg, J. W. (2008). Diffuse Large B-Cell Lymphoma. *Hematol Oncol Clin North Am.*, *22*(5), 941–949. https://doi.org/10.1016/j.hoc.2008.07.002.

Gopal, S., & Gross, T. G. (2018). How I treat Burkitt lymphoma in children , adolescents , and young adults in sub-Saharan Africa. *BLOOD*, *132*(3), 254–264.

Gopal, S., Wood, W. A., Lee, S. J., Shea, T. C., Naresh, K. N., Kazembe, P. N., … Mitsuyasu, R. T. (2012). Meeting the challenge of hematologic malignancies in sub-Saharan Africa. *BLOOD*, *119*(22), 5078–5088. https://doi.org/10.1182/blood-2012-02-387092.

Goteri, G., Lucarini, G., Zizzi, A., Costagliola, A., Giantomassi, F., Stramazzotti, D., … Leoni, P. (2011). Comparison of germinal center markers CD10, BCL6 and human germinal center-associated lymphoma (HGAL) in follicular lymphomas. *Diagnostic Pathology*, *6*(97), 1–7.

Hafez, R., Hussein, S., & Ismail, M. (2018). Definitive salvage chemotherapy for the treatment of refractory / relapsed non-Hodgkin lymphoma , a single center experience. *Alexandria Journal of Medicine*, *54*, 679–683.

Harris, N. L., Jaffe, E. S., Diebold, J., Flandrin, G., & Vardiman, J. (2000). Lymphoma classification - from controversy to consensus :The R.E.A.L. and WHO Classification of lymphoid neoplasms N. *Annals of Oncology*, *11*(suppl. 1), 4–9.

Hazarika, M., Iqbal, A., Krishnatreya, M., Dev, J., Bhuyan, C., Saikia, B. J., … Kataki, A. C.

(2015). Adult Non-Hodgkin's Lymphoma Patients: Experience from a Tertiary Care Cancer Centre in North East India. *Asian Pac J Cancer Prev*, *16*(7), 2879–2881. https://doi.org/10.7314/APJCP.2015.16.7.2879

Higgins, R. A., Blankenship, J. E., & Kinney, M. C. (2008). Application of Immunohistochemistry in the Diagnosis of Non-Hodgkin and Hodgkin Lymphoma. *Arch Pathol Lab Med*, *132*, 441–461.

Hjalgrim, H., & Engels, E. A. (2008). Infectious aetiology of Hodgkin and non-Hodgkin lymphomas: A review of the epidemiological evidence. *Journal of Internal Medicine*, *264*, 537–548. https://doi.org/10.1111/j.1365-2796.2008.02031.x

Hoffbrand, A. V., & Moss, A. H. P. (2016). *Hoffbrand's Essential Haematology* (7th Edit.). West Sussex, UK: John Wiley & Sons Ltd.

Horesh, N., & Horowitz, N. A. (2014). Does Gender Matter in Non-Hodgkin Lymphoma ? Differences in Epidemiology , Clinical Behavior , and Therapy. *Rambam Maimonides Med J*, *5*(4), 1–8. https://doi.org/10.5041/RMMJ.10172

Horvat, M., Zadnik, V., Šetina, T. J., & Boltežar, L. (2018). Diffuse large B-cell lymphoma : 10 years' real-world clinical experience with rituximab plus cyclophosphamide , doxorubicin , vincristine and prednisolone. *ONCOLOGY LETTERS*, *15*, 3602–3609. https://doi.org/10.3892/ol.2018.7774

Howard, R. M., & Hamilton, J. P. (2013). *Haematology: An illustrated colour text* (4th Edit.). Edinburgh: Churchill Livingstone Elsevier Ltd.

Jaffe, E. S., Campo, E., Harris, N. L., Pileri, S. A., Stein, H., & Swerdlow, S. H. (2017). Introduction and overview of the classification of lymphoid neoplasms. In H. S. Swerdlow, E. Campo, N. L. Harris, S. E. Jaffe, A. S. Pileri, H. Stein, & J. Thiele (Eds.), *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (4th Edit., pp. 190–342). Lyon: International Agency for Research on Cancer.

Jaffe, Elaine S. (2006). T-cell Lymphoma Pathobiology of Peripheral T-cell Lymphomas. *Hematology Am Soc Hematol Educ Program*, *2006*(1), 317–322. https://doi.org/10.1182/asheducation-2006.1.317

Jaffe, S. E., Harris, N. L., Stein, H., & Isaacson, G. P. (2008). Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. *BLOOD*, *112*(12), 4384–4399. https://doi.org/10.1182/blood-2008- 07-077982.

Jairajpuri, Z. S., Ghai, R., Saluja, S., Kapur, S., & Bhowmik, K. T. (2017). Expression of apoptosis related and proliferative proteins in malignant Lympho-proliferative disorders. *Iran J Pathol.*, *12*(3), 231–240.

Jia, K., Zhang, D., Wang, Y., Liu, Y., Kong, X., Yang, Q., … Wang, S. (2019). Generation and characterization of a monoclonal antibody against human BCL6 for immunohistochemical diagnosis. *PLoS ONE*, *15*(5), 1–12. https://doi.org/10.1371/journal.pone.0216470

Karin, E. (2006). Epidemiology and etiology of non-Hodgkin lymphoma – a review. *Acta Oncologica*, *45*(3), 258–271. https://doi.org/10.1080/02841860500531682

Katchi, T., & Liu, D. (2017). Diagnosis and treatment of CD20 negative B cell lymphomas. *Biomarker Research*, *5*(5), 1–5. https://doi.org/10.1186/s40364-017-0088-5

Khubchandani, S., & Czuczman, M. S. (2009). Targeted therapies in B-cell non-Hodgkin lymphomas. *Therapy*, *6*(3), 371–380. https://doi.org/10.2217/THY.09.19

Klener, P., & Klanova, M. (2020). Drug Resistance in Non-Hodgkin Lymphomas. *Int. J. Mol. Sci.*, *21*(2081), 1–25. https://doi.org/10.3390/ijms21062081

Kpessa-Whyte, M. (2018). Aging and Demographic Transition in Ghana : State of the Elderly and Emerging Issues. *The Gerontologist*, *58*(3), 403–408. https://doi.org/10.1093/geront/gnx205

Kristinsson, S. Y., Goldin, R., & Turesson, I. (2012). Familial Aggregation of Lymphoplasmacytic Lymphoma/Waldenström Macroglobulinemia with Solid Tumors and Myeloid Malignancies. *Acta Haematol*, *127*, 173–177. https://doi.org/10.1159/000335618

Küppers, R. (2005). Mechanisms of B-Cell lymphoma pathogenesis. *Cancer*, *5*, 251–262. https://doi.org/10.1038/nrc1589

Kuppers, R., & Dalla-favera, R. (2001). Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene*, *20*, 5580–5594.

Lakhtakia, R., & Burney, I. (2015). A Historical Tale of Two Lymphomas. *Sultan Qaboos University Med J*, *15*(3), 317–321. https://doi.org/10.18295/squmj.2015.15.03.003

Laurini, J. A., Perry, A. M., Boilesen, E., Diebold, J., Maclennan, K. A., Mu, H. K., … Weisenburger, D. D. (2012). Classification of non-Hodgkin lymphoma in Central and South America : a review of 1028 cases. *Blood*, *120*(24), 4795–4802. https://doi.org/10.1182/blood-2012-07-440073

Leukaemia and Lymphoma Society. (2013). Non-Hodgkin Lymphoma. Retrieved January 20, 2020, from https://www.llscanada.org/sites/default/files/National/CANADA/Pdf/InfoBooklets/Non-Hodgkin Lymphoma.pdf

Liu, C., Richard, K., Melvin, W., Zhu, X., & Conrad, D. H. (2016). CD23 can negatively regulate B-cell receptor signaling. *Scientific Reports*, *6*(25629), 1–8. https://doi.org/10.1038/srep25629

Macdonald, V. (2009). Chemotherapy : Managing side effects and safe handling. *CVJ*, *50*, 1–4.

McLaughlin, P. (2000). Biotherapy for lymphoma. *Current Oncology Reports*, *2*, 157–162.

Ministry of Health-Ghana. (2011). National strategy on cancer control in Ghana (2012-2016). Retrieved September 25, 2019, from https://www.iccp-portal.org/sites/default/files/plans/Cancer Plan Ghana 2012-2016.pdf

Miranda-Filho, A., Piñeros, M., Znaor, A., Marcos-Gragera, R., Steliarova-Foucher, E., & Bray, F. (2019). Global patterns and trends in the incidence of non-Hodgkin lymphoma. https://doi.org/10.1007/s10552-019-01155-5

Mishra, D., Singh, S., & Narayan, G. (2016). Role of B Cell Development Marker CD10 in Cancer Progression and Prognosis. *Molecular Biology International*, *2016*, 2–9. https://doi.org/10.1155/2016/4328697

Molyneux, E. M., Rochford, R., Griffi, B., Newton, R., Jackson, G., Menon, G., … Barr, Y. (2012). Burkit's lymphoma. *Lancet*, *379*, 1234–1244. https://doi.org/10.1016/S0140-6736(11)61177-X

Mosalpuria, K., Bociek, R. G., & Vose, J. M. (2014). Angioimmunoblastic T-Cell Lymphoma Management. *Semin Hematol*, *51*, 52–58. https://doi.org/10.1053/j.seminhematol.2013.11.008

Mundada, M., Ahmed, F., & Santa, A. (2017). A challenging case of anaplastic large - cell lymphoma with primary bony presentation. *Asian J Oncol*, *3*, 155–157. https://doi.org/10.4103/ASJO.ASJO_122_16

Naderi, N., & Yang, D. T. (2013). Lymphoplasmacytic Lymphoma. *Arch Pathol Lab Med*, *137*, 580–585. https://doi.org/10.5858/ arpa.2012-0034-RS

Naing, L., Winn, T., & Rusli, B. N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences*, *1*, 9–14.

Ning, Y., Foss, A., Kimball, A. S., Neill, N., Matz, T., & Schultz, R. (2013). Characterization of a case of follicular lymphoma transformed into B-lymphoblastic leukemia. *Molecular Cytogenetics*, *6*(34), 1–7.

Nogai, H., Dorken, B., & Lenz, G. (2011). Pathogenesis of Non-Hodgkin's Lymphoma. *J Clin Oncol*, *29*(14), 1803–1811. https://doi.org/10.1200/JCO.2010.33.3252

Okosun, J., & Cwynarski, K. (2016). Non-Hodgkin lymphoma: high grade. In A. V. Hoffbrand, D. R. Higgs, D. M. Keeling, & A. B. Mehta (Eds.), *Postgraduate Haematology* (7th Edit., pp. 631–650). West Sussex, UK: JohnWiley & Sons Ltd.

Olaniyi, J. A. (2012). Burkitt Lymphoma: A Review. *Scientific Reports*, *1*(6), 1–4. https://doi.org/10.4172/scientificreports.337

Olszewski, J. A., & Castillo, J. J. (2013). Survival of Patients With Marginal Zone Lymphoma. *Cancer*, *101*, 629–638.

Ondrejka, S. L., & Hsi, E. D. (2015). Pathology of B-Cell Lymphomas : Diagnosis and Biomarker Discovery. In A. M. Evens & K. A. Blum (Eds.), *Non-Hodgkin Lymphoma, Cancer Treatment and Research* (Vol. 165, pp. 27–51). Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-319-13150-4_2

Onwubuya, I. M., Adelusola, K. A., Durosinmi, M. A., Sabageh, D., & Ezike, K. N. (2015).

Lymphomas in Ile-Ife , Nigeria : Immunohistochemical Characterization and Detection of Epstein-Barr virus Encoded RNA. *Journal of Clinical and Diagnostic Research.*, *9*(6), 14–19. https://doi.org/10.7860/JCDR/2015/12085.6127

Paepe, P. De, & Wolf-Peetrs, C. De. (2007). Diffuse large B-cell lymphoma: a heterogeneous group of non-Hodgkin lymphomas comprising several distinct clinicopathological entities. *Leukemia*, *21*, 37–43.

Pallister, C., & Watson, M. (2011). *Haematology* (2nd Edit.). East Kilbride, UK: Scion publishing Ltd.

Perry, A. M., Perner, Y., Diebold, J., Nathwani, B. N., Maclennan, K. A., Hans, K., & Weisenburger, D. D. (2016). Non-Hodgkin lymphoma in Southern Africa: review of 487 cases from The International Non-Hodgkin Lymphoma Classification Project. *British Journal of Haematology*, *172*, 716–723. https://doi.org/10.1111/bjh.13885

Poggio, T., Duyster, J., & Illert, L. A. (2018). Current Immunotherapeutic Approaches in T Cell. *Cancers*, *339*(10), 1–17. https://doi.org/10.3390/cancers10090339

Portier, B. P., & Taglialatela, G. (2006). Bcl-2 Localized at the Nuclear Compartment Induces Apoptosis after Transient Overexpression. *Journal of Biological Chemistry*, *281*(52), 40493–40502. https://doi.org/10.1074/jbc.M606181200

Pourhoseingholi, M. A., Vahedi, M., & Rahimzadeh, M. (2013). Sample size calculation in medical studies. *Gastroenterol Hepatol Bed Bench*, *6*(1), 14–17.

Pramoda, C., Jorgensen, J. L., Kanagal-Shamanna, R., Gurevich, I., Diane, P. M., Alessandra, F., … Miranda, R. N. (2014). Utility of Quantitative Flow Cytometry Immunophenotypic Analysis of CD5 Expression in Small B-Cell Neoplasms. *Arch Pathol Lab Med*, *138*, 903–909. https://doi.org/10.5858/ arpa.2013-0367-OA

Rizvi, M. A., Evens, A. M., Tallman, M. S., Nelson, B. P., & Rosen, S. T. (2006). T-cell non-Hodgkin lymphoma. *Blood*, *107*(4), 1255–1264. https://doi.org/10.1182/blood-2005-03-1306

Rizzo, K., & Nassiri, M. (2012). Diagnostic Workup of Small B Cell Lymphomas: A Laboratory Perspective. *Hindawi Pub. Corp.*, *2012*, 3–15. https://doi.org/10.1155/2012/346084

Sagaert, X., Tousseyn, T., & Yantiss, R. K. (2012). Gastrointestinal B-cell lymphomas : From understanding B-cell physiology to classification and molecular pathology. *World J Gastrointest Oncol*, *4*(12), 238–249. https://doi.org/10.4251/wjgo.v4.i12.238

Sandlund, J. T., & Martin, M. G. (2016). Non-Hodgkin lymphoma across the pediatric and adolescent and young adult age spectrum. *Hematology Am Soc Hematol Educ Program*, *2016*(1), 589–597. https://doi.org/10.1182/asheducation-2016.1.589

Sathiya, M., & Muthuchelian, K. (2009). Significance of Immunologic Markers in the Diagnosis of Lymphoma. *Academic J. Cancer Res*, *2*(1), 40–50.

Schieber, M., Gordon, L. I., & Karmali, R. (2018). Current overview and treatment of mantle cell lymphoma. *F1000Research*, *7*, 1–10. https://doi.org/10.12688/f1000research.14122.1

Schlette, E., Fu, K., & Medeiros, L. J. (2003). CD23 Expression in Mantle Cell Lymphoma : Clinicopathologic Features of 18 Cases. *Am J Clin Pathol*, *120*, 760–766. https://doi.org/10.1309/XV4AG7EMWQU7ER67

Segbefia, C. I., Renner, L. A., Dei-Adomakoh, Y., & Welbeck, J. (2013). Changing pattern of childhood cancers at Korle Bu Teaching Hospital, Accra, Ghana. *Postgraduate Medical Journal of Ghana*, *2*(2), 65–67.

Seifert, M., Scholtysik, R., & Küppers, R. (2013). Origin and Pathogenesis of B Cell Lymphomas. In R. Küppers (Ed.), *Lymphoma: Methods and Protocols, Methods in Molecular Biology* (Vol. 971, pp. 1–24). Springer Science+Business Media, LLC. https://doi.org/10.1007/978-1-62703-269-8

Sharma, M., Mannan, R., Madhukar, M., & Navani, S. (2014). Immunohistochemical (IHC) Analysis of Non-Hodgkin's Lymphoma (NHL) Spectrum According to WHO / REAL Classification : A Single Centre Experience from Punjab , India. *Journal of Clinical and Diagnostic Research*, *8*(1), 46–49. https://doi.org/10.7860/JCDR/2014/8173.3988

Shimizu, K., Yoshida, J., Kakegawa, S., Astumi, J., Kaira, K., Oshima, K., … Takeyoshi, I. (2010). Primary thymic mucosa-associated lymphoid tissue lymphoma. *J Thorac Oncol*, *5*(1), 117–121.

Skarin, A. T., & Dorfman, M. D. (1997). Non-Hodgkin's lymphomas: Current classification and

management. *CA Cancer J Clin*, *47*(6), 351–372.

Stathis, A., & Owens, C. (2016). Risk factors, etiology, and pathogenesis. In A. Younes (Ed.), *Handbook of Lymphoma* (pp. 11–19). Switzerland: Springer International Publishing. https://doi.org/10.1007/978-3-319-08467-1_2

Tageja, N., Padheye, S., Dandawate, P., Al-katib, A., & Mohammad, R. M. (2009). New targets for the treatment of follicular lymphoma. *Journal of Hematology & Oncology*, *2*(50), 1–14. https://doi.org/10.1186/1756-8722-2-50

Teras, L. R., Desantis, C. E., Cerhan, J. R., Morton, L. M., Jemal, A., & Flowers, C. R. (2016). 2016 US Lymphoid Malignancy Statistics by World Health Organization Subtypes. *CA Cancer J Clin*, *66*(6), 443–459. https://doi.org/10.3322/caac.21357.

Townsend, W., & Marcus, R. (2016). Non-Hodgkin lymphoma: low grade. In A. V. Hoffbrand, D. R. Higgs, D. M. Keeling, & A. B. Mehta (Eds.), *Postgraduate Haematology* (7th Edit., pp. 614–630). West Sussex, UK: John Wiley & Sons Ltd.

Turner, J. J., Hughes, A. M., Kricker, A., Milliken, S., Grulich, A., Kaldor, J., & Armstrong, B. (2004). Use of the WHO lymphoma classification in a population-based epidemiological study. *Annals of Oncology*, *15*, 631–637. https://doi.org/10.1093/annonc/mdh140

Wagner, S. D., Ahearne, M., & Ferrigno, P. K. (2010). The role of BCL6 in lymphomas and routes to therapy. *British Journal of Haematology*, *152*, 3–12. https://doi.org/10.1111/j.1365-2141.2010.08420.x

Xie, Y., Pittaluga, S., & Jaffe, S. E. (2015). The Histological Classification of Diffuse Large B-cell Lymphomas. *Semin Hematol*, *52*(2), 57–66. https://doi.org/10.1053/j.seminhematol.2015.01.006.

Yakubu, M., Ahmadu, B. U., Yerima, T. S., Simon, P., Hezekiah, I. A., & Pwavimbo, A. J. (2015). Prevalence and clinical manifestation of lymphomas in North Eastern Nigeria. *Indian J Cancer*, *52*(4), 551–555. https://doi.org/10.4103/0019-509X.178435

Yin, C. C., Lin, P., Carney, D. A., Handy, B. C., Rassidakis, G. Z., Admirand, J. H., … Medeiros, L. J. (2005). Chronic Lymphocytic Leukemia/ Small Lymphocytic Lymphoma Associated With IgM Paraprotein. *Am J Clin Pathol*, *123*, 594–602.

https://doi.org/10.1309/FDGWB5C2MYRYXH2E

Younes, Anas. (2011). Beyond chemotherapy: new agents for targeted treatment of lymphoma. *Nat Rev Clin Oncol*, *8*(2), 85–96. https://doi.org/10.1038/nrclinonc.2010.189

Zelenetz, A. D., Abramson;, J. S., Advani;, R. H., Andreadis;, C. B., Bartlett, N., Bellam, N., … Nademanee, A. (2011). Non-Hodgkin ' s Lymphomas Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*, *9*(5), 484–560.

Zelenetz, A. D., Gordon, L. I., Wierda, W. G., Abramson, J. S., Advani, R. H., Andreadis, C. B., … Sundar, H. (2015). Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, Version 1.2015: Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*, *13*(3), 326–362.

Zimmermann, M., Oehler, C., Mey, U., Ghadjar, P., & Zwahlen, D. R. (2016). Radiotherapy for Non-Hodgkin's lymphoma: still standard practice and not an outdated treatment option. *Radiation Oncology*, *110*(11), 1–10. https://doi.org/10.1186/s13014-016-0690-y

APPENDIX A - DATA ABSTRACTION FORM

PERSONAL INFORMATION

Folder #: Unique#: Age: Sex:

Referral date:

CLINICAL MANIFESTATION (Circle A or B if present or absent respectively)

1. B-symptoms	2. Lymphadenopathy	3. Splenomegaly	4. Hepatomegaly
A. Yes B. No	A. Yes B. No	A. Yes B. No	A. Yes B. No

SITE OF INVOLVEMENT (Circle the applicable alphabet)

1. Nodal	2. Extranodal
A. Yes B. No	A. Yes B. No

Histology diagnosis: **Clinical outcome:** **Follow up:**

APPENDIX B

PROTOCOL FOR DEHYDRATION AND CLEARING

Container	**Fluid**	**Time (mins)**
1	70% alcohol	2
2	80% alcohol	2
3	90% alcohol	2
4	100% alcohol	3
5	100% alcohol	3
7	Xylene	5
8	Xylene	5
9	Xylene	5

APPENDIX C

PROTOCOL FOR IMMUNOHISTOCHEMISTRY

Procedure type	**Method**
Deparaffinization	Selected
Cell conditioning (Antigen unmasking)	92 mins at 95^0C
Pre-primary peroxidase inhibition	Selected
Primary antibody	Anti-CD20 - 8 mins at 37^0C Anti-CD3 - 16 mins at 37^0C Anti-CD5 - 16 mins at 37^0C Anti-CD23 - 16 mins at 37^0C
Optiview HQ linker incubation	8 mins
Optiview HRP multimer incubation	8 mins
DAB and H_2O_2 incubation	8 mins
Counterstain (hematoxylin II)	4 mins
Post counterstain (Bluing)	4 mins

APPENDIX D

LIST OF REAGENTS

Reagents and materials for Immunohistochemical studies are listed below:

1. Primary antibody
    a. CONFIRM anti-CD3
    b. CONFIRM anti-CD5
    c. CONFIRM anti-CD20
    d. CONFIRM anti-CD23
2. Hematoxylin II
3. Bluing reagent
4. Liquid coverslip (LCS)
5. EZ prep concentrate (10X)
6. Cell conditioning solution (CC1)
7. Distilled water
8. Reaction buffer concentrate (10X)
9. Optiview DAB IHC detection kit
    a. Peroxidase inhibitor (3.0%)
    b. HQ Universal linker (<50 ug/mL)
    c. HRP Multimer (<40 ug/mL)
    d. 0.2% 3,3-Diaminobenzidine tetrahydrochloride (DAB)
    e. H_2O_2 (0.04%)
    f. Copper sulfate (5.0 g/L)
10. Mild dishwashing detergent (sunlight detergent)
11. Alcohol
12. Xylene
13. Mountant
14 Cover slip

APPENDIX E

HISTOPATHOLOGICAL DIAGNOSIS (MORPHOLOGY)

Histology diagnosis	Frequency	Percent
? Burkitt Lymphoma	1	1.5
Diffuse large and small cell lymphoma	4	6.1
Diffuse large cell lymphoma	22	33.3
Diffuse large cell lymphoma with CNS involvement	1	1.5
MALT lymphoma	3	4.5
NHL -Anaplastic large cell type	1	1.5
NHL with CNS involvement	2	3.0
NHL, lymphoplasmacytic type	1	1.5
Non-Hodgkin lymphoma (follicular type)	7	10.6
Non-Hodgkin lymphoma (large cell type)	1	1.5
Non-Hodgkin lymphoma (NHL)	10	15.2
Non-Hodgkin lymphoma (small cell type)	12	18.2
Small and large cell lymphoma (follicular)	1	1.5
Total	66	100

APPENDIX F

ETHICAL APPROVAL

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/APRIL/2019 April 3, 2019

Mr. Joseph Adomako
Department of Haematology
School of Biomedical and Allied Health Sciences
Korle Bu

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-Et/M.8 – 5.16/2018-2019***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its March 28, 2019 full board meeting reviewed and approved your research protocol.

Title of Protocol: "Immunophenotypic Characterization of Non-Hodgkin Lymphomas"

Principal Investigator: **Mr. Joseph Adomako**

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till April 4, 2020.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....

Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, SBAHS
Head, Dept. of Haematology

• P. O. Box KB 52, Korle Bu, Accra, Ghana. **Telephone:** +233 (0) 302 665103/4 • **Fax:** +233 (0) 302 660762
• **Email:** admin.chs@ug.edu.gh /provost.chs@ug.edu.gh • **Website:** www.chs.ug.edu.gh