The Use of Immunophenotypic Biomarkers and Quantitative Polymerase Chain Reaction as Diagnostic And Prognostic Indicators of Diffuse Large B Cell Non-Hodgkins lymphoma in Sudan

Thesis for a Ph.D. Degree in the Department of Periodontology and Oral Medicine

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"Our ASPIRATIONS are our possibilities."

Robert Browning

"Great accomplishments start with great ASPIRATIONS."

Gary Hamel

Attestation of Authorship

"I hereby declare that the scientific work I have submitted to the University of the Western Cape is mine and that, to the best of my knowledge and certainty, it contains no material that was previously published or written by another person except where explicitly defined in the references, all external references and sources are clearly acknowledged and identified within the contents, nor part of this work has been submitted for the award of any other degree or diploma of a university or other institution of higher learning. I empower the University of the Western Cape to reproduce for the purpose of research either the whole or any portion of this content".

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01/ 11 / 2021

Abstract

Background: The incidence of Diffuse large B cell Lymphoma has been increasing lately at an alarming rate especially, in developing countries like Sudan. The standard therapy in Sudan is based solely on the R-CHOP chemotherapy regimen, yet it has been noticed that Diffuse Large B cell Lymphoma prognosis remains unfavorable. The late diagnosis and the consequent side-effects of the therapy directly affected the disease's poor outcome. There is a scarcity of scientific publications regarding DLBCL in Sudan, but the increased burden necessitates the need for further research.

The treatment of DLBCL in Sudan is routinely determined according to the histopathological evaluation of the tumor and not on the phenotypic sub-classification of cancer, which was recently updated in 2016 by the WHO classification of Hematopoietic diseases. Hence, the current diagnostic methods and general approach for DLBCL have failed to reflect the biological behavior or accurately predict the prognosis of the DLBCL.

Therefore, there is an urgent need for the phenotypic sub-classification of the DLBCL by using more specific molecular markers (MYC and BCL2) and investigating the prognostic prediction potential of other clinicopathological variables. In this way, a targeted therapeutic approach can be implemented, which would result in an improved prognostic outcome.

Moreover, there is a clear gap in the literature regarding the genomic screening of DLBCL, especially given the extensive genetic diversity of the African population that could be of potential oncogenicity relevance. Consequently, the author investigated and identified the molecular and genomic signature of DLBCL in Sudanese patients using

the Quantitative Polymerase Chain Reaction technique. Furthermore, there was no official registry that we could access any information about DLBCL patients.

Aims: The study aimed at investigating the diagnostic, prognostic, and molecular prediction potential of MYC and BCL2 oncogenes in DLBCL. **Objectives:** To quantify the diagnostic and prognostic value of MYC and BCL2 oncogenes in Double Expresser Lymphoma phenotype of DLBCL as predictors of patients' survival, in addition to exploring the molecular and genomic signature via capturing the oncogenic dosage and translocations in DLBCL. Furthermore, we intended to develop a tumor software registry that captures cancer patients' essential clinical and epidemiological data.

Methods: The study was conducted at the Radiation and Isotope Center in Khartoum, Sudan, using retrospective center-based clinical and histopathological data of 151 patients with DLBCL from 2013-2018. The expression levels of MYC and BCL2 biomarkers were visualized and quantified using Immunohistochemistry and Quantitative Polymerase Chain Reaction. The clinicopathological data related to the expression level of the selected biomarkers were then collated.

Results: Sixty-five of the study samples were females, while eighty-six were males. The mean age for patients was 57.25 years. 46% were stage IV at the time of diagnosis. 83% of the samples had concurrent high expression rate of MYC and BCL2 diagnosed as Double Expresser Subtype of DLBCLD. The highest tumor site was cervical Lymph nodes.

Eighty-four patients (55%) passed on of this malignancy. The mean survival time (time from diagnosis to the last follow-up) was 46 months (3.8 years). The highest death of this malignancy was among the male gender, 'married' group, residents of western Sudan, retired, non-smokers, patients with high lactase dehydrogenase enzyme, Ann Arbor stage IV patients, DEL positive subgroup.

Univariate and multivariate Cox regression analyses were used to identify the parameters with prognostic values. In addition, the Disease-specific five-year survival rate of DLBCL was estimated using the Kaplan Meier survival estimate. Quantitative Real-time PCR has identified an elevated oncogenic dosage of MYC and translocations within the BCL2 biomolecule in DLBCL. Moreover, a Tumor Registry Software was developed, enabling digital entry, storage, and stratification of patients' clinical data.

Conclusions: Simultaneous detection of high co-expression of MYC and BCL2 biomarkers determined a positive Double Expresser Lymphoma subtype of DLBCL. This was an independent predictor of inferior prognostic outcome for DLBCL lymphoma patients. This information suggests that detecting the diagnostic and prognostic potential of MYC and BCL2 biomarkers could serve as the foundation for developing a valuable tool for patient classification and target-based treatment. In addition, since the majority of the patients presented at late stages of the disease, various recommendations were proposed to avoid the diagnostic delay and subsequent unfavorable outcomes.

Dedication

To my late father, Abubaker Abbas, for always believing in me

To my mother, Safia Salih Eissa, the greatest love of all, my idol

To my husband, Monzer, for your endless love and support

To my children, Zeena and Khalid, my joy and source of motivation

To my one and only sister, Reem, my everything

To my brothers, Yassir and Ammar, my rock

To my mentor, Professor Abdalsalam Salih, thank you for everything

To Nahla Satti, my best friend, sister, and my comfort

To Aziz Ibn Omer for introducing me to UWC

To my friends all over the world, each one of you counts!

To the brave cancer patients who still have a smile on their faces

To my beloved homeland SUDAN

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I would like to acknowledge The Radiation and Isotope Center in Sudan for giving me the chance to conduct the study at the institution. I appreciate the assistance and flexibility I experienced from the administration and the technical staff throughout the study timeline. My sincere gratitude goes to Mrs. Zulfa Smith at The University of the Western Cape for her tremendous administrative help throughout the years at UWC.

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"He, who leaves home in search of knowledge, walks in the path of God."

Prophet Mohamed Peace Be Upon Him

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List of Abbreviations

AHSCT Allogeneic hematopoietic stem cell transplantation

ALP Alkaline phosphatase

ALT Alanine transaminase

ARL Aids-related lymphoma

ART Antiretroviral Therapy

ASCO American Society of Clinical Oncology

ASCT Autologous stem cell transplantation

AST Aspartate Aminotransferase

BCL2 B cell Lymphoma 2

BET The Bromodomain and Extra-terminal

BMREC Biomedical Research Ethics Committee

BSFH The British Society for Haematology

CAR-T Chimeric antigen receptor T cell

CINV Chemotherapy-induced nausea and vomiting

CMP Comprehensive metabolic panel

CMR Complete metabolic response

CT Cycle Threshold

CV Coefficient of Variation

DEL Double Expresser Lymphoma

DHL Double Hit Lymphoma

DLBCL Diffuse Large B cell Lymphoma

DMP Data Management Plan

DNA Deoxyribonucleic Acid

DSS Disease-Specific Survival

FDG-PET Fluorodeoxyglucose Positron Emission Tomography

FMOH Federal Ministry of Health

EFR External Beam Radiation in radiotherapy

ESMO European Society of Clinical Oncology

ECOH-PS Eastern Cooperative Oncology Group Performance Status

FFPE Formalin Fixed Paraffin Embedded

FDG-PET Fluorodeoxyglucose

GCBL Germinal center B-cell Lymphoma

GDP Gross Domestic Product

GIT Gastrointestinal tract

GSK-3 Glycogen Synthase kinase-3

HepB Hepatitis B virus

HIS Health Information System

HIV Human immunodeficiency virus

HSV1 Herpes Simplex Virus 1

IDF International Diabetes Federation

IHC Immunohistochemistry

IFRT Involved-field radiation therapy

ILROG International Lymphoma Radiation Oncology Group

IPI International Prognostic Index

LDH Lactate dehydrogenase

MASCC Multinational Association for Supportive Care in Cancer

MBR Major Breakpoint region

MCR Minor cluster region

MDR-TB Multi-drug-resistant tuberculosis

MRNA Messenger ribonucleic acid

MRI Magnetic Resonance Imaging

MYC Myelocytomatosis Oncogene Product

NHL Non-Hodgkins Lymphoma

NMR Nuclear magnetic resonance

OS Overall survival

PBS Phosphate Buffered Saline

PCR Polymerase Chain Reaction

PFS Progression-free survival

PPE Personal Protective Equipment

Q-PCR Quantitative Polymerase Chain Reaction

RCHOP Rituximab Cyclophosphamide Doxorubicin Hydrochloride Vincristine Sulfate Oncovin Prednisone

REMARK Reporting Recommendations for Tumor Marker Prognostic Studies

RICK Radiation and Isotope Center Khartoum

RIF Radiotherapy Induced Fatigue

RINV Nausea and vomiting induced by radiotherapy

RT-PCR Real-time qualitative polymerase Chain reaction

TAMRA Tetramethyl carboxyrhodamine dye

THL Triple Hit Lymphoma

UN-HDR United Nations High Commissioner for Refugees

VTE Venous Thromboembolism

WHO World Health Organization

SECTION I: Background, Literature Review, and Project Overview

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Preamble:

Section I is composed of seven chapters. The first three chapters elaborate on the Republic of Sudan in terms of geographical setting, health care system, health information system, and cancer statistics. Chapter four is the literature review of the study that elaborates on Lymphomas; Non-Hodgkins Lymphoma in terms of statistics, epidemiology, etiology, and risk factors, Diffuse Large B cell Lymphoma with regard to etiology, clinical presentation, and classification. Chapter five thoroughly illustrates the specification of the project's rationale, aims, objectives, and hypothesis. Chapter six demonstrates the thesis structure, and lastly, chapter seven states the ethical considerations related to the project.

Contents of Section I

CHAPTER 1: Republic of Sudan

1.1 Geographical Setting

Sudan ranks as the third-largest country in Africa, with an area of 728,215 square miles, following Algeria and the Democratic Republic of Congo. It is located in the northeastern part of the continent and is bound by seven countries, namely Egypt, Chad, Central Africa, Libya, South Sudan, Ethiopia, and Eretria, Figure (I.1.1). Sudan was the largest country in Africa and the Arab world before South Sudan declared political independence in 2011(Sørbø and Ahmed, 2013) (CIA, 2016).

The department of economic and social affairs of the United Nations, via World meter e-measure, has estimated the population of Sudan to be 43.9 million as of August 2020, with a median age of 19.7 years (Worldometer, 2020). Almost one-third of the population (35%) lives in an urban setting, while 65% are rural dwellers. The country is divided into eighteen states with the highest population density of 7.9 million inhabitants residing in the metropolitan Khartoum, the political and financial center of the country (Mayada et al., 2020).

Sudan is a nation of diverse cultures, dialects, and ethnicities (Deng, 2011). The geographical landscape varies from flat deserts in the north to rich soils of the savannah in the Nubian Mountains. The distribution of tribes throughout the country depends on the nature of their living habits, ranging from agriculture, grazing, or trade (Zambakari, 2015). The climate conditions vary between the states, from intermittent floods during the rainy season to severe droughts; this has affected the nutritional status of the agriculture-dependant population (Ahmed, 2020). The infrastructure of the majority of the states in Sudan is considered poor, with inadequate designs and conditions of roads, scarcity of transportation vehicles, and costly travel expenses to and from the capital.

This has enormously affected the accessibility of the affected communities to health care facilities (Charani et al., 2019a) (Fadlallah et al., 2020).

Figure I.1.1 Geographical location of Sudan showing the states and major cities. Available at https://www.dreamstime.com/rosevite2000_info ID: 173475475 Accessed 04.11.2021.

Sudan is currently facing enormous political fractures and economic challenges that have resulted in a decline in the quality of life of the majority of the population (Khan et al.). The internal displacement of the population due to war conflicts or the deteriorating living circumstances regarding health, education, and income has created massive pressure on the already limited capacity of the big cities (Saeed and Ali, 2013). The internal displacement monitoring center has estimated the total number of IDPs in Sudan to be 2,134,000 persons, with 272,000 and 84,000 new displacements due to disasters and war conflicts, respectively, in 2019 alone (Mohamed, 2020). The geographical location of Sudan, having open borders with countries of unstable political conditions such as South Sudan, Libya, and Eretria, has added to the burden; this has resulted in a large extent of immigration into the country (Fadlallah et al., 2020). Moreover, Sudan's

open borders have led to massive healthcare challenges in controlling the recent COVID-19 pandemic (Fadul et al., 2021).

In 2018, the international organization for migration had indicated that 695,000 refugees, 105,000 south Sudanese have returned to Sudan from South Sudan, and additional migrants need urgent humanitarian assistance, including residential camps, water, food supply, and health care facilities (Frowd, 2018) (UNOCHA, 2018). Moreover, the net migration rate of Sudan in 2018 was -1.2% in 1000 people, reflecting the high number of emigrants, mainly professional skilled workers outside the country (Adesina, 2020).

1.2 Health Care Indicators

The economic sanctions imposed by the United States on Sudan since 1997 have significantly affected the fundamentals of health, education, and banking sectors, complicating the importation of medications, medical equipment, and technology (Hamid, 2012) (O'Driscoll, 2017). The negative impact of the sanctions was further amplified by the secession of South Sudan in 2011, seizing almost two-thirds of the oil revenues (Elbeely, 2013).

The latest update of the World Bank classification of countries by income in July 2020 has lowered the category of Sudan from lower middle income to the low-income country, with 8.4 million (19.5%) of the population living in extreme poverty, earning a daily income of less than 1.9 US dollars (HAMADEH, 2020).

In 2020 the United Nations development program estimated the human development value of Sudan to be 0.510 in its human development report, listing Sudan as 170th among 189 countries (Table I.1). This UN report also revealed low values of health indicators in Sudan, i.e., 38.2% of children under the age of five suffer from moderate or severe forms of malnutrition, the mortality rate in under-five children was shown as (60.5 in every 1000 live births). The maternal mortality ratio was 295 deaths in every

100.000 live births. The life expectancy at birth was estimated to be 65.3 years, while the health sector expenditure was only 6.3% of the GDP in 2020 as per the world datasheet of the Human Development Report 2020 (UNDP, 2020) (Mahgoub, 2020).

Table I.1 Indicators of The Human Development Index in Sudan according to the World Human Development Report 2013, 2020 and World data Sheet 2020 (UNDP, 2020).

Human Development Indicators and components	**2012**	**2020**
Human Development Index (HDI)	0.414	0.510
Sudan ranking among world countries	171	170
Life expectancy at birth (years)	61.8	65.3
Expected years of schooling (years)	3.1	7.9
Mean years of schooling (years)	4.1	3.8
Gross national income GNI per capita	1848 (SDG)	3829 (SDG)

In the education sector, the UN-HDR 2020 indicated the rate of primary school dropout (24.8%) as 2,443,016 children are out of school; the education index of Sudan is 0.345. An adult literacy rate has shown a slight improvement compared to the (65.81%) indicated in 2008 to 60.7% in 2020. The literacy rate is higher among adult females aged (15 to 24 years), with a percentage of (73.49%), while adult males attained (72.51%) literacy rate. Education inequality was estimated to be 42.5%, reflecting the unequal distribution of educational resources, including public school funding, highly qualified

teachers, and books. Only 30.9% of the population has access to internet services (UNDP, 2020).

CHAPTER 2: Health Care System in Sudan

2.1 General Overview

The health care system in Sudan is composed of complex interlinked elements that comprehensively interact to deliver health service to the Sudanese population eventually. These baseline components include resources, management, organization, infrastructure, and funding (Ebrahim et al., 2017a).

Sudan has a decentralized health care system; strategic plans and health financing policies are produced at three administrative levels federal, state, and locality-based. The Sudanese Federal Ministry of Health (FMOH) is the body that is responsible for the specification of national health policies, development and implementation of strategies, coordination between states, and international collaborations. State Ministries of Health work at a micro-level organization; they implement FMOH guidelines. In contrast, localities deliver the services to the people. However, states and localities are authorized to regulate their areas according to the emerging need (Noory et al., 2020).

In addition to the public sector, which falls under the administration of the Ministry of Health, medical care services in Sudan are provided to the population by peripheral systems like insurance companies, hospitals, and university clinics of the armed forces and private medical colleges (Salim and Hamed, 2018). Furthermore, multiple international non-governmental organizations in Sudan provide substantial health services specific to underdeveloped states and minorities. Unfortunately, there is marked disintegration between the health care providers in Sudan. Health care policies are fragmented, with numerous bodies developing uncoordinated health strategies with minimal synchronization with other partners. Regulations to secure extensive

stakeholder contribution and engagement and improve the coordination between health care policymakers are deficient and ineffective (Charani et al., 2019b).

Health delivery services are structured into three levels: primary, secondary, and tertiary planes. In its latest health profile report, the Sudanese Federal Ministry of Health has stated that 86-93% of Sudanese people have primary health care facilities near their residential areas; however, only 24–40% of them have access to the basic package. This insurance package does not cover the diagnosis of cancer nor therapy. Patients can only utilize it for emergency medical care, i.e., road traffic accidents and maternal delivery; moreover, the report indicated that 36% of the primary medical care facilities are in suboptimal conditions in terms of workers, equipment, and overall capacity (Organization, 2017). The public health sector serves people via fifty-five hospitals, 3,726 family health centers, and 141 locality units. Regarding the medical insurance coverage, the National Health Insurance Fund barely covers 35 – 40% of the Sudanese affiliated with the governmental sector. Faith-oriented organizations, i.e., Islamic Daiwan AlZakah and missionary churches, provide partial and occasionally complete medical coverage to the poor distributed within the country (Adesina, 2020).

The private health sector in Sudan is rapidly growing; yet, its services are curative and are mainly concentrated in big cities like Khartoum. The number of private investor-owned ‘for profit’ hospitals is estimated to be 17 in total. On the contrary, non-profit hospitals, medical care facilities regularly funded by charity organizations, and educational institutions that do not pay taxes are 32 in addition to 319 health centers (Kheder et al., 2020).

2.2 Challenges with regards to the Health Care System in Sudan

The health care system in Sudan faces enormous challenges related to the country's complex political and socioeconomic variables. The most critical constraints of health delivery in Sudan include the poor infrastructure of health care facilities, fragmentation, and disorganization between the three levels of the ministry of health. In addition to the fast-growing population, the deficient number of accredited, trained medical staff, weak monitoring schemes, and financial restrictions (Charani et al., 2019a).

***Funding*:** The health financing plan has been negatively impacted by the economic sanctions, particularly regarding the importation of medications, equipment, and care-providing utilities. Almost half of the health amenities are underequipped with no active maintenance contracts. The financial resources customized to the health sector are limited. Despite the rising demand, health sector expenditure in Sudan is estimated to be 6.3% of the GDP, with an almost 10% shortage. This allocated percentage is inefficient and does not cover the fundamental priorities of the health care delivery system. In 2015, the 'out of pocket expenditure' of total health expenditure was estimated to be 78.9% (Organization, 2017).

In Sudan, the national revenue is produced via tax and non-tax sources, and Petroleum derivatives products constitute the significant Non–tax revenues resource. While trade and service tax contributes more to the tax revenue source, the tax to GDP ratio is approximately 8%. Hence, the health care sector lacks the financial needed support expected to be received from tax revenues. (Pedersen and Bazilian, 2014).

***Medical workforce*:** Furthermore, Sudan faces massive forms of external migration of skilled personnel with specific regard to health care workers, with a net migration rate of -1.2% in 1000 people in 2018. This has been mainly attributed to poor payments, ill-defined job descriptions, and insufficient compensations compared to neighboring

African countries and the Gulf area. In 2003 the magnitude of Sudanese medical diaspora was estimated to be 12,000 out of 21,000 graduates of different medical colleges accounting for 60% of the medical doctors registered in the Sudanese Medical Council (Sudanese et al., 2006), almost 3426 specialists outmigration Sudan to work for the international market. Despite the negative impact of skilled workers' migration, the country has benefited from the diaspora as these doctors provide their families with regular financial remittances, contributing substantially to the foreign revenue for their country. Moreover, physicians and pharmacists have established numerous development programs working abroad with initiatives that significantly impacted Sudanese people's lives (Abdalla et al., 2016).

In 2017, the health workforce statistics via the WHO illustrated the country's profile, which showed only 19 doctors, three pharmacists, 79 nurses/midwives, and only one dentist per 100,000 population (Figure I.2.1). These figures are significantly lower than the WHO targets for low-income countries, which is 230 doctors per 100,000 population. The shortage in the human workforce is substantial, and the deficiency of sufficiently trained medical and laboratory technicians has adversely affected health care delivery in Sudan (UNOCHA, 2020).

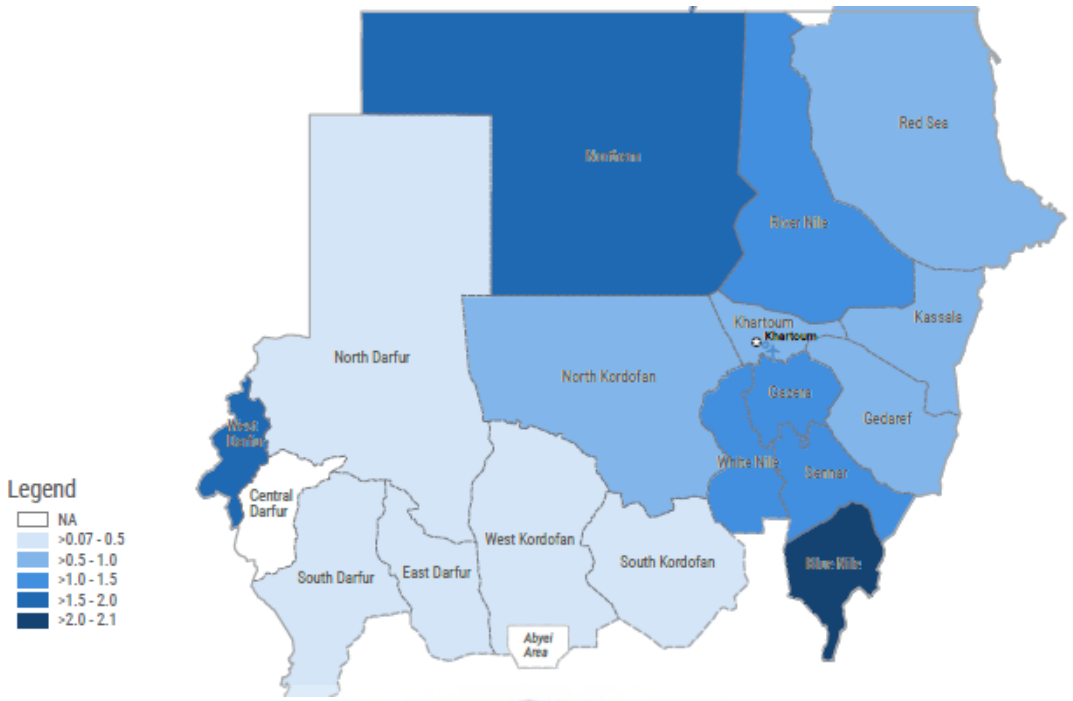


Figure I.2.1 The density of health workforce per State in Sudan (doctors, nurses, and midwives) per 1,000 population per state (UNOCHA, 2020).

Inequality of distribution of health care: Sixty-five percent of the Sudanese population are rural inhabitants where accessibility to health care services is minimal. Most states in Sudan suffer from insufficient health care utilities and trained medical staff. Primary health care is regularly provided by uncertified medical and dental assistants and traditional healers who lack the knowledge or experience to address complex medical diagnoses such as malignancies (Ismail, 2020).

There is marked inequality in the distribution of health care therapeutic facilities and histo-pathological diagnostic centers and medications in Sudan. Sixty percent of the centers providing secondary and tertiary care are in the capital Khartoum, leaving the remaining 40% distributed throughout the seventeen states. In 2013, the availability of essential drugs and medical products in care providing centers in the public sector was 53.7%, while only 1 CT scanner and 0.3 MRI machines were available per million population. Health profile statistics (2016) of Sudan indicated that 15 primary health

care centers and 74 hospital beds were available for every 100.000 population (Mansour et al., 2021).

Medical colleges and teaching hospitals are located in the urban areas creating a shortage of healthcare services in the rural areas where 65% of the population resides. Furthermore, this creates a substantial financial and logistical burden on the people living in rural areas. Frequently they need to bear the high costs of trips to and from big cities, the cost of treatment in cases not covered by medical insurance, in addition to living costs throughout the treatment journey (Al Mahdi, 2019). This aspect has a well-established effect on individuals' health-seeking behavior. Some patients tend to ignore their medical needs in favor of not facing massive financial burdens, especially in lower socioeconomic areas.

***The burden of disease*:** Communicable and non-communicable diseases form a considerable burden for the health sector in Sudan, with an estimate of 52.8% and 47.2%, respectively (Figure I.2.2) (Charani et al., 2019c). Malaria forms a tremendous burden of communicable diseases, with an expected 41 million Sudanese people at risk of developing malaria and an estimated 1.5 million new cases in 2017 alone, accounting for 35% of the combined malaria burden in the Eastern Mediterranean area (Organization, 2020). Governmental financial cuts have adversely affected the capacity and extension of control programs; moreover, the availability of insecticide treat nets and malaria testing have also been extensively jeopardized (Phillips et al., 2017).

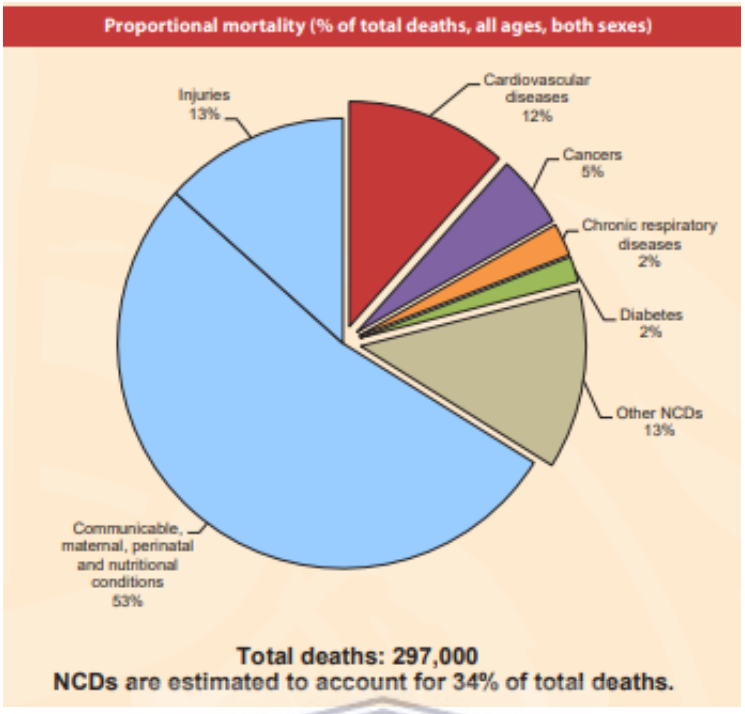


Figure I.2.2 Causes of mortality in Sudan (2018) reproduced from Sudan Health system profile 2018

The estimated incidence of tuberculosis in Sudan is 50 per 100 000 population in 2017, accounting for 21054 reported cases. People with a lower socioeconomic status, internally displaced, and refugees crossing the open borders are at higher risks and more aggressive TB morbidity and mortality rates (Hassanain et al., 2018). Moreover, Sudan is suffering from Multidrug-resistant tuberculosis (MDR TB) that commonly occurs due to malpractice in dose prescription and patients' compromised adherence to medications; MDR TB has an estimated prevalence of 19% in re-treatment cases and 1.8% in new cases in 2012 (Ali et al., 2019).

Schistosomiasis represents the most prevalent parasitic infection in Sudan with varying degrees of prevalence throughout the states; the prevalence of Schistosomiasis crossed 25%, 27.4%, and 46.5% in Eastern Darfur state, New Halfa, and White Nile state, respectively (Afifi et al., 2016). This prevalence is mainly related to environmental

hazards such as undrained water and contaminated food attributed to poor personal hygiene (Cha et al., 2019).

Sudan is considered a low HIV prevalent country in the sub-Saharan countries. The number of newly reported cases of HIV in 2019 was estimated to be 3500, while the total number of adult and children patients living with HIV is estimated to be 46000, marking the prevalence of 0.2 in the general population. Only 22% of these patients are under the ART regimen coverage (Bashir et al., 2019).

The latest update of the International Diabetes Federation (IDF) in Sudan indicates that the prevalence of Diabetes in adults to be 17.9% (Cho et al., 2018). This prevalence varies between the states ranging from 20% to 20.8% in northern and eastern Sudan (Omar et al., 2019). This has been mainly attributed to the urbanization of the lifestyle, i.e., obesity and increased consumption of carbohydrates and sugar products (Forouhi and Wareham, 2014).

The average yearly expenditure of the care of childhood and adult diabetes was estimated to be 283 and 175 in 2017 USD (23% and 9% of incomes of the patients' families) respectively, 36% of the first figure accounted for the cost of insulin (Eliadarous, 2017).

2.3 Health Information System in Sudan

The comprehensive and reliable data constitutes the foundation of decision-making across the entire health system and is considered crucial for the specification, implementation, and monitoring of health care policies worldwide. Such policies will directly impact the appropriate distribution of financial resources and, hence, cost-effective delivery of healthcare services. Health Information System (HIS) software is developed to digitalize the recorded data, decrease the burden of manual paper-based methods, and fix a regular quarterly or annual reporting rate.

The health information system is responsible for data, generation, collection, analysis, and communicative utilization. HIS comprises data at different levels of the health care system; the individual level is concerned with the patient's health profile, diagnostic and therapeutic needs. The health facility level guides decisions like purchasing drugs, equipment, and utilities within the public and private facilities; the population level covers information on peoples' viewpoints, behaviors, and health-seeking determinants. Lastly, the public health level is mainly concerned with identifying challenges and creating timelines for potential solutions.

In Sudan, the Federal Ministry of Health has specified four years (2012–2016) national health information strategy that focuses mainly on digitalizing HIS, improving the data quality, and stabilizing reporting rates via developing a standardized integrated reporting system. Moreover, FMOH has expanded the coordination and collaboration between multiple sectors including, the United Nations, non-governmental organizations, and the private health care sector (El-Nour et al., 2016).

Lately, in 2017, the Sudanese government has specified a twenty-five-year strategic plan that has prioritized health system reform via reducing the burden of communicable and non-communicable diseases. The introduction of e-health programs, supporting the local pharmaceutical industry, categorical free health care services, e.g., under-five children, pregnancy, emergency care, and renal dialyzes (Ebrahim et al., 2017b).

The Health Information system in Sudan is currently facing significant challenges. Low budget and unequally distributed human resources among the localities, fragmentation within programs regarding the data collection, parallel implementation channels, and a low number of reports, particularly at the level of primary health care centers. Furthermore, the quality of the generated data is jeopardized, especially with the manual handling of the data in terms of collection, storage, and management. HIS primarily

reflects the indicators developed from the public sector, whereas private data is seldom shown (El-Nour et al., 2016, Al-Said, 2010).

CHAPTER 3: Cancer in Sudan

Cancer is a broad, non-specific medical term that refers to the uncontrolled mitotic division and cellular transformation of healthy cells into malignant ones. Moreover, the cancer cells originate in a primary tumor site, and they may move to secondary organs via a process known as metastasis (Ferlay et al., 2019b). The outcome of aggressive forms of cancers may lead to morbidity and mortality depending on the type and stage of the tumor and patient's factors (Zhang et al., 2020). The various phenotypes of cancer exhibit a disparate incidence with diverse aetiological factors and unpredictable survival outcomes (Duraker and Hot, 2020).

The prevalence of cancer is rapidly increasing in Sudan, with 25,746 new cases and 17160 deaths in 2018. In females, breast cancer was the most prevalent, with 5677 cases accounting for 36.6%, followed by cancer of the cervix, as 1084 (7%) new cases were registered in 2018. However, Non-Hodgkins Lymphoma (NHL) (10.9%), and Leukaemia (10.2%), ranked first and second as the most frequently registered cancer types in males (Figure I.3.1).

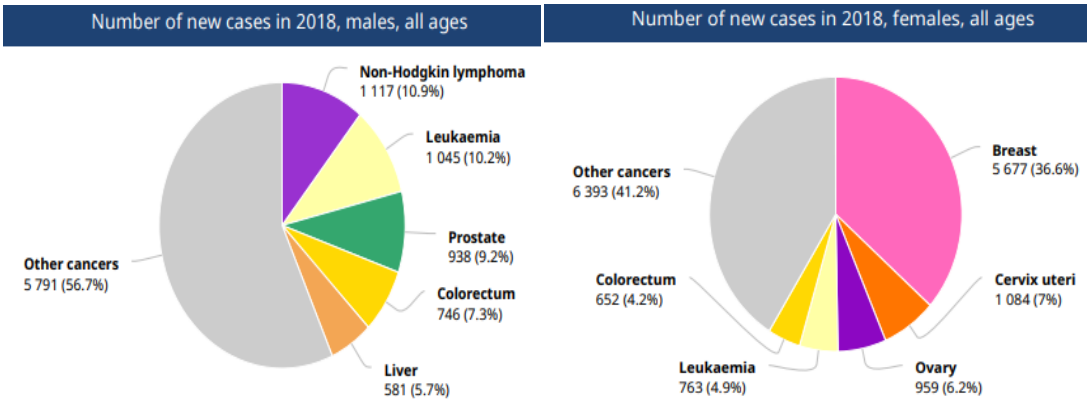


Figure I.3.1 Incidence of Cancer by gender in Sudan (Bray et al., 2018).

The increased incidence of cancer in Sudan has been attributed to multiple causes depending on the phenotype of the malignant tumor and patient's related factors. The usage of tobacco was associated with increased risk for Oral Cancer (Idris et al., 1994) (Ahmed, 2013), coexisting infections like Epstein Barr Virus and Human papilloma virus were found to have a positive correlation with tumor genesis in the breast (Ahmed et al., 2019a) (Elhasan et al., 2019).

Cancer ranks as one of the ten causes of hospital admission in Sudan. Nevertheless, as discussed earlier, the health care system in Sudan suffers from tremendous financial and logistical shortages in addition to the enormous burden of distressing infectious diseases such as cholera and TB. This has resulted in limited utilized budget care for cancer care.

Despite the rapidly expanding population approaching 43 million, Sudan has very few oncology centers that perform histopathological investigations and onco-therapy in the capital Khartoum and a single center located in the central state of Gezira. Furthermore, Sudan lacks efficient registration systems for cancer-related data. Patients' record files are still manually written, stored, and retrieved due to the non-availability of either digital software or trained personnel to aid in patients' data collection and central storage. Even though a national registry in Sudan was established in the early sixties and was reinstated in 2009, the non-availability of a unified surveillance data collection system has adversely affected the efficiency, reliability, and sustainability of cancer-related data.

Moreover, the deficit in the coverage of the health information system leaves considerable gaps in the rural states; this endangers the quality and quantity of the collected data as it might not reflect the accurate figures and the magnitude of cancer at the national level. Moreover, there is an apparent scarcity in molecular and biological

studies that enhance a comprehensive understanding of the etiology of various types of cancer in Sudan. This could be attributed to the non-availability of a national cancer biobank which would allow optimum tissue storage units and advanced laboratory bioengineering devices.

CHAPTER 4: Literature Review

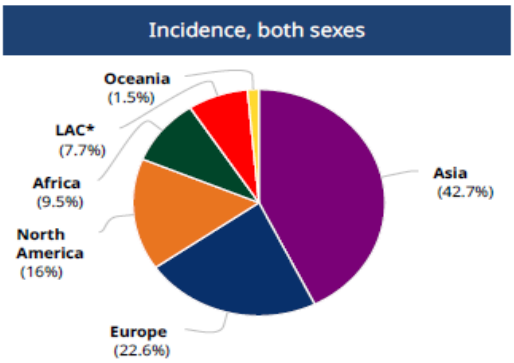
4.1 Non-Hodgkins Lymphoma

4.1.1 General Introduction

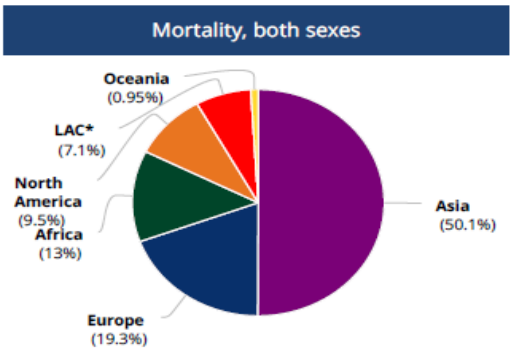
Non-Hodgkin's Lymphoma (NHL) is a group of malignant neoplasms of the lymphoid system that encompasses malignant B or T lymphocytes within their cytological configuration (Singh et al., 2020). These heterogeneous lymphomas vary in etiology and exhibit diverse pathogenetic mechanisms depending on their genetic, environmental, and host-dependent influences (Chiu and Hou, 2015). The various subtypes of Non-Hodgkin Lymphoma have diverse clinical presentations and behavioral patterns (Sapkota and Shaikh, 2020). Furthermore, prognosis and treatment response depends on tumor host-related factors (Nair et al., 2018).

4.1.2 Epidemiology

According to the most recent GLOBOCAN data, 509,600 new cases of NHL were diagnosed globally in 2018, which encompasses 2.8% of worldwide cancer diagnoses. Asia had the highest incidence of NHL with 42.7% of the world's detected cases. Africa ranked fourth with a total of 7.7%, subsequent to Europe at 22.6% and North America with 16% of the globally detected Non-Hodgkin Lymphoma cases. With regards to mortality, Africa ranks third in the global mortality rate, followed by Asia and Europe (Figure I.4.1) (Myneni, 2021).



Population	Number
Asia	217 695
Europe	115 118
North America	81 361
Africa	48 587
*Latin America and the Caribbean	39 410
Oceania	7 419
Total	509 590



Population	Number
Asia	124 585
Europe	48 096
Africa	32 395
North America	23 565
*Latin America and the Caribbean	17 729
Oceania	2 354
Total	248 724

Figure I.4.1 Global Incidence and Mortality rates of Non-Hodgkins Lymphoma in 2018 (Bray et al., 2018).

In Africa, NHL ranks fifth of the most prevalent cancer with an incidence rate of 4.6%, following breast cancer that accounted for 16%, cervix uteri cancer 11.3%, prostate 7.7%, and liver with 6.1% (de Martel et al., 2020). The mortality rate for NHL was estimated to be 4.7%, placing it as sixth-highest cancer in Africa in 2018. Furthermore, in the Northern African region, NHL had a 5.8% incidence rate and a 5.7% mortality rate in 2018. These rates are escalating at an alarming rate in Africa. This is attributed to various factors, such as the growing population, aging of populations, human immunodeficiency virus HIV positive status, and Malaria (Tomoka et al., 2018).

In Sudan, NHL comprised the third-highest new cancer cases following breast cancer and leukemia in 2018, with 1675 (6.5%) new cases, 1014 deaths, and a 5.9% mortality rate (Ferlay et al., 2019a) (Figure I.4.2). In males of all ages, NHL constituted the highest among other types of tumors with the percentage of (10.9%), while in females, breast cancer exceeded all other cancers with an incidence of (36.6%).

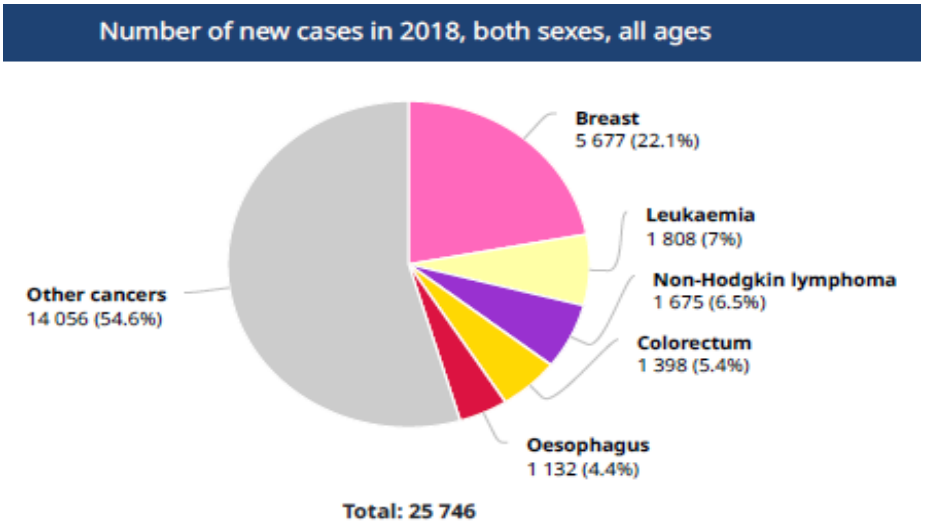


Figure I.4.2 Number of new cases of cancer in Sudan Source: (GLOBOCAN) (Ferlay et al., 2019a)

The age-standardized incidence rate per sex of NHL in Sudan was shown to be 8.3 per 100.000 in males and 3.9 per 100.000 in females, while the Age-standardized mortality rate was estimated to be 4.2 per 100.000 (Figure I.4.3) (Ferlay et al., 2019c, Bray et al., 2018).

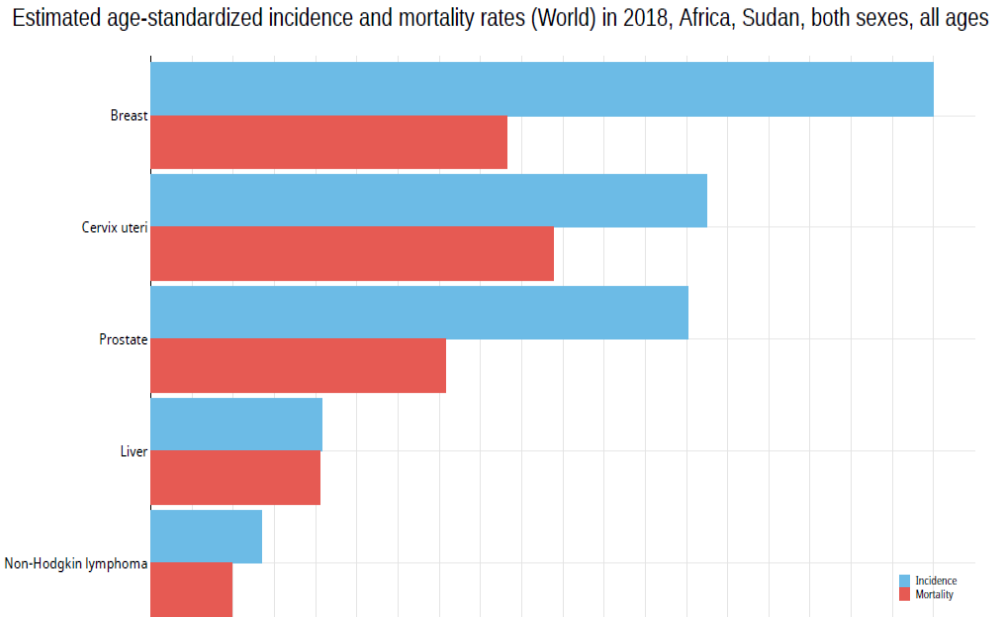


Figure I.4.3 Age-standardized incidence and mortality rates of Non-Hodgkins Lymphoma in Sudan 2018 (Miranda-Filho et al., 2020).

4.1.3 Etiology and Risk factors

Over the last few years, there has been a marked increase in the registered number of new cases of NHL worldwide (Miranda-Filho et al., 2019) (Figure I.4.4). This was attributed to various etiological factors related to immunodeficiency and congenital disorders, such as Rheumatoid Arthritis, which was shown to double the risk for developing Non-Hodgkin Lymphoma than healthy individuals. This was attributed to the prolonged inflammatory activity and comprehensive immune system stimulation, mainly B lymphocytes (Mercer et al., 2017). Rheumatoid Arthritis medications, i.e., methotrexate and tumor necrosis factor (TNF) inhibitors, have not been shown to increase the risk for Non-Hodgkin Lymphoma (Klein et al., 2018).

Acquired immunodeficiency etiological factors that could impact the increased risk for Non-Hodgkin lymphoma are best illustrated in Acquired Immunodeficiency Syndrome (AIDS). The impaired immune response caused by the Human Immunodeficiency Virus (HIV) directly impacted the Non-Hodgkin Lymphoma risk. NHLs in AIDS patients are usually of B cell origin, diffuse large B cell (DLBCL) being the most frequent subtype of NHL, followed by Burkitt's Lymphoma and AIDS-related Lymphoma (ARL) (Gibson et al., 2014).

Furthermore, a growing number of viruses and microbial agents increased the risk for lymphomagenesis in adults. Reactivation of the Epstein-Barr virus (EBV) in immune-compromised patients has reflected a positive correlation with increased risk to NHL (Teras et al., 2015). A 2018 metanalysis study has investigated the correlation between Non-Hodgkin Lymphoma and Hepatitis B virus. It concluded that Hepatitis B is significantly associated with DLBCL, and patients with positive Hepatitis B serotype have a two to three folds higher chance of developing Non-Hodgkin Lymphoma.

Moreover, HepB positive patients diagnosed with Non-Hodgkin Lymphoma presented at a younger age and more recurrent hepatic dysfunction before and following chemotherapy (Li et al., 2018a). The two mechanisms suggested for the pathological role of Hepatitis B virus in lymphomagenesis are the integration of the virus into the host genome driving molecular oncogenes to be overexpressed or to the downregulation of tumor suppressor genes expression. The second mechanism shows that chronically infected hepatocytes produce viral antigens, stimulating B lymphocytes' proliferation. This might result in molecular translocations or overexpression of tumor oncogenes resulting in malignant transformation (Marcucci et al., 2012).

In addition, some studies have illustrated the role of Helicobacter pylori (H Pylori) bacteria in the activation of gastrointestinal tract Diffuse Large B cell Lymphoma in adults via immunological cross-reactivity between the neoplastic B lymphocytes and the H Pylori bacteria (Cheng et al., 2019).

The male gender was more susceptible to Non-Hodgkin Lymphoma (Horesh and Horowitz, 2014). Moreover, aging has been suggested to increase the risk of NHL; this has been attributed to various hypotheses, i.e., accumulative genetic changes; likewise, hematopoietic stem cells exhibit clonal restraint with aging resulting in the proliferation of malignant B lymphocytes (Sarkozy et al., 2015b).

Family history of Lymphomas has a substantial impact on the risk for NHLs; it has been shown that individuals who have first-degree relatives suffering from Non-Hodgkin Lymphoma have a 1.7 fold increased risk for developing NHL themselves.

Additional environmental factors have a varying etiological impact on Non-Hodgkin Lymphoma. Occupational hazards, i.e., exposure to pesticides, chemical solvents like xylene and benzene, ultraviolet radiation, increased animal fat consumption, organ

transplants, and blood transfusion, were substantial in increased risk NHLs (Zhang et al., 2011).

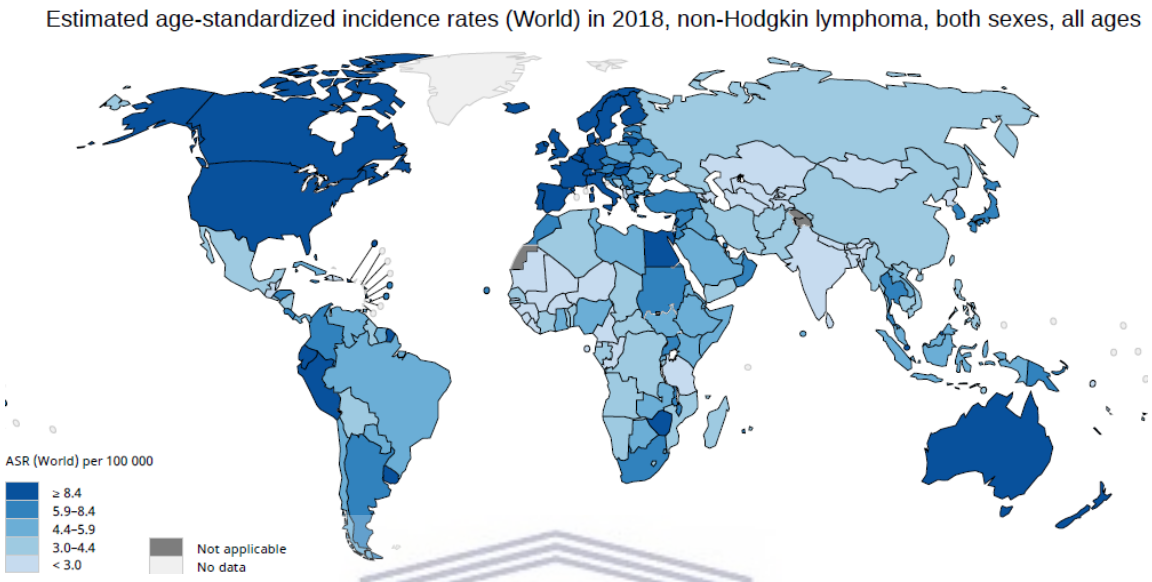


Figure I.4.4 World map showing age-standardized NHL incidence rates in both sexes. Source (Globocan 2018) (Bray et al., 2018).

4.2 Diffuse Large B cell Lymphoma

4.2.1 Etiology and Risk Factors

Lymphoid cells originate primarily in primary lymphoid organs. The Lymphoid predecessors, which are fated to become T-lymphocytes complete their maturation in the thymus hence termed T-cells. Whereas B-lymphocytes develop in the bone marrow, therefore they are called B-cells (Figure I.4.5). Peripheral blood T and B lymphocytes circulate in a distinct pattern via secondary lymphoid organs, i.e., Lymph nodes, spleen, and mucosa-associated lymphoid tissues. Cellular circulation mode is mainly controlled by adhesion molecules and cytokine receptors (Metodiev, 2015).

Diffuse Large B Cell Lymphoma (DLBCL) is the most common type of non-Hodgkin Lymphoma globally, with an estimate of 30-40% of the entire cases worldwide. The annual incidence of DLBCL is estimated to be 7 to 8 cases per 100,000 per year and is considered to be one of the highest ten causes of cancer mortality (Sukswai et al., 2020).

This malignancy is termed "Diffuse large B cell Lymphoma" as tumor cells exhibit a diffuse distribution pattern when seen under the microscope. Furthermore, neoplastic B lymphocytes are morphologically atypical and appear larger than normal. They infiltrate the target tissues and do not act in response to anti-proliferative cellular signals (Niitsu et al., 2009). DLBCL encompasses a broad spectrum of heterogeneous entities in terms of their histological immunophenotypes. The site of tumor presentation is nodal or extranodal (Swerdlow et al., 2016).

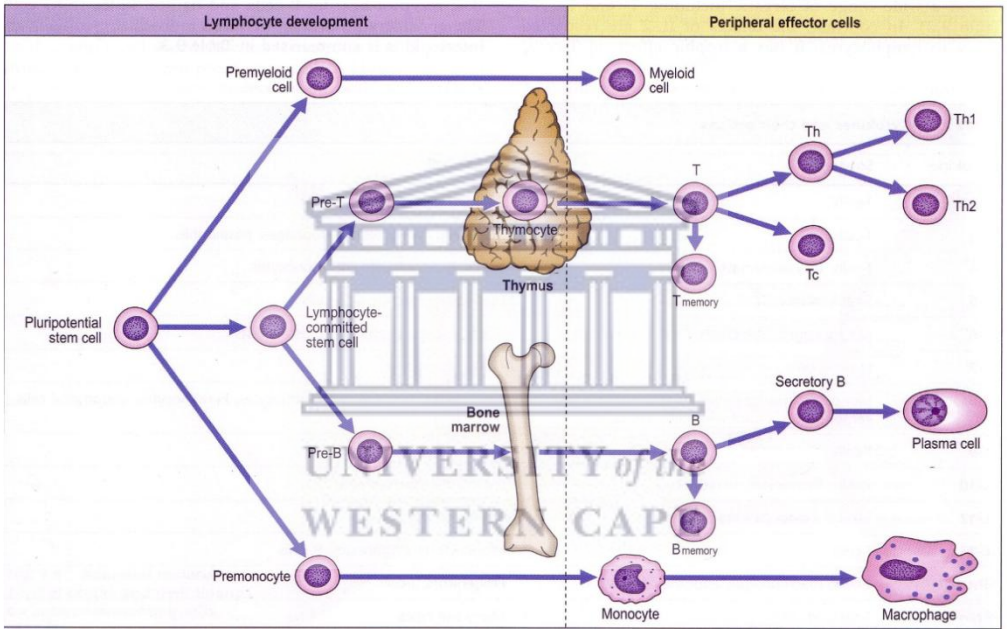


Figure I.4.5 The development of Lymphocytes from a pluripotent stem cell in the bone marrow (Metodiev, 2015)

Several potential risk factors are thought to increase the risk for DLBCL. These include a suppressed immune response caused by Human Immunodeficiency Virus HIV (Mezger et al., 2020) (Mezger et al.), autoimmune diseases, i.e., Rheumatoid arthritis (Gorodetskiy et al., 2020), and organ transplantation (Blinder and Fisher, 2008, Cassidy et al., 2017). Moreover, an established family history of Non-Hodgkin Lymphoma, Hepatitis C seropositivity, an increased Body Mass Index (BMI) in young adults was associated with an increased risk for DLBCL (Cerhan et al., 2014). Some

factors were shown to have gender specificity for females; these included hormonal therapy, low adult Body Mass Index, hair dyes, and exposure to ultraviolet emission. Alcohol consumption and previous blood transfusion were identified as potential risk factors for males (Flowers and Skibola, 2016). Advanced age (Above 64 years old), slight male gender predilection, single unmarried and advanced tumor stage was shown to have a high-risk potential for Diffuse Large B cell Lymphoma (Çağlayan et al., 2019).

4.2.2 Clinical Presentation

4.2.2.1 General Overview

Diffuse Large B cell lymphoma commonly shows a rapid and aggressive behavior that presents clinically as a fast-growing non-painful tumor mass, lymphadenopathy in about two-thirds of cases, hence termed nodal tumor. The remaining tumor sites show a clinical presentation in organs rather than lymph nodes; this is termed extranodal disease. The stomach, gastrointestinal tract, and groin are the most frequent anatomical sites of extranodal disease. Fever, night sweating, extreme fatigue, loss of appetite, and sudden massive weight loss are common symptoms of DLBCL, in addition to symptoms that may relate to the site of the tumor, i.e., abdominal pain, bloody stool, and diarrhea (Niitsu et al., 2009).

4.2.2.2 Diffuse Large B cell Lymphoma in the Oral Cavity

Lymphomas represent 3.5% of the entire intraoral malignancies, constituting the second most frequent neoplasms in the head and neck region following squamous cell carcinoma (Silva et al., 2016b). Diffuse large B-cell Lymphoma is the most common hematological subtype of Non-Hodgkin Lymphoma involving the oral cavity. Almost 50% of the cases tend to de novo growth (Bhattacharyya et al., 2010). In the head and neck region, the tonsils are the most affected site (Kolokotronis et al., 2005), followed

by the parotid gland, while intraoral sites include the gingival (Manjunatha et al., 2011), palate (Souto et al., 2013) and the buccal mucosa (Batta et al., 2019), tongue, the floor of the mouth, retro molar area and the maxilla respectively (Zou et al., 2018b).

The most frequent intraoral manifestations of Diffuse Large B cell Lymphoma are oral ulcers, pain, lip numbness, facial swelling, tooth mobility, and pathological fractures (Pereira et al., 2015). Extraoral findings may include lymphadenopathy and facial asymmetry (Silva et al., 2016a). In advanced cases, with delayed or misdiagnosis of Diffuse Large B cell Lymphoma in intraoral structures, the tumor may act aggressively and proliferate, causing subsequent bone expansion (Abuaffan, 2017), cortical bone perforation, and intraoral soft tissue mass formation (Djavanmardi et al., 2008). Radiographically, the lesion presents as an ill-defined radiolucency with irregular margins. This radiological picture mimics several intraoral neoplasms making the diagnosis of Lymphoma in the jaws quite challenging and frequently delayed. DLBCL can be misdiagnosed as an odontogenic tumor, periapical granuloma, cyst, or a dental abscess (Bugshan et al., 2015).

4.2.2.3 The WHO Classification of Diffuse Large B cell Lymphoma

The World Health Organization (WHO) (2008) has classified hematopoietic and lymphoid tumors according to clinical, histological, and biologic implications. (Swerdlow, 2008). This classification has categorized DLBCL according to the tumor cell of origin mainly two molecular phenotypes, germinal center B-cell (GCB) and activated B-cell (ABC), in addition to a category of cases that could not be otherwise classified. These subtypes vary in their molecular chromosomal alterations, protein induction pathways, and clinical outcome. The five years overall survival for Germinal B cell was estimated to be 50-60% of the cases while drops to 15-30% for patients with Activated B cell (Young et al., 2015).

However, in 2016, there has been an upgrade and a revision of the classification represented by recognized guidelines for categorizing malignant lymphomas in specific (Swerdlow et al., 2016). The revised profile has included upgraded scientific data regarding existing tumors that integrated vital diagnostic and prognostic implications (Swerdlow et al., 2016).

The revised classification of DLBCL has identified a distinctive novel subtype that bases its segregation on the co-expression of two biomarkers, MYC, and BCL2 oncogenes. Scientific literature has shown that almost 30-50% of Diffuse Large B cell Lymphomas exhibit MYC oncogene expression (Karube and Campo, 2015, Ziepert et al., 2020).

In 25-35% of DLBCL positive MYC cases, there is a simultaneous co-expression of BCL2 protein (Zhang et al., 2019). This phenotype has been termed the "Double Expresser Lymphoma (DEL)" with significant concurrent overexpression of both MYC/BCL2 proteins, in the absence of genetic translocations as the majority of DEL-DLBCL cases do not express underlying chromosomal rearrangements (Johnson et al., 2012b).

The 2016 WHO classification has stated that Double Expresser Lymphoma "DEL" is considered a novel indicator for the prognosis of Diffuse Large B cell Lymphoma cases. Overexpression of MYC and BCL2 proteins is measured using a cutoff point of >40% and 50% of positive cellular/ nuclear staining, respectively; these figures are recommended for Double Expresser Lymphoma diagnosis (Hu et al., 2013). Double Expresser Lymphoma has shown inferior prognostic and treatment outcomes than other DLBCL phenotypes (Aggarwal et al., 2016).

It is estimated that 30% of Diffuse Large B cell Lymphoma cases exhibit concurrent overexpression of MYC and BCL2, defined as Double Expresser Lymphoma (Green et al., 2012). Patients with Double Expresser Diffuse Large B cell Lymphoma are older at

diagnosis and present with advanced stages of the tumor. They experience B symptoms that include fever, night sweating, and drastic weight loss. Moreover, Double Expresser subtype patients present with marked advanced international prognostic index (IPI) and inferior suboptimal prognosis upon R-CHOP chemotherapy regimen (Nowakowski et al., 2016).

Furthermore, it has been shown that over 50% of recurrence presented as relapsed/refractory Diffuse Large B cell Lymphoma were patients of Double Expresser Lymphoma subtype (Liu and Barta, 2019). Savage et al. (2016) have stated that with six years median follow up of Double Expresser Lymphoma patients who underwent R-CHOP regimen. These individuals had an increased potential risk for CNS relapse without considering either the IPI score or the cell of origin (Savage et al., 2016).

The overall survival of Double Expresser subtype patients over five years was 30–45%; on the contrary, the five-year survival was higher for the control group accounting for 66–75% (Wight et al., 2018). Furthermore, the Double Expresser Lymphoma subtype of Diffuse Large B cell Lymphoma was associated with a subordinate complete response rate, shorter overall survival, and lesser progression-free survival (Green et al., 2012). Hence, several studies have emphasized the prognostic value of subclassifying DLBCL into the Double Expresser Lymphoma subtype (Kim et al., 2016b, Shi et al., 2017).

Utilizing Immunohistochemical (IHC) detection of Double Expresser Lymphoma is considered uncomplicated and efficient and is essential for routine diagnosis. According to various studies, the cutoff point “positive expression” of immunohistochemistry results for MYC and BCL2 ranges above 40% and 50% respectively is considered the high expression, especially with their close correlation with clinical outcomes (Johnson et al., 2012a).

CHAPTER 5: Rationale, Aims, Objectives, and Hypothesis of the study

5.1 Rationale of the Study

The incidence of Diffuse large B cell Lymphoma has been increasing lately at an alarming rate especially, in developing countries (Martijn et al., 2017). The standard therapy in Sudan is based solely on the R-CHOP chemotherapy regimen. It has been noticed that the prognosis of Diffuse Large B cell Lymphoma is not favorable. There is a scarcity of published scientific data on DLBCL in Sudan; hence, further research in this domain was identified. Globally, late diagnosis and side-effects related to the treatment directly affected the disease's poor prognosis (Stefan, 2015).

In Sudan, the treatment of DLBCL is routinely planned according to the histopathological assessment of the tissue specimen and not on the phenotypic sub-classification of the tumor, as recently updated in 2016 WHO classification of Hematopoietic diseases (Swerdlow et al., 2016). Hence, the current diagnostic methods and general approach for the DLBCL have failed to reflect the biological behavior or accurately predict the prognosis of the DLBCL. Therefore, there is an urgent need for phenotypic sub-classification of the DLBCL, which can be done by utilizing MYC and BCL2 molecular biomarkers of patients' prognosis to direct the therapeutic approach and assign patients to the optimum targeted treatment.

Moreover, there is a clear gap in the literature regarding the genomic screening of Diffuse Large B cell Lymphoma, especially with the extensive genetic diversity of the population that could be of potential oncogenicity relevance. Consequently, in this project, we identified the molecular and genomic signature of the tumor in Sudanese patients using the Quantitative Polymerase Chain Reaction technique (qPCR).

DLBCL has often been noticed in dental settings, as it frequently presents in the cervical lymph nodes of the head and neck area. To the best of the author's knowledge, apart from published case reports (Abuaffan, 2017), there has been minimal intense research on Diffuse Large B cell Lymphoma in the Sudanese heritage. Thus, the purpose of this study was to bridge these gaps in the literature. This would inform policy-makers, health professionals, and researchers with a comprehensive baseline profile on the diagnostic and prognostic values of DLBCL biomarkers.

5.2 General Aims

- To investigate the diagnostic value of MYC and BCL2 oncogenes in Double Expresser Lymphoma phenotype of Diffuse Large B cell Lymphoma patients.

- To identify the prognostic value of MYC and BCL2 biomarkers in Double Expresser Lymphoma subtype of Diffuse Large B cell Lymphoma as predictors of patients' survival.

- To explore the molecular and genomic signature of the DLBCL in Sudanese patients via quantitative polymerase chain reaction molecular technique.

5.3 Specific Objectives

1. To sub-classify the Diffuse Large B cell Lymphoma samples into Double Expresser Lymphoma phenotype using the expression levels of MYC and BCL2 proteins.

2. To correlate the clinico-pathological parameters and the expression levels of MYC/BCL2 levels with the Disease-Specific Survival of Diffuse Large B cell Lymphoma patients.

3. To investigate the dependant and independent survival prediction of the clinicopathological parameters and the expression levels of MYC and BCL2 proteins in Diffuse Large B cell Lymphoma patients.

4. To quantify the relative expression level of messenger RNA of MYC and BCL2 oncogenes and correlate it to the protein expression level of the molecules and investigate its effect on the survival of Diffuse Large B cell Lymphoma patients.

5. To assess and measure the oncogenic dosage of MYC molecule and the translocations related to BCL2 oncogene using Quantitative Real-time PCR.

6. Develop a specification or data standard technology for a "Tumor Registry Software" that captures essential clinical and epidemiological data of cancer patients.

5.4 Hypothesis

High Expression levels of MYC and BCL2 biomarkers in the phenotypic subtype of Diffuse Large B cell lymphoma (Double Expresser Lymphoma) predicts poor prognostic outcome in RCHOP treated patients.

CHAPTER 6: Thesis Structure

The thesis formatter consists of the cover page, attestation of authorship, the abstract, dedication, acknowledgment, table of contents, attached list of figures and tables, and lastly, a list of abbreviations.

This document is divided into seven sections and twenty-one chapters; each section commences by listing the included chapters, introductory preamble, and contents list. At the closing of the thesis, a list of ten appendices was outlined.

Section I comprises seven chapters; Chapters 1, 2, and 3 outline the geography, ethnicity, health care system, and cancer statistics of The Republic of Sudan. Chapter four thoroughly elaborates on the literature review of the project, focusing on the Non-Hodgkins Lymphoma and Diffuse Large B cell Lymphoma in terms of epidemiology, etiology, risk factors, clinical presentation, and classification. The study's rationale and the general aims, specific objectives, and hypothesis of the study are stated in chapter 5. Whereas chapter 6 illustrates the structure of the thesis, and chapter 7 focuses on the ethical considerations of the project.

Section II illustrates the methodological considerations utilized in the project; chapter 8 discusses the study cohorts, design, setting, inclusion/exclusion criteria in addition to the selected study variables. Chapter 9 explains the technique used to achieve the study objectives, the diagnostic value of MYC and BCL2 molecules in sub-classifying DLBCL. Immunohistochemistry was the method of choice; steps of IHC, staining protocols, and slide quantification were illustrated in this chapter. Moreover, chapter 10 demonstrates the quantitative real-time PCR procedures, i.e., DNA extraction, mRNA quantification, oncogenic dose, and translocations. One of the project's main objectives was to develop tumor registry software; this is thoroughly explained in chapter 11. The statistical tests utilized in analyzing the data are shown in chapter 12.

The project results are shown in section III in four chapters; chapter 13 demonstrates the clinicopathological data of DLBCL patients. The IHC results and survival prediction are shown in chapter 14, while chapter 15 focuses on the quantitative real-time PCR findings. A summary of the study results is shown in chapter 16.

Section V of the thesis encompasses the discussion related to the study findings, survival rates of DLBCL in relation to the expression levels of MYC and BCL2 investigated via IHC and qPCR were shown and correlated to possible justifications in chapter 17. Specific parameters related to the development of the tumor registry software are discussed in chapter 18 of section V. The challenges and limitations faced during the study timeline are listed in chapter 19 of the thesis.

Section VI concludes the study findings and suggestions proposed by the author upon analysis of DLBCL data in chapters 20 and 21. References are listed in section VI, and lastly appendices are listed in section VII. The thesis document was assessed via 'Turnitin' software for plagiarism, and the result was considered very much acceptable.

CHAPTER 7: Ethics

This research project was conducted at the National Radiation and Isotope Center, the main histopathological and treatment center in Khartoum-Sudan. All investigations were carried out according to the declaration of Helsinki and the Hippocratic Oath. The Health Research Committee of the Sudanese Ministry of Health has approved the project (SR 5/9/18) (Appendix 1). Moreover, the Biomedical Science Research Ethics Committee (BMREC) at the University of the Western Cape has approved the scientific methodology and ethics of this project, code# (BM19/1/30) (Appendix 2 and 3 and 4).

Upon obtaining the ethical clearance from the Sudanese Ministry of Health, the Radiation and Isotope Center administration unit consented to collect their clinical data, medical records, and tissue samples of Diffuse Large B Cell Lymphoma patients (Appendix 5). It is noteworthy to mention that patients consented to use their data for research purposes at RICK records.

All clinical and histopathological data were secured in password-protected devices. Patients' data were de-identified at various stages of analysis and interpretation. There has been no hazard of physical harm to any alive study participants during this study timeline. No additional genetic analysis was done except for that related to the DLBCL tumor. Participation in the project was entirely voluntary. All participants or their related families were assured that they had the option to withdraw discontinue participation at any time they desired without any consequent actions.

SECTION II: Investigation Methodology

CHAPTER 8

CHAPTER 9

CHAPTER 10

CHAPTER 11

CHAPTER 12

Preamble

This section includes five chapters. Chapter 8 elaborates on the study variables, including the study design, setting, population, and the patient cohort. Chapters 9 and 10 thoroughly detail the study's methodological techniques: the paraffin blocks, immunohistochemistry, and qPCR. Chapter 11 delineates on developing the tumor registry software. Chapter 12 enumerates the statistical tests used for the analysis of the recorded data.

Contents of Section II:

CHAPTER 8: The Study Cohorts

8.1 Study Design

This was a retrospective analytical study, in which medical records and formalin-fixed paraffin-embedded tissue (FFPE) blocks of tumor tissue of patients of DLBCLwere collected and retrieved from archival units. The selected cases were diagnosed between the years (2013 and 2018).

8.2 Study Setting

The study was performed at the Radiation and Isotope Center (RICK) in Khartoum, Sudan. RICK is the main Oncology and tumor biology center where the diagnosis, treatment, and research projects of cancer are conducted in Sudan (Figure II.8.1).

Figure II.8.1 Radiation and Isotope Center- Khartoum (https://www.uicc.org/membership/radiation-isotopes-centre-khartoum-rick) accessed 1.11.2021

8.3 Study Population

The medical records and tumor tissue blocks of 151 patients diagnosed and treated for Diffuse Large B Cell Lymphoma between the years (2013 and 2018) at RICK were analyzed. The National Radiation and Isotope Centre in Khartoum, Sudan, constituted the study samples of this project. Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria were used as a scientific foundation for the study design (Sauerbrei et al., 2018) (Altman et al., 2012). Epidemiological data were obtained from the archival unit of RICK. Retrieval of the patients' files and FFPE Tissue blocks were done manually, shelf by shelf. This center, though being the central referral histopathological center in Khartoum, lacked a digital registry.

Upon selecting the patients' records, FFPE were identified and collected, and a consultant pathologist selected the blocks with sufficient tissue from the tumor. These were then cut into three-four μm thickness sections and stained with Hematoxylin and Eosin (H&E) to confirm the diagnosis (Figure III.14.2). The diagnosis was confirmed by the researcher and endorsed by a consultant pathologist.

A detailed history, treatment, follow-up, survival data, and R-CHOP-based treatment regimen were obtained from the patients' files. This data was recorded in a customized designed data entry sheet (Appendix 6). During tissue sectioning and staining, 14 samples (8.5%) had technical faults or missing data and were excluded, so 165 was the total collected samples, and 151 was the final sample size.

The internationally recognized Lugano classification was followed for the staging of the study cases, previously known as the Ann Arbor system, in which the tumor is classified into four stages (I, II, III, and IV) (Swerdlow et al., 2016). Stage I, single lymph node/single organ, is affected by the tumor cells. Stage II Lymphoma hits two groups on the same diaphragm side, either above or above or below. Stage III is a more aggressive

form of DLBCL, as lymph nodes on two sides of the diaphragm might be involved or have reached the spleen. Stage IV has widely metastasized into organs other than lymph nodes, e.g., bone marrow, liver, and lung (Cheson, 2014).

8.4 Inclusion and Exclusion Criteria

Inclusion Criteria:

A) Patients aged 16 years and above.

B) Patients diagnosed with DLBCL that fits the WHO- ICD10 classification.

C) Patients who have not received any surgical treatment, chemotherapy, or radiotherapy before specimen collection.

D) The selected patient had to have adequate tissue material in their FFPE block.

E) Have signed an informed consent,

Exclusion Criteria:

A) Patients' files with insufficient or inconsistent medical data or mismatched tissue blocks.

B) Individuals under 16 years were excluded from the study as they are rarely seen at RICK.

C) Patients who sought treatment in private clinics.

D) Records of HIV or Hepatitis B surface antigen-positive patients or were under medication were also excluded.

8.5 Study Variables

A) Host related parameters:

- **Age**: Patients age range was between 17-81 years old.
- **Gender**: Male or Female.
- **Marital Status**: Single, married, widowed, or divorced.
- **Mode of referral**: Self-referred to RICK, referred by a general practitioner working for the governmental sector, e.g., primary health care centers, and lastly referred by a private physician.
- **Occupation**: Student, labor, employee (i.e., professionals), housewife, and retired.
- **Habitual smoking**: However, no details were available other than whether the patient was a smoker at the time of diagnosis or a former one or not a smoker at all.
- **Residence**: Residential areas were categorized according to the geographical distribution noted by the Department of Sociology & Social Anthropology, Faculty of Economic & Social Studies, the University of Khartoum, as this might have an etiological impact on the tumor parameters (Unruh and Abdul-Jalil, 2014). Khartoum is the capital, and its surroundings have the highest population density because of the accumulation of educational and medical services; Northern Sudan provinces are the areas from the Egyptian borders passing through the desert and the Nile valley. Western Sudan, which constitutes the provinces of Darfur with its five provinces, along with Kordofan, which includes three provinces and the areas of Blue Nile province. Eastern provinces include areas of PortSudan and Kassala. Patients who presented to RICK from South

Sudan were included in the study as they were few and had resided in Sudan before South Sudan's independence in 2011.

- The patients' socioeconomic status was not listed as a variable, as no data was available regarding the educational background or the salary range of the affected persons. Nevertheless, most patients could not afford the high cost of the private medical sector and hence seek governmental health care and thus are of middle or low socioeconomic status.

B) Tumor related parameters:

- **Specimen site**: Cervical Lymph nodes (C) / Submandibular Lymph nodes (SM)/ Axillary mass (AX)/ Trephine Specimen (TE)/ Inguinal mass (IN)/ Supraclavicular Lymph nodes (SC)/ Parotid mass (P) / Abdominal mass (AB) /Intestinal mass (IS) / Thyroid mass (TY)/ Groin mass (GR)/ Neck swelling (NC)/ Oropharyngeal mass (OP)/ Brain biopsy (B) / Scrotal (SR) / Tonsils (TO) /Ovarian mass (OV) /Skin (SK) / Submental Lymph node (SE).

- **Date of Diagnosis and last follow-up**: were recorded in month and year of the first diagnosis and last follow-up or death registered in RICK file records.

- **Lactate Dehydrogenase enzyme**: LDH has been directly linked to DLBCL; hence it was in our interest. The cutoff point for LDH was shown to be High (>280 U/L or 2.34 microkatals/L) and Low (<4.68 microkatals/L) (William et al., 2013).

- **Ann Arbor staging**: Though the terminology of the staging classification has been updated to 'Lugano classification,' the term AnnArbor is still widely used at the time of data collection. The tumor stage is ranked between stages I, II, III, and IV according to the cancer stage at the time of diagnosis.

Additionally, more recorded data was related to the immunohistochemistry results, including MYC and BCL2 staining positivity, whether the sample was Double Expresser Lymphoma or not, treatment modality, and the current status of the patients dead or censored; these variables were added to the sheet after obtaining the results of the study and prior to statistical analysis phase (Table II.1).

Table II.1 Representative Sample (13) showing the study variables recorded from patients' files and IHC stained paraffin blocks. M=male gender, W=Widowed, SF= Self referral, IV= stage four Ann Arbor.

Sample	Gender	Age	Marital Status	Occupation	Residence	Mode of Referral	Smoking	Specimen Site	LDH level
13	M	70	W	Retired	Western Sudan	SF	No	Cervical Lymph nodes	High

AA staging	Date of Diagnosis	Last Follow up	BCL2>50%	MYC >40%	Double Expresser Lymphoma	Treatment	Current Status
IV	7.2016	10.2018	Positive	Positive	Positive	R-CHOP	Dead

It is noteworthy that all the data was dealt with according to the data management plan (DMP) described by the University of the Western Cape. DMP thoroughly states the collected data's administrative, legislative, storage, ethical, confidentiality, and sharing guidelines (Appendix 10). Methodological techniques detailed in the next chapter were done in close consultation and supervision of an expert laboratory technician.

CHAPTER 9: Methodological Techniques

9.1 Formalin Fixed Paraffin Embedded Tissue Blocks

The handling of Formalin-Fixed Paraffin-Embedded (FFPE) blocks is of enormous importance to ensure optimum immunohistochemistry IHC results (Xie et al., 2011). Tissue that is over or under fixed or drying can lead to protein degradation or crosslinking (Engel and Moore, 2011).

Standardized protocols regularly utilized for FFPE blocks in the histopathology laboratory of RICK were employed in this project. For each of the two targeted biomarkers, the FFPF pre-staining protocol was optimized. All tissues that were damaged were excluded before immunohistochemistry.

9.2 Tissue Preparation

Formalin-Fixed Embedded tissue blocks were sectioned into 3-4 um sections using the (Leica Biosystems) rotary microtome, then transferred and mounted on coated positively charged glass slides and dried in a hot air oven at 60°C for 1 hour. The sections were dewaxed in xylene for 5 minutes for three cycles. They then were rehydrated through descending grades of ethyl alcohol, beginning with 100% ethyl alcohol, then 90% ethanol, 70% ethanol, and lastly to distilled water, 4 minutes for each concentration. The sections were then washed three times with phosphate buffer saline PBS. The sections were then retrieved in the Target Retrieval device PT-Link of Dako (Real Envision Detection Kit, China) (Ruzinova et al., 2010), then left to cool at room temperature and washed three times with phosphate buffer saline PBS. Afterward, the sections were cycled by cytomated pen, upon which 0.3% hydrogen peroxide in methanol was added to each section for 15 min to block endogenous peroxidase activity and then washed two times with PBS.

9.3 Immunohistochemistry

To visualize and scientifically quantify the biomarkers chosen in this study, immunohistochemistry (IHC) was our method of choice. This method utilizes the antigen-antibody interaction to provide information about the expression and distribution of the targeted protein. However, it preserves the composition, cellular characteristics, and morphology of the native tissues (Grillo et al., 2017). Nevertheless, immunohistochemistry has some well-recognized challenges, as obtaining the desired outcome depends mainly on the accurate execution of the procedure. The accuracy of the results might show a discrepancy according to various factors, such as tissue selection, deparaffinization, fixation, antibodies selection, antigen retrieval, and proper quantification method (Kim et al., 2016a).

Initially, in this study, we planned to duplicate the tissue sections on one slide to simultaneously check the expression level of two biomarkers. This method aimed to decrease the financial burden and optimize the method for further usage of such affordable methods in a low-income county like Sudan. However, the author experimentally found it challenging and changed the single section per slide protocol to avoid molecular interaction between the biomarkers.

9.4 Staining Protocol of MYC and BCL2 Biomarkers

The antibody used to obtain MYC expression levels is the monoclonal rabbit antihuman c-MYC antibody (no.A11911; ABclonal, Massachusetts, USA) (Lian et al., 2017). According to the manufacturer's instructions, the antibody was applied at 1:50 dilution in antibody diluents, and sections were incubated overnight at 4 degrees C.

Slides were then washed with phosphate-buffered saline (PBS) three times for 5 minutes each. Peroxidase-conjugated biotinylated goat anti-rabbit secondary antibody was applied to the sections and incubated for 30 minutes at room temperature, and then slides were rinsed with PBS. After washing, 3, 3'Diaminobenzidine chromogen was

added to the slides for 10 minutes, then washed in distilled water three times and counterstained with Mayer's hematoxylin for 2-3 minutes. Slides were then dehydrated with graded ethanol, 75 %, 85 %, 90 %, 100 % each for 1 minute and finally immersed in xylene for 5 minutes. Coverslips were added via adding DPX mounting solution and lowering the slips to visualize the slides under the microscope to avoid air bubbles.

To detect BCL2 oncogene expression levels (BCL2 M0887, mouse, monoclonal antibody, 1:100; Dako, Carpinteria, CA, USA) (Na et al., 2019) was used. According to the protocol received from the manufacturer, the antibody was applied at 1:100 dilutions in antibody diluents (CAT#1W-1000), and sections were incubated for 60 minutes. Slides were then washed with PBS three times for 5 minutes each.

Peroxidase-conjugated secondary antibody anti-IgG was applied to the sections and incubated for 30 minutes at room temperature, and then slides were rinsed with PBS. After washing, 3, 3'Diaminobenzidine chromogen was added to the slides for 10 minutes, then washed in distilled water three times and counterstained with Mayer's hematoxylin for 2-3 minutes. Slides were then dehydrated with graded ethanol, 75 %, 85 %, 90 %, and 100 % each for 1 minute and finally immersed in xylene for 5 minutes. Coverslips were secured via DPX mounting solution. Care was taken to avoid air bubbles.

9.5 Quantification of IHC stained sections

Initially, stained slides were coded to mask patients' information, manually annotated by three researchers calibrated before 15 slides for each biomarker. To standardize the calibration and minimize the interobserver variability, the records for each biomarker were completed by a single observer blinded for the sample's information. The intensity of immunohistochemistry staining was classified as described by (Lian et al., 2017) as 1, 2, and 3, which represented a weak, moderate, and strong stain, respectively. This

correspondingly, the positively stained cells were graded as 0, 1, 2, 3, and 4. 0 score represents ≤5%, 1 signifies (6-25%), 2 indicates (26-50%), 3 shows (51-75%) and finally 4 illustrates (76-100%) of positively stained slides. To assess the immunoreactivity of each sample, the intensity was multiplied by the percentage of positively stained slides, and samples which showed ≥4 were considered as (High) expression; on the contrary, less than 4 were labeled as (Low) expressers. Consequently, areas that illustrated diagnosis of interest in the sections were pictured and saved (Figure II.9.1/2).

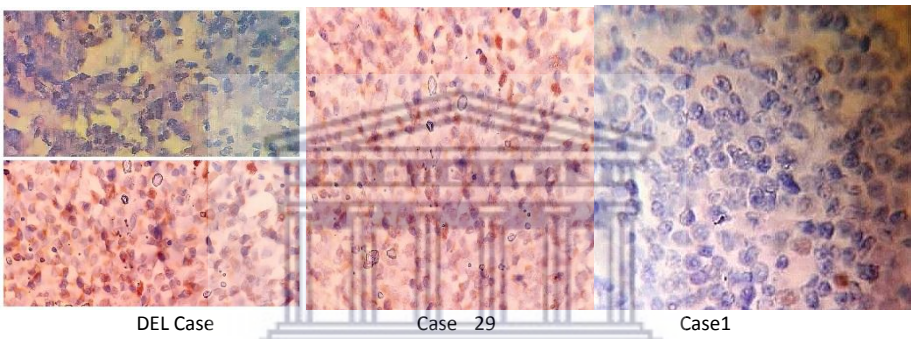


Figure II.9.1) Representative photomicrographs showing immunohistochemical expression of MYC and BCl2 biomarkers in DLBCL cases: Case 1, 90% MYC+ Bcl2 (High); Case 29 (75% Bcl2+).

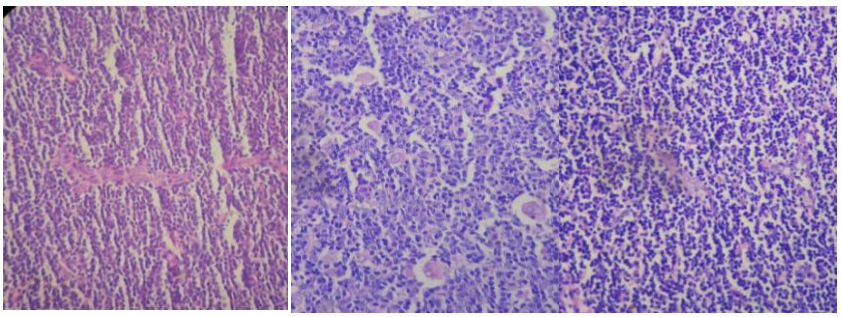


Figure II.9.2) H&E stained Diffuse large B-cell Lymphoma sections. Masses of atypical lymphocytes uneven chromatin in a necrotic background

CHAPTER 10: Quantitative Real-time Polymerase Chain Reaction

10.1 Overview

Quantitative real-time PCR is considered one of the most extensively utilized methods of gene quantification because of its enormous dynamic range, remarkable sensitivity, and ability to be very sequence-specific. Moreover, real-time PCR features little post-amplification processing and is acquiescent to escalating sample throughput (Wong and Medrano, 2005).

The introduction of Quantitative real-time PCR has revolutionized the field of gene expression analysis. This technology collects live data during the PCR process, simultaneously merging amplification and detection processes (Udvardi et al., 2008). There are numerous advantages to employing quantitative real-time PCR to quantify gene expression over other molecular techniques. It does not necessitate post-amplification processing (Derveaux et al., 2010); additionally, RT-qPCR has superior sensitivity over blot hybridization and RNase protection assays (Hsu et al., 2005).

Moreover, qPCR can detect a single and diminutive copy of DNA transcript with significantly lower variation coefficients than other techniques, e.g., q-PCR coefficient of variation may range from 14.2%-24%. At the same time, probe hybridization CV might reach up to 45.1% (Ricchi et al., 2017). Quantitative real-time PCR requires a reduced amount of RNA template than other molecular analysis techniques. Moreover, in case of availability of the appropriate equipment, qPCR can be a significantly high-throughput technique in which thousands of experimental genetic samples are tested and processed concurrently (Waseem et al., 2019).

Nevertheless, quantitative PCR is considerably expensive in terms of equipment, primers, and reagents; and due to its enormously superior sensitivity, solid experimental planning and a comprehensive understanding of the normalization techniques are crucial

for precise conclusions (Salipante and Jerome, 2020). In this study, the relative expression levels of MYC and BCL2 biomarkers, the oncogenic dose of the MYC gene, in addition to the translocations detected in the BCL2 biomarker were investigated.

10.2 Extraction of Deoxyribonucleic Acid (DNA)

10.2.1 Specimen Deparaffinization

QIAamp DNA FFPE Tissue Kit (cat. no. 56404) was used (Press et al., 2008). To achieve optimum dewaxation, FFPE blocks of DLBCL were cut into 20µm sections and placed into 1.5 ml centrifuge tubes. 800 µl of xylene was then added to each tube which was gently oscillated for 30 minutes. The samples were then centrifuged at 14000 rpm for 5 minutes, and the supernatant was removed using a Pasteur pipette. This was followed by the rehydration of the sections through different grades of ethyl alcohol, beginning with adding 800 µl of 100% ethanol and centrifugation of the sample at 14.000 rpm for three minutes, likewise for 80%, 60%, 40% ethyl alcohol, and finally placed in deionized water, 10 seconds in each of these rehydrating solutions followed by centrifugation at 13000 rpm for 5 minutes. Ultimately, the tissues were ready for the DNA extraction step.

Figure II.10.1 QIAamp DNA FFPE Tissue Kit (50)**,** DNA Extraction Kit (Janecka et al., 2015)

10.2.2 DNA Extraction Procedure

DNA was extracted from re-hydrated tissue using a DNA extraction Kit according to the protocol of the manufacturer (Qiagen DNA Extraction kit, Germany). Briefly, 200 µl of the buffer ATL was added to the re-hydrated tissue specimen. This buffer is composed of sodium dodecyl sulfate anionic surfactant, which assists in the fast lysis of the specimen cells by unfolding the non-covalent bonds in proteins (Gunaratna et al., 2018).

Followed by this step, 200 µl proteinase K enzyme (20mg/ml) was added and incubated at 56^{o}C 2hours, then 200 µl of AL buffer was added and incubated at 55^{o}Cfor 1-2 hours, 200 µl of ethanol 99% was added, and tubes were vortexed at 30 seconds. The mixture was then applied to the spin column and centrifuged at 12,000 rpm for 1 min. The collection tube waste was discarded, and 500µl of AW1 washing buffer solution was added and centrifuged for 60 sec at 12000 rpm. Another 500µl of washing buffer AW2 was added and centrifuged for 1 min at 12000 rpm, the tubes were then centrifuged for high speed to 3 min for ethanol drying, the columns were placed in clean 1.5 ml tubes, and 100 ul of AE buffer were added and incubated for 2 min at room temperature followed by centrifugation for 60 sec at 12000 rpm to allow stable storage of the extracted at DNA-20 C till further tests (Table II.2).

Table II.2 Sequences of MYC, BCL2, and normalization gene primers used in Quantitative RT-PCR

Investigated Genes	**Forward Sequence (5´–3´)**	**Reverse Sequence (5´–3´)**
MYC	CCTCCACTCGGAAGGACTATC	TGTTCGCCTCTTGACATTCTC
BCL2	GTGGATGACTGAGTACCTGAACC	AGACAGCCAGGAGAAATCAAAC
β-globulin (Normalization)gene	CCTGGCACCCAGCACAAT	GGGCCGGACTCGTCATAC

10.3 Quantification of the Expression of MYC and BCL2 proteins

Linking the expression level and the expression pattern of specific genes to a biological process or phenotype assists researchers in a clear understanding of the gene function, the pathogenesis, control of the development, cellular behavior, cell signaling pathways, and tumorigenesis.

The quantitative detection system was carried out using real-time PCR (Anlytika Jena, Germany) (Baruah et al., 2019). Quantitative real-time PCR master mix for one reaction was prepared according to the manufacturer guidelines as follows: 10µl of Real mod^{R} green 2X (Intron Biotechnology, South Korea), 2 µl of each 10 P mol/ml forward and reverse primer, 5 µl of DNA, the volume was completed to 20 µl by nuclease-free water. The reaction mix was cycled at 94°C for 30sec, 60°C for 30 sec, and 72°C for 30sec (40 cycles). The gene expression was determined by using relative analysis by beta globulin as a housekeeping gene for normalization.

10.4 Quantification and Determination of the Oncogenic Dosage of MYC

The genetic dose is the precise determination of the number of copies of a gene in the genome; the oncogenic dosage of a biomarker is identified via its ability to transform a cell in terms of deregulation of its expression or function. However, alteration in gene dosage, i.e., a number of gene copies resulting from gene mutations, might have considerable consequences on the expression level of genes responsible for the tumor phenotype. Hence, exploring such defects and a complete understanding of their biological significance can enhance cancer therapeutic modalities (Heideman et al., 2013).

Gene dosage investigations are crucial for the molecular diagnosis of malignant tumors caused by deleting or amplifying certain DNA regions containing specific genes**.** The quantitative real-time PCR technique calculates the copy number of each oncogene. This

method has shown advantages over traditional molecular techniques, i.e., the Southern-blot technique. Thus, qPCR is considered a superior molecular technique for quantitating the oncogenic dosage in clinical and genetic research domains (Dziadziuszko et al., 2006).

The real-time PCR quantitative detection system was carried out using real-time PCR (Anlitka Jena, Germany). The MYC oncogenic dosage was estimated, utilizing the Beta globulin gene as a reference; via the kit TaqMan Fast Universal PCR Master Mix in the Applied Biosystem 7500 instrument, the oncogenic dosage was calculated according to the manufacturer's instructions (Weihe, 2014).

The Real-time PCR master mix for one reaction was prepared as follows: 10 µl of (Analytica jene, Germany) probe master mix, 2 µl of each 10 P mol/ml forward, reverse primer and probe targeting to MYC oncogene, 5 µl of DNA, The volume was completed to 20 µl by nuclease-free water. The reaction mix was cycled 95°C for 5minute, 95°C for 15sec, 60°C for 30 minutes (40cycles); the MYC oncogene dosage was determined by using absolute analysis by generating a standard curve to determine the quantity of MYC oncogene dose, Taqman probes evaluated the number of MYC gene copies in each sample (Table II.3).

Table II.3 Sequence of primers and probes used in quantification of the oncogenic dose of MYC

MYC Gene	**PROBES (5'-3')**	**FORWARD PRIMER (5'-3')**	**REVERSE PRIMER (5'-3´)**
	ACCAGCAGCAGCAGCAGAGCGA (rox)	TCTACTGCGACGAGGAGGAG	GCAGCAGCTCGAATTTCTTCC

10.5 Detection of BCL2-IGH Translocations

A total of 40 specimens from patients with positive overexpression of MYC and BCL2 over expression elucidated as Double Expresser Lymphoma (DEL) were analyzed. The real-time PCR qualitative detection system was carried out using real-time PCR (Anlitka Jena, Germany). Real-time PCR master mix for one reaction was prepared as follows: 10 µl of (Analytica jene, Germany) probe master mix, 2 µl of each 10 P mol/ml forward, reverse primer and probe to detect BCL2-IGH Translocations, 5 µl of DNA, The volume was completed to 20 µl by nuclease-free water. The reaction mix was cycled 95°C for 5minute, 95°C for 15sec, 60°C for 30 minutes (40cycles). For optimum analysis of translocations within BCL2 oncogene, PRISM 7700 translocations detector (Applied Biosystems) (Luthra et al., 1998) was used.

CHAPTER 11: Development of Tumor Registry Software

Currently, there is no established Cancer Registry in Sudan that covers the population on a large scale; this is a direct result of the challenges faced by a restrained health care system. The health system in Sudan is greatly affected by political-economical sanctions, inadequate infrastructure, and a long-lasting war. While the system is decentralized, we find that majority of the health care facilities are based in Khartoum state; these centers suffer from insufficiently trained health care providers due to external migration. Moreover, a substantial displaced population in Sudan due to war circumstances, creating a massive burden on the health system.

In order to address the lack of a tumor registry, the author sought a practical resolution for the storage and stratification of patient data. A Tumor Registry Software (TRS) was developed, which incorporates updated variables that were found to be of enormous importance in the cancer research domain. Variables included in the software provide a link between the histopathological diagnostic center and the treatment health care facility.

At RICK, both diagnostic and therapeutic services are provided within the center premises. Hence, all necessary patients parameters will be accessible for registrars and data entry units. This software will be also available for use in multiple hospitals such as Khartoum Dental Teaching Hospital.

11.1 Standardized Parameters and Specifications of the Tumor Registry Software

The software variables covered all aspects related to cancer patients and included demographics, related tumor parameters, referral mode, clinical remarks, diagnosis, outcome, follow-up and survival data, covid19 status, and the personnel responsible for the diagnosis.

Patients' demographics will include file #, biopsy block #, National Identification Number, Passport number if available, and place of birth. These parameters will enable further linkage of the patients' data with the ministry of interior for future collaborations. Furthermore, patients' personal data variables in the software included gender, age, marital status, ethnic tribe, residence, contact number, next of kin, occupation, habits, and medical history.

Figure II.11.1 Password-Coded Introduction Page of Tumor Registry Software

The ethnic background of the Sudanese population is diverse. The tribes stratified by the Department of Sociology and Social Anthropology at the University of Khartoum were

used (Casciarri and Babiker, 2018). Social habits like smoking, snuff dipping, and alcohol consumption have an entry. The commencement, duration, and frequency of a particular social habit are of particular interest to as many cancers have proved to directly correlate with practices mentioned earlier (Jiang et al., 2019, Janbaz et al., 2014). As discussed in previous chapters, Diffuse Large B cell Lymphoma in specific had shown a significant association with many viruses and comorbidities (Cesarman, 2014). The patient's medical comorbidity is essential in our software (Wästerlid et al., 2019).

Figure II.11.2 Parameters Illustration from Tumor Registry Software 1

The mode of referral of the patient, such as self-referral, governmental, or private health care sector, can be noted. The main complaint can be recorded as well as the clinical

presentation. Further variables like the date of diagnosis, tumor site, tumor size, clinical remarks, differential diagnosis, and definitive diagnosis can be recorded. The International Classification of Disease (ICD10 2019) was used to stratify the tumor variables (Mainor et al., 2019). Furthermore, additional parameters which can be recorded in the registry include the tumor stage, treatment (chemotherapy, radiotherapy, combined), follow up (date of the last visit, lost for follow up), recurrence, outcome (cure, death, and currently on treatment), COVID 19 infection/vaccination status, in addition to the lab technician and the pathologist in charge.

Some data sets were registered in numerical, i.e., age, date of diagnosis, and last follow up; others had optional entry categories, i.e., tribe, ICD classification. Some variables like 'clinical remarks' can be entered manually. The software has double-entry validity checkpoints; it requires no internet connection for data entry. The confidentiality of the patients will be ensured according to the international association of cancer registries (Storm et al., 2005) (Figure II.11.1). The software developer will provide training workshops for the registry unit staff, assigned by the hospital administration, software support, update, and administration will be provided by the developer.

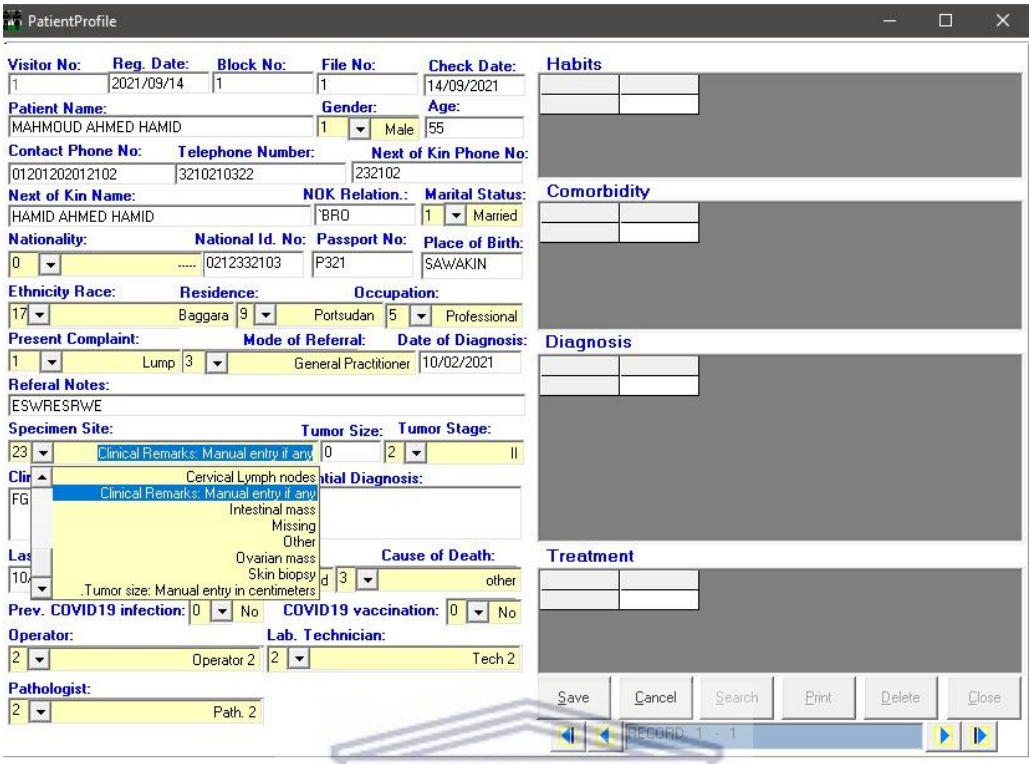


Figure II.11.3 Parameters illustration from Tumor Registry Software 2

11.2 Technical Aspects related to the Tumor Registry Software

A specialized software engineer developed the Tumor Registry Software. The programming language used was Visual Basic Language (Saymote, 2014). The database utilized was Microsoft Access for easy installation within computers of hospitals and histopathological centers (Eckstein and Schultz, 2018). Seagate Crystal Reports was used as a report writer (Wisniewski et al., 2003).

CHAPTER 12: Statistical Analysis

The first objective of this project was to quantify the Double Expresser Lymphoma cases among the Diffuse Large B cell Lymphoma samples using the concurrent co-expression levels of MYC and BCL2 biomarkers in addition to exploring the clinico-pathological parameters of the patients using descriptive tables.

The second objective was to evaluate the prognostic value of the clinico-pathological variables and the expression levels of the MYC and BCL2 biomarkers in Diffuse Large B cell lymphoma patients; the correlation between these elements and the Disease-specific survival rate was investigated (DSS).

To correlate the clinico-pathological parameters and the expression levels of the selected biomarkers to prognosis and patient disease-specific survival (DSS), the Cox Regression proportional hazard models correlation were used. The two-tailed Pearson Chi-square (Fisher Exact) test was used to estimate the correlation between the categorical clinico-pathological parameters and the expression levels of MYC/BCL2 biomarkers.

The effect of the survival predictors was detected with multivariate-adjusted Cox models. The multivariate models were adjusted for the independent clinico-pathological predictors, identified via multivariate non-adjusted models such as Double Expresser Lymphoma (DEL).

Survival estimates were schemed using the Kaplan Meier method and compared using the log-rank statistical test. The hazard ratios and 95% confidence intervals were calculated using univariate and multivariate Cox proportional-hazards models. A backward selection method was utilized to eliminate the non-significant terms from the model. A two-sided *P* value less than 0.05 was considered statistically significant.

The Disease-specific survival time was calculated in months from the date of the first diagnosis to the last follow-up or death. All patients who were alive or lost to follow-up at the end of the collection phase were censored. "Disease-specific survival rate refers to the percentage of people in a study or treatment group who have not died from a specific disease in a defined period. The period usually begins at the time of diagnosis or at the start of treatment and ends at the time of death" (Cheng and Chang, 2017).

In this study, using the Disease-Specific survival 'DSS' rate to estimate the survival rate of Diffuse Large B cell Lymphoma was the only viable option. A population life table containing demographic parameters, i.e., gender, age, etc., matched to Diffuse Large B cell Lymphoma could have been an effective tool to estimate patients' survival. However, unfortunately, it is not available in Sudan. Moreover, in Sudan, the information provided in the death certificates is minimal and inaccurate. Furthermore, no discharge letters are given to patients upon them leaving the hospital.

Two sources were used to obtain accurate information on the survival/death of the patients included in the sample. These included: (I) a review of death certificates stored at RICK for some of the study samples; (II) where necessary; patients were contacted by phone to ascertain the outcome. In the survival analysis, some patients/cases were recorded as censored observations; these are the cases where patients were still alive or when a patient's information is only known for a limited duration (loss of follow-up).

For the quantitative real-time PCR study**,** two tests were used to compare the investigated groups, the T-test, and Wilcox-test to test for the difference between the target mRNA relative expression of the normalized gene compared to MYC and BCL2 biomolecules. The results were summarized as hazard ratio (HR) and a 95% confidence interval. The p-value was considered statistically significant at ≤ 0.05. The analysis was performed using Statistical Package for Social Sciences (SPSS) version 24.

SECTION III: RESULTS

CHAPTER 13

CHAPTER 14

CHAPTER 15

CHAPTER 16

Preamble:

The results of this project are detailed in Section III, subdivided into 4 Chapters. Chapter 13 documents the clinic-pathological data of DLBCL patients. The diagnostic and prognostic implications of MYC and BCL2, in addition, the survival rates of patients with DLBCL, are listed in Chapter 14. In contrast, Chapter 15 focuses on the quantitative real-time PCR findings. A comprehensive summary of the results is illustrated in Chapter 16. All Chapters are laid out as per the objectives of the study.

Contents of Section III

CHAPTER 13: Clinico-Pathological Characteristics of Patients with DLBCL

The clinical data of 151 patients with DLBCL at the RICK between the years 2013 and 2018 constituted the study samples. The sample size was calculated according to the requirements of the intended predictor model construction. This calculation was based on the recommendations of Harrell's survival predictor model, which states that "The number of included cases should be ten times greater than the number of the event predictors" (Iasonos et al., 2008). Hence, the sample size was adequate in order to investigate fifteen predictors. The distribution of DLBCL cases between the years 2013-2018 is illustrated in figure (III.13.1). Among the samples, 65 were females representing (43.05%) while 86 were males accounting for (56.95%) of the study participants (Figure III.13.2). The mean age of the patients was 57.25 years, with a minimum of 17 years and a maximum of 81 years old.

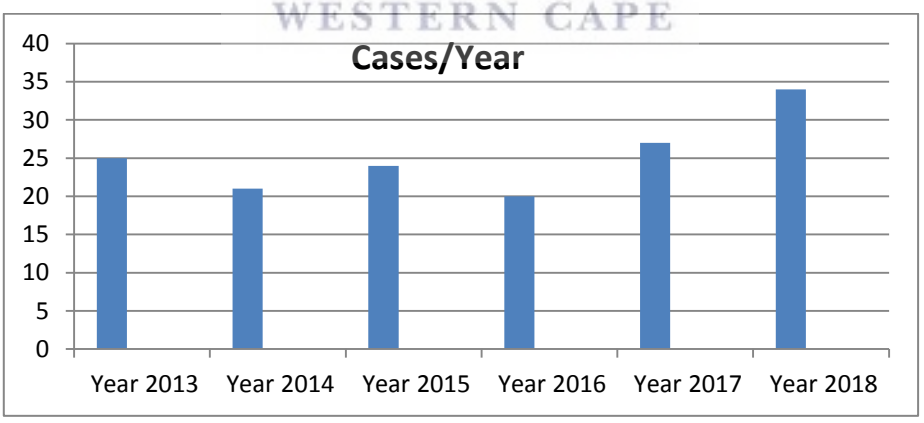


Figure III.13.1 Distribution of DLBCL cases per year

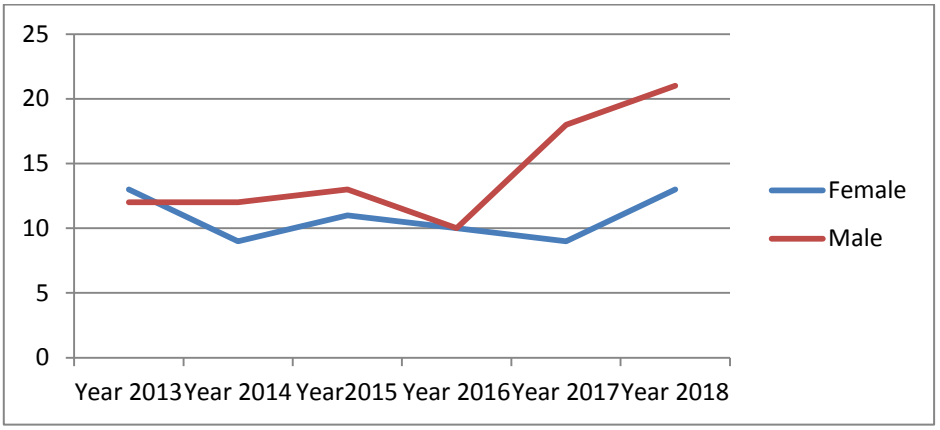


Figure III.13.2 Distribution of DLBCL cases by gender and year

The majority of the study samples were from married individuals (68.87%), followed by widowed (15.89%), single (9.27%), and divorced (5.96%). Within the study cohort, the highest proportion of patients were "Employees" being professionals (N=46, 30.46%), e.g., school teachers, full-timers, and office workers. "Retired" patients constituted (29%), while housewives represented (21.19%) of the patients and finally labor like factory workers and street cleaners shared almost the same distribution with students representing a (10%) percentage each Figure (III.13.3).

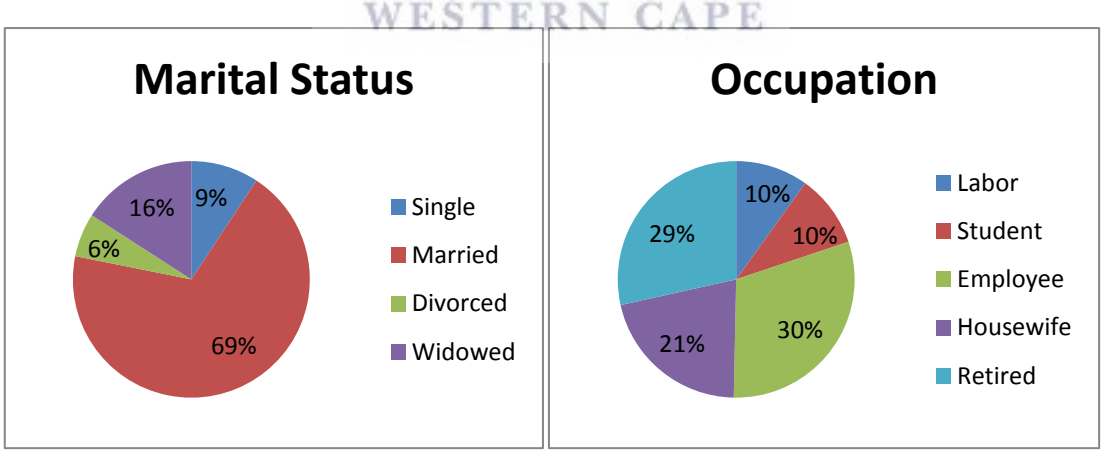


Figure III.13.3 Marital status and Occupation distribution among DLBCL patients

The mode of referral was stratified into three categories, as shown in (Figure III.13.4). Self-referral (SF) patients identified a specific medical concern, which initiated their seeking medical care. Hence, they voluntarily came to RICK to seek medical advice and management. This group constituted half of the study cohort (51%). The second group

(GP) represented patients referred by a general practitioner from a primary health care facility or dental center facility. This group of patients constituted (31.79% of the patient pool). The third group (PP) represented patients (17.22%) who attended a private physician and were referred to RICK for further investigations.

The original residential areas of the patients are indicative of their tribal ethnicity, and these were categorized into five major regions due to the massive heterogeneity of the Sudanese people. Over one-third of the patients (33.11%) were from the western provinces of Sudan, where the Arab Negros and the Pure Negros are the significant inhabitants. (31.13%) of the study, participants resided in Khartoum, the capital of Sudan, and its surroundings. This was followed by patients who were from the northern part of Sudan (24.50%), an area occupied by the Nubians, the lowest percentages among the study groups were the easterners (8.61%) and patients from South Sudan (2.65%).

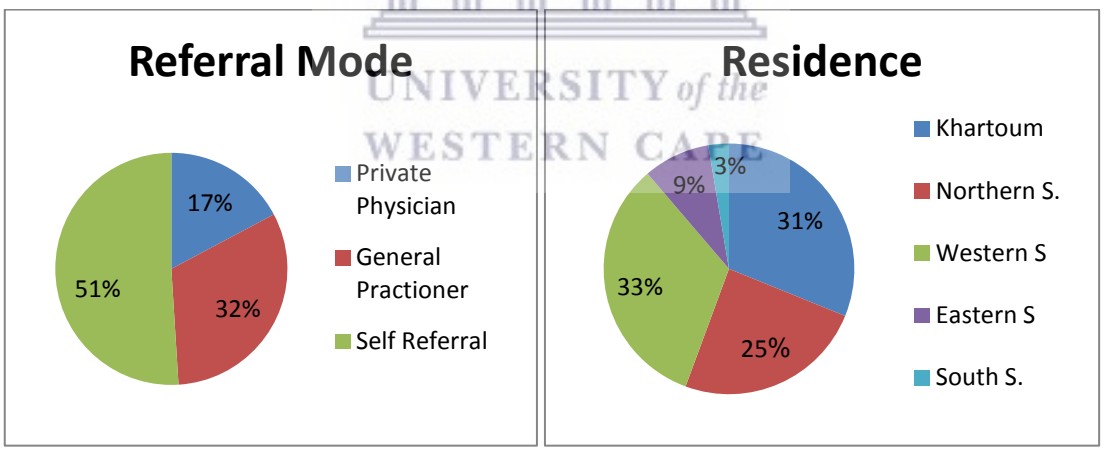


Figure III.13.4 Referral mode and Residential distribution among DLBCL patients

Patients who had their blood tests done at RICK laboratories had their results saved, so the author could obtain the level of Lactate Dehydrogenase enzyme for its prognostic value, as was discussed earlier. (63.58%) of patients had a "High" level of LDH enzyme, which was described to be above >280 U/L or 2.34 microkatals/L (William et al., 2013).

Tobacco smoking was recorded for patients at the time of diagnosis. It was sub-grouped into "current smoker," where the patient reported using tobacco until he attended RICK. This showed a minor frequency with only 27 patients and a percentage of (17.88%), "former smoker" where the patient had ceased this habit before any symptoms appeared, and this group also showed a lower rank with (21.85%) and non-smokers, which constituted (60.26%) of the study samples. There was no data on the mode or the frequency of smoking (Figure III.13.5).

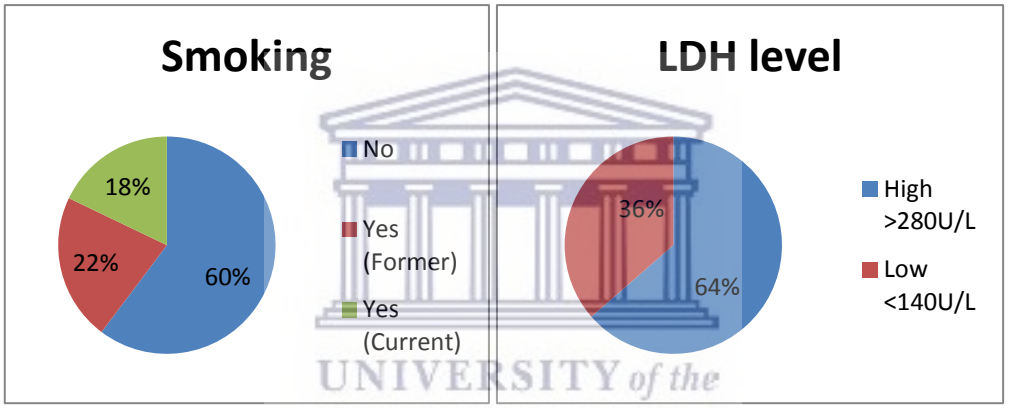


Figure III.13.5 Habitual Smoking and LDH level distribution among the participants

In this study, most cases were diagnosed at the late stages of the tumor Figure (III.13.6). Stage IV accounted for 46.36% of the patients, and only 15.23% of patients managed to get an early diagnosis of the tumor. Stage II had a higher percentage of 21.85% of the total number of cases, and late-stage III showed 16.56% of the tumor stage.

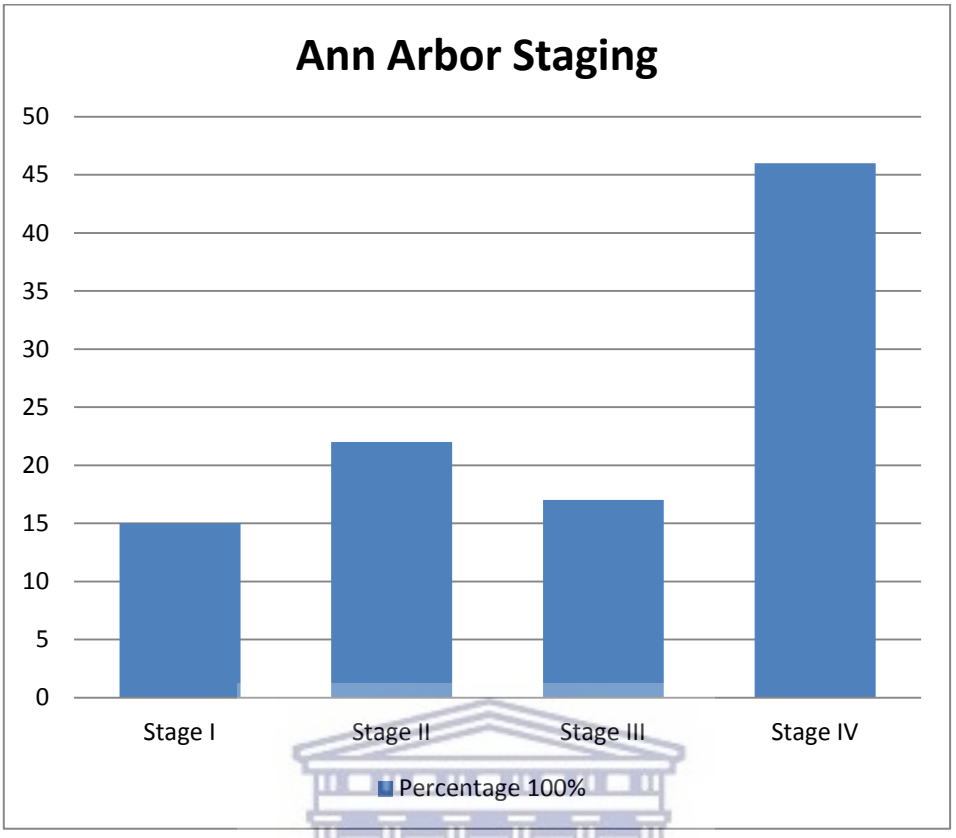


Figure III.13.6 Ann Arbor Staging among DLBCL patients

The tumor site showed varying distribution percentages Figure (III.13.7), with the highest located at the cervical lymph nodes (31.13%), followed by (13.25%) in submandibular lymph nodes.

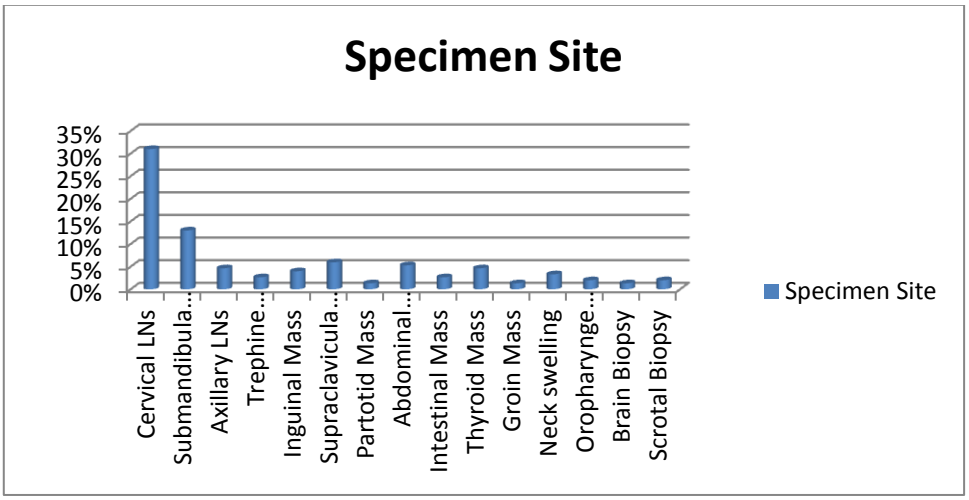


Figure III.13.7 Tumor Site among the Study Participants

Table III.1 Tumor Site in relation to Gender and Age

Specimen site	Gender						Age	
	Female		Male		Total		Mean	SD
Cervical Lymph Nodes	23	35.4%	24	27.9%	47	31.1%	58.89	14.88
Submandibular L.Nodes	10	15.4%	10	11.6%	20	13.2%	57.95	13.08
Auxiallary Lymph Nodes	2	3.1%	5	5.8%	7	4.6%	55.29	12.62
Trephine	3	4.6%	1	1.2%	4	2.6%	48.75	13.15
Inguinal mass	0	0.0%	6	7.0%	6	4.0%	57.50	16.13
Supraclavicular Lymph N.	3	4.6%	6	7.0%	9	6.0%	61.22	16.48
Parotid mass	1	1.5%	1	1.2%	2	1.3%	59.00	1.41
Abdominal mass	2	3.1%	6	7.0%	8	5.3%	64.00	17.86
Intestinal mass	0	0.0%	4	4.7%	4	2.6%	72.50	2.89
Thyroid mass	0	0.0%	7	8.1%	7	4.6%	49.29	9.93
Groin mass	0	0.0%	2	2.3%	2	1.3%	47.00	9.90
Neck swelling	2	3.1%	3	3.5%	5	3.3%	64.40	17.20
Oropharyngeal mass	1	1.5%	2	2.3%	3	2.0%	56.00	13.53
Brain mass	2	3.1%	0	0.0%	2	1.3%	62.50	24.75
Scrotal mass	0	0.0%	3	3.5%	3	2.0%	60.67	20.65
Tonsils	2	3.1%	2	2.3%	4	2.6%	22.00	6.16
Ovarian mass	9	13.8%	0	0.0%	9	6.0%	60.56	7.91
Skin biopsy	2	3.1%	1	1.2%	3	2.0%	62.67	17.04
Submental L.Nodes	1	1.5%	2	2.3%	3	2.0%	45.00	11.36
Tongue base mass	2	3.1%	1	1.2%	3	2.0%	42.33	11.15
Total	65	100.0%	86	100.0%	151	100.0%	57.25	60.00
P_value	0.001						0.001	

Table III.2 Ann Arbor Stage in relation to Gender

A A staging (Lugano)	Gender						Age		
	Female		Male		Total		mean	SD	Median
Stage I	8	12.3%	15	17.4%	23	15.2%	43.35	11.32	45.00
Stage II	18	27.7%	15	17.4%	33	21.9%	46.42	11.75	49.00
Stage III	13	20.0%	12	14.0%	25	16.6%	59.20	12.62	58.00
Stage IV	26	40.0%	44	51.2%	70	46.4%	66.21	12.12	70.00
Total	65	100.0%	86	100.0%	151	100.0%	57.25	15.32	60.00
P_value	0.237						**0.000**		

CHAPTER 14: Diagnostic and Prognostic Indicators of DLBCL

14.1 MYC and BCL2 as Diagnostic Indicators of DLBCL Double Expresser Lymphoma

One of the objectives of this project was to use the expression levels of MYC and BCL2 biomarkers to diagnose Double Expresser Lymphoma phenotype of Diffuse Large B cell Lymphoma and correlate the findings with the prognostic values of the proteins. For the sample to be considered "DEL" Double Expresser Lymphoma, there must be a simultaneous co-expression of the MYC and BCL2 proteins above 40% and 50% respectively of positive cellular staining, respectively. Among the 151 DLBCL patients, hundred twenty-six samples (N=126/83%) showed concomitant overexpression of MYC and BCL2 proteins and hence classified as Double Expresser Lymphoma. The rest of the samples illustrated either under-expression or solo overexpression of one of the biomarkers.

Table III.3 Double Expresser Lymphoma in relation to Gender

Double Expresser Lymphoma	**Gender**						**Age**	
	Female		**Male**		**Total**		**Mean**	**SD**
Negative	13	20.0%	12	14.0%	25	16.6%	46.32	12.38
Positive	52	80.0%	74	86.0%	126	83.4%	59.41	14.96
Total	65	100.0%	86	100.0%	151	100.0%	57.25	60.00
P_value	0.322						0.000	

MYC protein has shown an overexpression beyond the 40% cutoff point in 134 cases (88.7%), and 17 cases (11.3%) showed no or minimal expression level. Figure (III.14.1A) shows representative IHC staining samples of DLBCL cells expressed MYC and BCL2 biomarkers. BCL2 has shown negative or underexpression in 11 cases

(7.28%) of the total 151, while most of the cases (N=140/ 93%) showed overexpression above 50% of cellular staining. Figure (III.14.1B) and Figure (III.14.2) show representative staining samples of BCL2 protein in DLBCL sections.

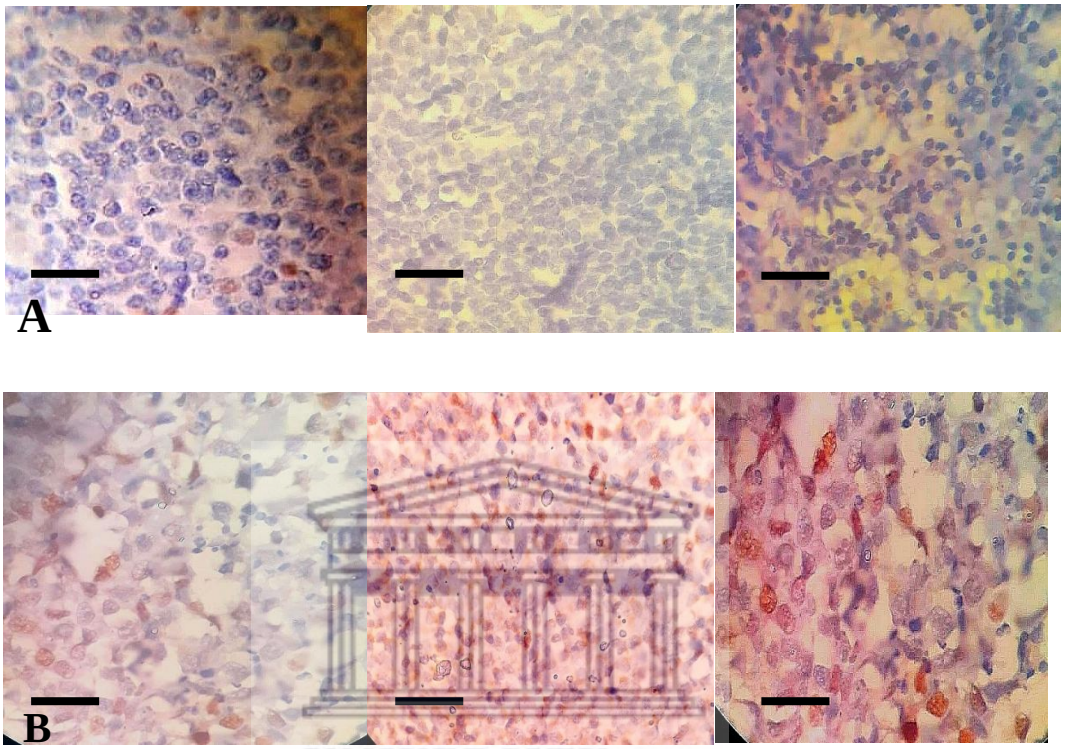


Figure III.14.1 Representative Photomicrographs showing IHC expression of MYC and BCl2 biomarkers in DLBCL cases: A: Case 1, 90% MYC+, B: Case 29, 75% Bcl2. Scale bar represents 50µm.

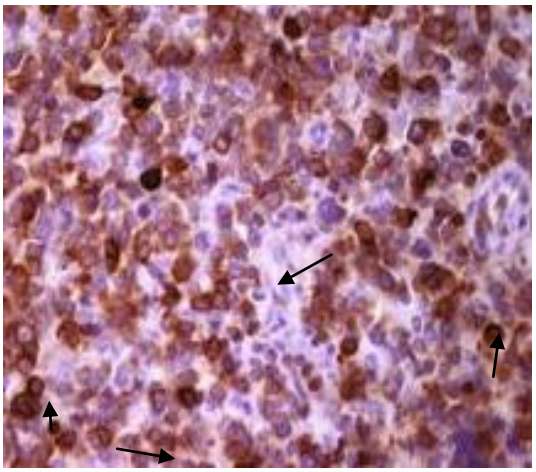


Figure III.14.2 DLBCL case stained by BCL2 showing typical appearance of neoplastic cells showing two times the size of normal small lymphocytes (*black arrows*), (original magnification ×40)

14.2 Clinico-Pathological Parameters as Prognostic Indicators of Diffuse Large B cell Lymphoma

During this study period, Disease-Specific Survival (DSS) was used to evaluate the prognosis of DLBCL. DSS is defined as "The percentage of people in a study or treatment group who have not died from a specific disease in a defined period. The period usually begins at the time of diagnosis or at the start of treatment and ends at the time of death." (Cheng and Chang, 2017).

During the study period, 84 patients passed on as a result of DLBCL, accounting for 55.63% of the total cohort. The restricted mean survival time was estimated to be the longest follow-up time and was found to be 42.97 months from the date of diagnosis. Statistically, an extended mean survival time was calculated hypothetically by exponentially extending the survival curve to zero and was estimated to be 46.95 months. The highest death rates were among the male gender, 'married' group, residents of western Sudan, retired, non-smokers, patients with high lactase dehydrogenase enzyme, Ann Arbor stage IV patients, DEL positive subgroup, and samples with tumors located at the ovaries, intestinal masses, and groins.

14.3 Survival Distribution among the Study Variables

Using the Log-rank test, the equality of survival distribution among the different levels of the study variables was tested. There was a statistically significant difference in the survival for the marital status, occupation, LDH, Ann Arbor staging (Lugano), MYC, DEL, at a significance level of 0.05 (Table III.4).

Table III.4 Log-rank table showing the equality of the survival distribution for the different levels of variables of DLBCL patients, values highlighted in pink were statistically significant.

Variable	Chi^2	P_value
Gender	0.479	0.489
Marital status	10.744	0.013
Occupation	13.786	0.008
Residence	6.763	0.149
LDH	38.180	0.001
Mode of Referral	2.082	0.353
A A staging (Lugano)	51.293	0.001
MYC	7.345	0.007
BCL	0.784	0.376
DEL	8.769	0.003

14.4 Association between Clinico-Pathological Parameters and Disease-Specific Survival using Univariate Cox Regression Analysis

To assess the prognostic independent effect of each variable, ignoring the influence of other covariates, a non-adjusted Univariate Cox Regression model was created using Wald statistics. Multiple clinical parameters showed statistically significant association with Disease-specific survival in the univariate analysis (Table III.5).

Age was found to be statistically significant at the level of 0.05 (p=0.001), as one year increase in age increased the risk of death by 5% (HR=1.052) Figure (IV.19.2). The level of Lactase Dehydrogenase enzyme positively correlated with disease-specific survival (p=0.001) as the hazard to death increased almost 7.5 times in the LDH 'high group' (HR= 7.463).

Regarding Ann Arbor staging, stage I was used as a reference. The data showed statistical significance in correlation to DSS (p=0.002) as the hazard increased as the tumor stage advanced (HR= 2.6, 21.1, 31.4) respectively for stages II, III, and IV.

Furthermore, the expression level of MYC biomolecular elevated the hazard 4.3 times in MYC positive group (HR=4.27) and showed a statistically significant correlation with DSS (p=0.014). The Double Expresser positive group showed an increased hazard to death compared to the DEL negative group (HR=4.010) and correlated significantly to DSS with a p-value of (0.007).

Table III.5 Association between clinic-pathological parameters and DSS time using univariate Cox Regression model, variables highlighted in pink showed a statistical significance, yet could not independently predict DSS.

Parameter	Disease-Specific Survival		95% Confidence Interval for HR	
	P value	Hazard Ratio HR	Lower	Upper
Gender	0.496	1.163	0.753	1.796
Age	0.001	1.052	1.032	1.072
Marital Status	0.021			
Married	0.206	2.48	0.606	10.193
Divorced	0.812	1.23	0.206	7.430
Widowed	0.40	4.59	1.071	19.685
Occupation	0.014			
Student	0.330	0.517	0.137	1.953
Employee	0.955	0.976	0.412	2.310
Housewife	0.620	1.232	0.541	2.804
Retired	0.055	2.127	0.985	4.594
Residence	0.177			
Northern	0.016	0.454	0.238	0.865
Western	0.398	0.801	0.479	1.339
Eastern	0.401	0.700	0.304	1.611
South	0.880	1.086	0.371	3.176
Smoking	0.203			
Former	0.130	1.469	0.892	2.418
Current	0.598	0.848	0.458	1.596

LDH	0.001	7.463	3.543	15.720
Referral Mode	0.368			
GP SF	0.384 0.584	0.749 1.058	0.390 0.578	1.436 1.937
A A Staging	0.001			
II III IV	0.403 0.003 0.001	2.629 21.152 31.431	0.273 2.827 4.326	25.304 158.258 228.346
MYC	0.014	4.274	1.341	13.622
BCL2	0.390	1.854	0.454	7.575
DEL	0.007	4.010	1.463	10.989

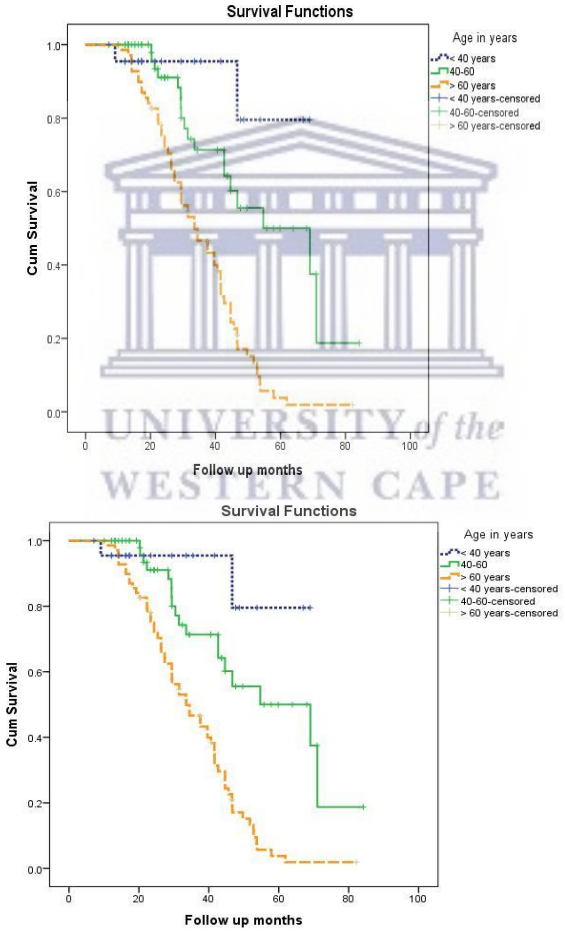


Figure III.14.3 Age effect on survival of DLBCL patients was statistically significant at the level of 0.05 (p=0.001), as a one-year increase in age increased the risk of death by 5% (HR=1.052).

When the independent effect of gender was tested, the females were the reference. Being a male had almost 1.2 times the hazard than female (HR= 1.163), yet, gender had no

statistically significant correlation with DSS. BCL2 expression level showed a 1.9 increased hazard in BCL2 positive patients (HR= 1.854). However, there was no statistically significant correlation with DSS.

14.5 Predictors of Disease-Specific Survival of DLBCL in NON Adjusted Multivariate Cox Regression Model

When the effect of each variable on the survival of DLBCL was measured and controlling for the impact of other covariates using multivariate analysis (backward method), Double Expresser Lymphoma (p=0.035) and Ann Arbor staging (p=0.003) had a statistically significant effect. They were considered independent predictors of the disease-specific survival DSS of DLBCL patients at a 95% confidence interval (Table III.6).

Table III.6 Clinico-Pathological Variables and Biomolecular expression levels as predictors of Disease-specific survival using multivariate Cox Regression model (Backward method). Variables highlighted in pink showed a statistical significance and could independently predict DSS.

Variable	P_value	HR	95% CI for HR	
			Lower	Upper
DEL	0.014	0.102	0.017	0.624
Gender	.193	1.712	.761	3.849
Age	.149	1.027	.990	1.066
Marital status	.079			
Married	.157	7.265	.465	113.580
Divorced	.878	.789	.038	16.416
Widowed	.167	7.395	.433	126.171
Occupation	.126			
Student	.049	10.754	1.007	114.865
Employee	.044	3.246	1.035	10.186
House wife	.641	1.291	.441	3.776
Retired	.732	1.177	.462	3.000
Residence	.217			
Northern	.647	.812	.332	1.982
Western	.804	1.089	.556	2.134
Eastern	.071	2.626	.921	7.492
South	.214	2.843	.547	14.786

Smoking	.248			
Former	.096	.529	.250	1.119
Current	.456	.695	.267	1.809
LDH	.073	3.147	.897	11.046
Mode of Referral	.282			
GP	.257	.591	.238	1.467
SF	.997	.998	.472	2.111
A A staging (Lugano)	0.003			
II	.466	2.476	.216	28.362
III	.002	47.735	4.073	559.457
IV	.002	51.394	4.155	635.693

14.6 Identification of Disease-Specific Survival predictors of DLBCL in Adjusted Multivariate Cox Regression Model

The multivariate Cox Regression was adjusted for the predictors previously identified via the non-adjusted Cox regression model. When the model was adjusted for Double Expresser Lymphoma DEL, Ann Arbor staging was found to be a predictor of Disease-Specific Survival (p=0.001) (Table III.7). The hazard to death in Ann Arbor stage II was 3.2 times the hazard for group A stage I, which increased to 59 times for group III and 91 times in Ann Arbor stage IV patients.

When the multivariate model was adjusted for MYC expression level once more, Ann Arbor staging (p= 0.001) showed a significant effect and an independent predictor of the disease-specific survival of DLBCL patients in multivariate analysis. The stage II group's death risk was four times the risk for the stage I group (HR=3.970), and it drastically elevated to eighty-three and hundred twenty-three times for stage III and IV groups (HR= 83.9, 123.5 respectively).

Table III.7 DEL and AA staging as independent predictors of Disease-specific survival using multivariate Cox Regression models 'Final Cox Model'

	P-value	HR	95% CI for HR	
			Lower	Upper
DEL	.001	.198	.044	.895
A A staging (Lugano)	.0015			
II	.311	3.234	.335	31.262
III	.001	59.118	5.794	603.224
IV	.000	91.865	8.833	955.358
MYC	.035	.130	.013	1.288
A A staging (Lugano)	.001			
II	.238	3.970	.402	39.207
III	.002	83.980	5.030	1402.236
IV	.001	123.594	7.598	2010.504

Comment: DEL and Ann Arbor staging have proven to have a statistical significance; in addition, they could INDEPENDENTLY predict Disease-Specific Survival of DLBCL.

14.7 Correlation between Double Expresser Lymphoma Phenotype, MYC/ BCL2 Expressions Levels, and Clinico-Pathological Variables

Using Chai squared χ^2 (Exact Fisher) statistical test, the association between Double Expresser Lymphoma and the clinicopathological variables of the study was investigated. The parameters tested were the age, gender, marital status, occupation, residence, smoking habit, mode of patient's referral, specimen site, level of the lactose dehydrogenase enzyme, and Ann Arbor tumor stage.

Occupation (p=0.014), smoking (p=0.041), Lactase dehydrogenase level (p=0.001), and Ann Arbor staging (p=0.001) were all found to have a statistically significant association with Double Expresser Lymphoma at the 0.05 confidence interval level.

When the association between the expression level of the MYC protein and the clinico-pathological features of DLBCL patients was investigated, Occupation (p= 0.10), LDH level (p= 0.001), and Ann Arbor staging (p= 0.001) showed a strong correlation with

MYC expression level. The expression intensity of the BCL2 oncogene's correlation with the clinicopathological variables was tested. Only LDH level (p=0.001) and Ann Arbor staging (p=0.001) were statistically associated with it.

14.8 Yearly Survival Rates of Diffuse Large B cell Lymphoma

The overall 5-year survival of Diffuse Large B cell Lymphoma was 19% with a 95% confidence interval. The disease-specific survival rates were estimated using the Kaplan Meier method from the histo-pathological diagnosis to the date of death or censoring. The overall 1, 2, 3, and 5-year survival rates were 98.7%, 80%, 58.9%, and 19%, respectively (Figure III.14.4).

Comment: To the best of the author's knowledge, this is first study that indicates the five years survival rate of DLBCL in Sudan. Note the drastic deterioration from the first to the fifth year of survival. Justifications will be discussed in Section IV.

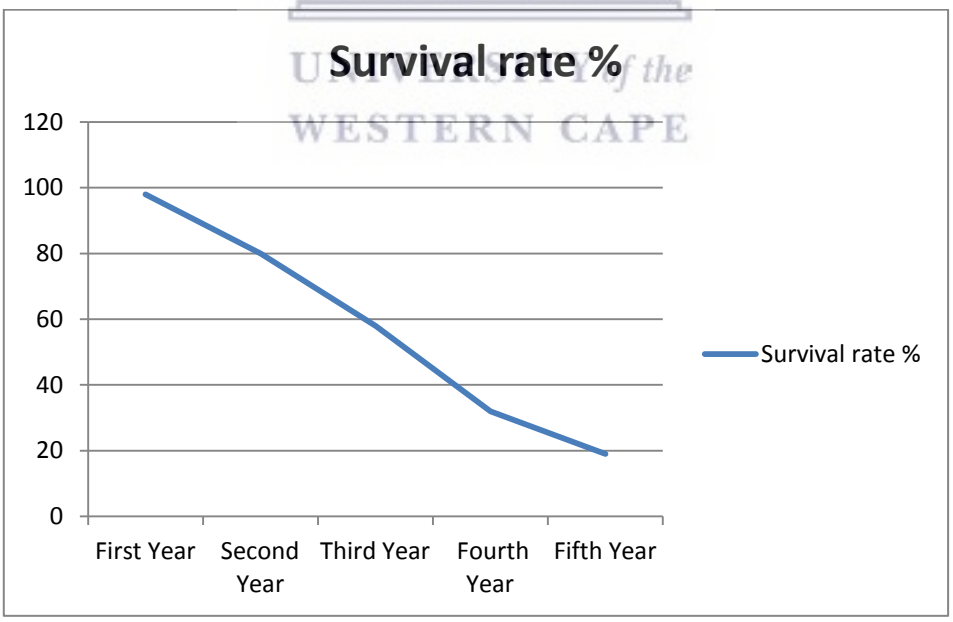


Figure III.14.4 Disease-Specific 5-yr survival rate for Sudanese DLBCL patients Kaplan Meier survival estimate

CHAPTER 15: Quantitative Real-Time Polymerase Chain Reaction

15.1 Quantification of the Relative Expression of MYC and BCL2 mRNA using Quantitative Real-time PCR

MYC and BCL2 biomarkers' relative expression rate was detected using q-PCR as described in chapter III. The qPCR threshold cycles (CT) of MYC had an average of 27 cycles, with the highest of 31 cycles and a minimum of 21 for MYC protein. The value of CT was calculated via the PCR curve that crossed the threshold of the curve. While BCL2 mRNA had 32 cycles on average, with the highest being 44 and the lowest of 24 cycles.

While the expression level of MYC protein detected via immunohistochemistry was investigated for the whole study samples, which were 151 and 134 samples showed MYC over expression, 70 (46.3%) samples were used for the purpose of retrieving MYC messenger RNA (mRNA) using qPCR. This was attributed to the quality and amount of the extracted DNA that varied between the samples. The ones with the best DNA features were selected.

Similarly, 140 samples showed positive BLC2 protein expression via IHC, whereas 40 had a sufficient amount of detectable mRNA accounting for (26.5) % of the total samples. Moreover, we included 30 lymphoid tissues from reactive cervical lymph nodes of matching gender and age patients in this study and were used as control samples for the qPCR experiment.

Quantitative real-time PCR in Diffuse Large B cell group illustrated that the expression level of input mRNA of MYC biomolecule was 4.32 ± 0.72 Figure (III.15.1). In contrast, the mRNA of the BCL-2 biomolecule was indicated as 2.10 ± 0.89 (Figure III.15.2). This result has demonstrated a significantly higher expression level than those of the

control group for both molecules that showed 0.72 and 0.69 for MYC and BCL2, respectively (P-value = 0.00031, 0.0043 respectively).

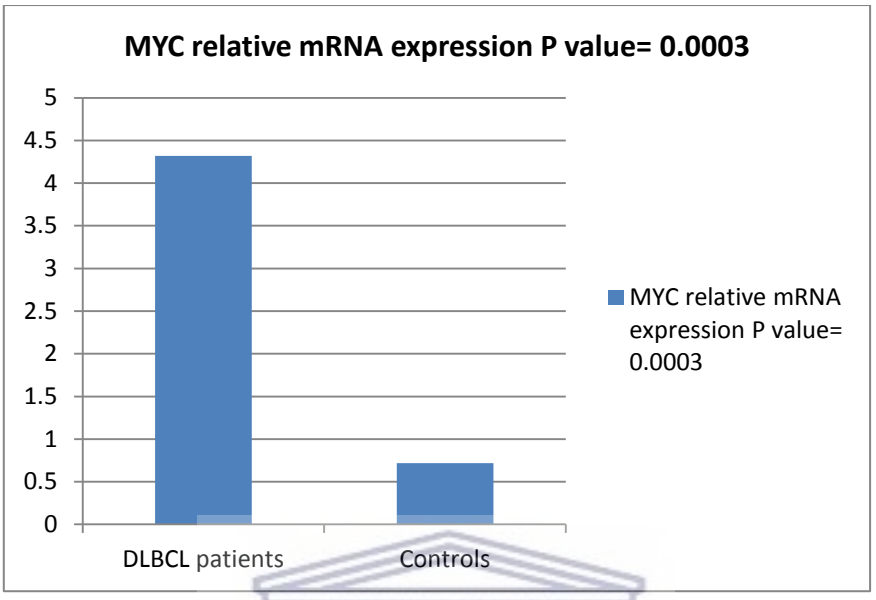


Figure III.15.1 Relative Expression of MYC messenger RNA in Double Expresser Lymphoma DLBCL. Separate reactions were run for MYC and BCL2, yet the samples chosen were DEL positive.

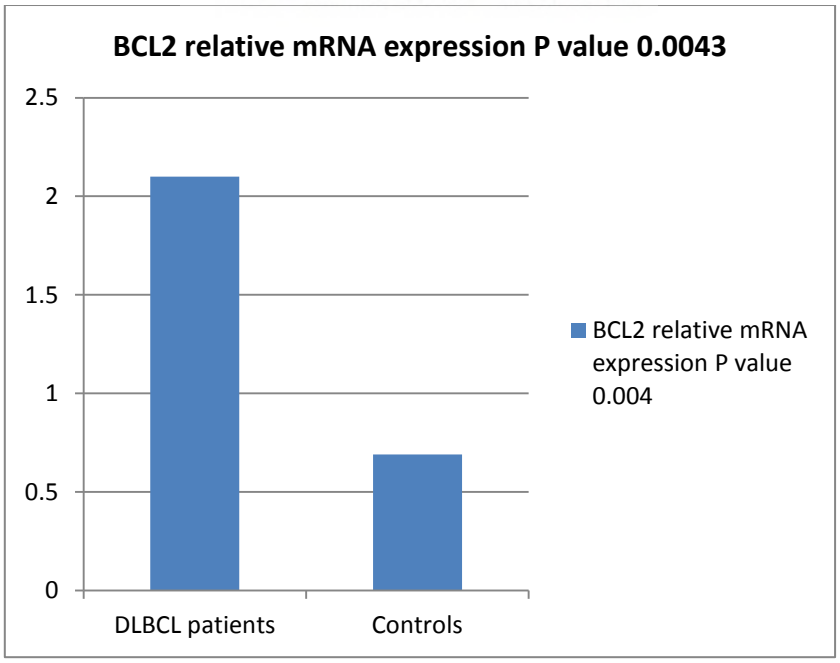


Figure III.15.2 Relative Expression of BCL2 messenger RNA in Double Expresser Lymphoma. Separate reactions were run for MYC and BCL2, yet the samples chosen were DEL positive.

Relative quantification quantifies the amount of mRNA in a sample by comparing it to an internal control reference gene. In this study, Beta globulin was used as an internal control for normalization, and we used the standard curve method in mRNA quantification. CT values were used to calculate the expression of MYC and BCL2 in DLBCL patients normalized by the B-Globulin table (III.8).

Table III.8 Relative quantification of mRNA expression of MYC and BCL2 using the standard curve method

DLBCL	**MYC (ng)**	**Beta Globulin (ng)**	**Myc normalization to Beta Globulin**	**MYC relative mRNA**
Average	0.98±0.0038	0.54±0.0043	0.07±0.006	4.32± 0.72
DLBCL	**BCL2 (ng)**	**Beta Globulin (ng)**	**BCL2 normalization to Beta Globulin**	**BCL2 relative mRNA**
Average	0.786±0.0034	0.47±0.0038	0.05±0.003	2.10 ± 0.89

Comment: Control for the relative quantification of mRNA of MYC and BCL2 was via using an internal normalization gene termed *Beta Globulin*.

Moreover, a significant statistical correlation was observed between the expression level of the messenger RNA and the high protein expression level that was shown via IHC in both investigated molecules in the Diffuse Large B cell Lymphoma group. MYC mRNA indicated via qPCR was correlated with high MYC protein level detected via IHC (Spearman correlation of 0.530, P-value = 0.0002). At the same time, mRNA and the protein expression level of BCL2 showed a Spearman correlation of 0.640, P-value = 0.0001; Table III.9).

Table III.9 Association between MYC mRNA and MYC protein expression and BCL2 mRNA and BCL2 protein expression

MYC	**MYC Protein level**	**MYC mRNA**	**Spearman Correlation R**	**P value**
DLBCL	N=134	N=70 4.32 ± 0.72	0.530	0.0002
Control	N=30	N=30 0.72		
BCL2	**BCL2 mRNA**		**R**	**P value**
DLBCL	N=140	N=40 2.10 ± 0.89	0.640	0.0001
Control	N=30	N=30 0.69		

15.2 Quantification of the Oncogenic Dose of MYC biomolecule

Concurrent quantitative real-time PCR cycles were run for the MYC gene and the reference gene Beta globulin using a range of amounts of template DNA per reaction**.** Fold change values were indicated and plotted for both genes. We assessed the relative number of MYC oncogene copies in relation to the copy number of a reference gene B-globulin which is assumed to be one per haploid genome (Multiplex TaqMan assay, relative quantification) described in section III. Change in MYC oncogenic dosage was observed in 50 out of 70 samples chosen for the qPCR experiment, accounting for (71.4%) of the DLBCL patients group (Figure III.15.3).

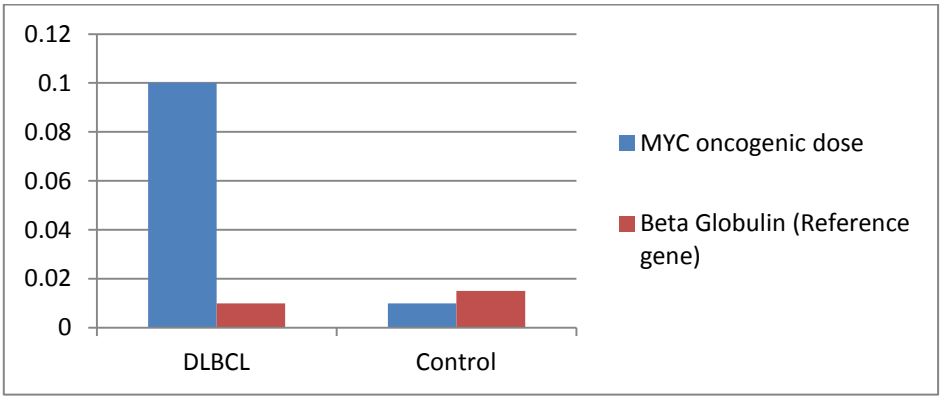


Figure III.15.3 Gene dosage (fold change) of MYC oncogene in Diffuse Large B cell Lymphoma; Beta globulin was used as a reference gene

15.3 Quantification of Translocations of BCL2 oncogene, Exploration of BCL2 Translocation in chromosome 18 t (14;18) via QRT PCR

The genetic detection of translocations in chromosome t(14;18) is extensively used to diagnose and monitor Diffuse Large B cell Lymphoma, displaying a high prevalence for this chromosomal alteration. It is commonly shown that the majority of the breakpoints take place at chromosome 18 at the (MCR) minor cluster region and the (MBR) major breakpoint region (Albinger-Hegyi et al., 2002).

Various studies have utilized quantitative real-time PCR protocols to identify translocations and breakpoints at a range of chromosomal clusters in Non-Hodgkins Lymphomas. Nevertheless, the detection ratios of BCL2 rearrangements varied significantly between these studies, and this has been attributed to the diverse methodologies techniques used (Salam et al., 2020).

In this study, 140 samples showed positive protein overexpression of BCL2 oncogene detected via IHC; cases were re confirmed as Diffuse Large B cell Lymphoma via H&E staining, among which 134 showed concurrent over expression of MYC and BCL2, illustrated as Double Expresser Lymphoma. To identify BCL2 translocations, we selected forty samples with confirmed BCL2 protein overexpression and showed a sufficient high-quality amount of extracted DNA. The target genomic strand was

chromosome 18 for the presence of a translocation in t(14;18) via quantitative real-time PCR. Primers used were MBR and MCR primer pairs as commonly demonstrated breakpoints**.**

As previously stated in section II, high molecular weight DNA was extracted from specimens for 40 samples of choice. The sequence of primers and probes utilized in the quantitative real-time PCR reaction is shown in Table III.10. Primers were labeled with 6-carboxyfluorescein (FAM). All probes were labeled at their 3 ends with the dye tetramethyl-6 carboxyrhodamine (TAMRA) (Yuan et al., 2001).

Table III.10 The Sequence of Primers and Probes utilized in the quantitative real-time PCR reaction for detection of BCL2 and Cyclophilin translocations.

Primer	Sequence
BCL2- mbr (Forward)	5_-GCT TTA CGT GGC CTG TTT CA-3_
BCL2 mcr (Forward)	5_-CCT GGC TTC CTT CCC TCT GT-3_
Immunoglobulin heavy chain gene JH	5_-NED-ACC TGA GGA GAC GGT GAC C-3_
cyclophilin. (Forward)	5_-TGA GAC AGC AGA TAG AGC CAA GC-3_
Probes	
Mbr probe	5_-FAM-AGG GCT CTG GGT GGG TCT GTG TTG-TAMRA-3
Mcr probe	5_-FAM-TCT CTG IGG AGG AGT GGA AAG GAA GG-TAMRA-3
Cyclophilin Probe (Forward)	5_-VIC-AGC ACC AAT ATT CAG TAC ACA GCT TAA AGC TAT AGG TAT-TAMRA-3
Cyclophilin. (Reverse)	5_-TCC CTG CCA ATT TGA CAT CTT C-3

Out of forty investigated samples, 14 showed translocation within t(14;18), 9 involving the major breakpoint cluster region (MBR), and 5 patients illustrated the minor breakpoint cluster region (MCR) of the BCL2 oncogene. DNA from normal cervical

lymph nodes was included as the negative control. The normalization gene utilized for this reaction was the Cyclophilin gene.

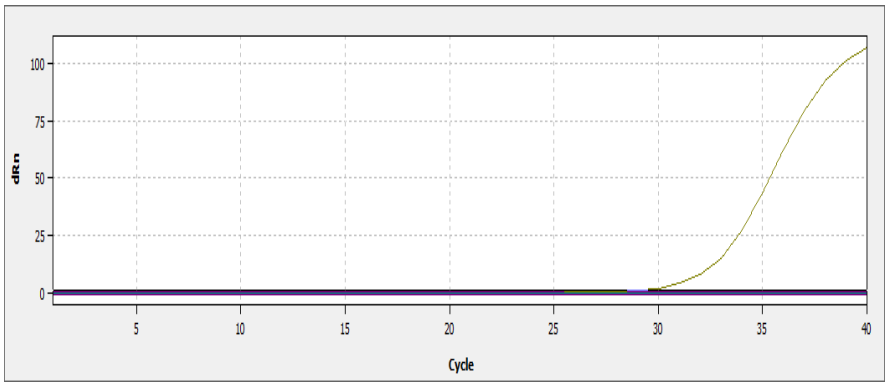


Figure III.15.4 Positive BCL2 translocations in DLBCL patients

Table III.11 Representative results of RT-PCR in Double Expresser Lymphoma samples showing translocations within BCL2 chromosome at18 t(14;18) normalized by Cyclophilin gene at MBR and MCR regions in the same reactions

Sample #	Mbr/Cy	Mcr/Cy	Size bp
4	Not detected	0.78098	122
9	0.72251	Not detected	154
13	0.93257	Not detected	129
16	1.40069	0.28478	170
21	0.62250	Not detected	144
28	0.83221	Not detected	153
33	0.34066	Not detected	188
51	0.61447	0.97123	205
61	1.09910	0.08307	241
69	0.84420	0.35481	219

Comment: With regard to the molecular analysis of the biomarkers, the oncogenic dosage was done for one of the oncogenes (MYC), while quantification of translocation was done for the other (BCL2); this is due to constraints with regards to finances.

CHAPTER 16: Summary of the study results

16.1 General overview

The study covered 151 DLBCL patients at RICK between the years 2013-2018. Sixty-five were females, while 86 were males. The mean age was 57.25 years. 68% were married, 30% were employees, 33% resided in western Sudan, 60% were non-smokers, 63% had high LDH, 50% had self-referral mode, 46% were stage IV at the time of diagnosis, 88% had high MYC, 92% had High BCL2, 83% had concurrent high MYC and BCL2 diagnosed as DEL. The highest tumor site was cervical Lymph nodes.

16.2 Prognosis of Diffuse Large B cell Lymphoma

The measurement unit was DSS Disease-specific survival; 84 patients, 55% died of DLBCL. The mean survival time (time from DX to the last follow-up) was 46 months (3.8 years). The highest death was among the male gender, 'married' group, residents of western Sudan, retired, non-smokers, patients with high lactase dehydrogenase enzyme, Ann Arbor stage IV patients, DEL positive subgroup, and samples with tumors located at the ovaries, intestinal masses, and Groins.

16.3 Prediction of Survival of Diffuse Large B cell Lymphoma

Cox Regression Models were used to assess the ability of the clinicopathological parameters and the expression levels of MYC/BCL2 to predict the Disease-specific survival in DLBCL patients. Results were as follows:

Univariate Analysis (The effect of each variable is assessed ignoring the influence of other covariates):

- Age, LDH level, Ann Arbor staging system, solo MYC over expression, and concurrent over expression of (MYC/BCL2) illustrated as (DEL) were

significantly associated with DSS Disease-Specific Survival of DLBCL in Univariate analysis.

Multivariate Analysis (The effect of each variable is assessed controlling for other covariates) showed:

- DEL (Over expression of both molecules) and Ann Arbor were independent predictors in the non-adjusted Multivariate Cox Regression model, so the concurrent expression of MYC and BCL2 could predict prognosis in DLBCL patients proving the study hypothesis.
- When adjusting for the independent predictors: DEL: Ann Arbor staging was a DLBCL predictor, MYC: AA again was a DLBCL predictor.
- Yearly survival rates of DLBCL from the first year to the fifth year were indicated as 98%, 80%. 58%, 32% and 19% respectively.

16.4 Association between clinicopathological parameters and expression levels of MYC/BCL2

- Occupation, Smoking, LDH level, and Ann Arbor staging were associated with concurrent overexpression of MYC/BCL2 illustrated as DEL.
- Occupation, LDH level, and AA staging were associated with solo MYC over expression.
- LDH level and Ann Arbor staging were associated with sol solo BCL2 over expression.

16.5 Quantitative real-time PCR

The relative expression of mRNA of MYC and BCL2 indicated via qPCR was statistically correlated with the protein level of MYC, and BCL2 detected via immunohistochemistry.

Moreover, DSS was significantly associated with the relative expression level of mRNA of MYC and BCL2. 67.4% of the samples showed a fold change in the oncogenic dosage of MYC biomolecule and were associated with poor survival as these samples were of dead patients. Out of forty samples investigated for the presence of BCL2 translocations, 14 samples (35%) showed marked translocations within chromosome 8 at MBR and MCR regions.

Consequently, we can conclude that evaluating the expression levels of MYC and BCL2 biomarkers gave a comprehensible insight into their diagnostic and precise prognostic impact on the survival of Diffuse Large B cell Lymphoma patients in Sudan.

SECTION IV: DISCUSSION

CHAPTER 17

CHAPTER 18

CHAPTER 19

Preamble:

This section includes Chapters 17, 18, 19, and 20. It embodies the discussion related to the study findings, survival rates of DLBCL in relation to the expression levels of MYC and BCL2 investigated via IHC and qPCR were shown to correlate, and a detailed discussion follows in this regard in Chapter 17. Specific parameters related to the development of the tumor registry software are discussed in Chapter 18. The challenges and limitations of this study and those faced during the study are enumerated in Chapter 19.

Contents of Section IV:

CHAPTER 17: General Overview

17.1 Overview

Research regarding the epidemiology of DLBCL is deficient in Sudan as minimal high-quality published data is revealed. This compared dismally to much research and published evidence undertaken in developed countries (Susanibar-Adaniya and Barta, 2021).

In this project, the author faced multiple challenges that made data collection a complex and challenging process. The lack of availability of a population-based cancer registry and population life tables in Sudan required that the patients' records were to be retrieved manually from the archive of the RICK. Furthermore, RICK had no software system for patients' data storage and stratification. Many file records were disorganized, and some vital data was missing. These files were excluded from the study.

Moreover, there was nominal coordination between medical care centers and facilities within the capital and the different states. Primary health care centers are not interconnected neither geographically distributed throughout neighborhoods. In addition, most death records were vague, and the exact cause of death was not clearly stated.

To the best of the author's knowledge, there are no studies on the diagnostic and prognostic value of MYC and BCL2 biomarkers in Sudan's Diffuse Large B cell Lymphoma. This study will provide pioneer scientific data on the expression levels of the targeted biomarkers, the prognostic prediction potential in addition to the clinic-pathological parameters of DLBCL patients.

The data provided by this study will have a considerable impact on Diffuse Large B cell Lymphoma research and offers a substantial contribution to the research domain in Sudan.

17.2 Clinicopathological Features of DLBCL Patients as Prognostic Indicators of Diffuse Large B cell Lymphoma

In this project, the investigated clinicopathological features of DLBCL patients included: gender, age, marital status, occupation, referral mode, residence, LDH level, tumor site Ann Arbor staging, date of diagnosis, and late follow up and treatment modality.

One of the study objectives was to determine the predictive potential of the clinico-pathological factors on the prognosis of Diffuse Large B cell Lymphoma. Using Cox Regression models, the study variables were tested and stratified according to their potential of predicting the prognosis of DLBCL. Univariate regression calculated the variable's prognostic potential, ignoring the effect of other co-variables, while multivariate regression controlled the impact of other investigated parameters.

17.2.1 Dependent Predictors of DLBCL Prognosis

Some variables were shown to have a statistically significant association to poor prognosis and increased the risk of death event in Univariate Cox Regression. Nevertheless, they could not independently predict the prognosis of Diffuse Large B cell Lymphoma. These included age, marital status, and Lactate Dehydrogenase level.

Age: The mean age of DLBCL patients in this study was 57 years. This result was lower than some studies, which showed higher ages of 70 years old (Smith et al., 2011, Smith et al., 2015a). Nevertheless, as shown in section III, age was found to be statistically significant at the 0.05 significance level with a (P value=0.001) as one year increase of age elevated the hazard for death by 5% (HR= 1.052).

A vital key used for predicting the survival of affected individuals is the International Prognostic Index (IPI). This tool states that the age of sixty years and above is a potential risk factor. It has been shown that patients who were 60 years and older

suffered increased tumor biological complexity (Smith et al., 2015a), in addition to accompanying co-morbidities associated with older ages (i.e., other tumors, arthritis, diabetes) and their ability to withstand chemotherapy (Janssen-Heijnen et al., 2005) (Varga et al., 2014).

Furthermore, several factors create a biological distinction within various age groups in Diffuse Large B cell Lymphoma resulting in inferior prognosis and poorer survival rates in older ages, some of which include patients' correlated comorbidities, the subtype of DLBCL known as activated B-cell (ABC) (Klapper et al., 2012) and the overexpression of BCL2 protein all of which increase in relation to age at diagnosis (Thunberg et al., 2009)

Since most cancer patients are considered elderly, it has been shown that the incidence rate of various types of malignancy increases with age, and patients of older ages suffer the most (Torre et al., 2015). For instance, in the United Kingdom, almost one-third of newly diagnosed cases of malignancy in 2010 were 75 years of age (Alessy et al., 2021) (Henson et al., 2020). Nevertheless, the latest studies have shown that the age at which the patient initially presents with the tumor is decreasing; moreover, the biological probability of malignancy in younger individuals may differ from that observed in older ones (Quintela et al., 2019). A Swedish population-based study has indicated that age has shown persistent significance as a potential unfavorable factor for individuals as youthful as forty years of age (Hedström et al., 2015b). A 2019 meta-analysis study (Bellardita et al., 2013) has shown that in young DLBCL patients, the 5-year mortality rate was two times higher than other causes of death. Nevertheless, in older patients, both DLBCL and other causes had almost a similar risk of death.

Marital status: The majority of the study participants were married. In our study, marital status was found to be statistically significant (P value=0.021). The hazard to death for

the divorced category was 1.2 times that the married patients (HR=1.237), whereas widowed patients had 4.6 times the probability of death compared to married patients (HR=4.591). However, in our project, marital status could not independently predict the outcome of Diffuse Large B cell Lymphoma (P value= 0.07).

The social status of Diffuse Large B cell Lymphoma has been shown to have a significant effect on the prognostic outcome of these patients, as 'married' patients showed superior prognosis to those who were widowed or divorced (Ahmed et al., 2019b). This result could be attributed to the health-seeking behavior of the patients who live with a spouse or partners usually have a more significant motive to seek treatment of their malignancy. Hence, they do it faster and more rapidly and do not delay that till the advanced stages of the tumor, resulting in a better outcome.

Moreover, this could also be explained by patients' adherence to healthier eating habits and the extensive supportive social network surrounding the cancer patient (Kravdal, 2013, Yang et al., 2019). The state of having a cancer patient within a family drastically changes the lives of all the family members, causing social and financial burdens, especially in populations with lower socioeconomic class and no medical insurance coverage (Sneha et al., 2017).

Zhai et al. (2019) found out that married patients with breast cancer had a better prognostic outcome than divorced and separated categories. This was most likely due to to the care provided to the patients by their partners in the daily activities, personal hygiene, and sometimes financial caregiving to the family (Zhai et al., 2019). Moreover, the quality of life of single prostate cancer patients was considered worse than married persons in a study that investigated the health-associated predictors of their quality of life (Bellardita et al., 2013).

Regarding Diffuse Large B cell Lymphoma, the psychological status of the patients is affected by the amount of emotional distress the patients have to suffer. Nevertheless, the presence of a partner and the provision of reciprocated care and support has been shown to decrease the stress levels and help the patients cope with the treatment journey throughout (Iii et al., 2013). In Sudan, there is a solid social network within the community. Families are extended; uncles, aunts, cousins, and even neighbors are expected to provide various kinds of support for the patient and his family.

Level of Lactate Dehydrogenase Enzyme: In this study, the level of lactate dehydrogenase enzyme was statistically significant (P value= 0.0001). The hazard of death increased seven times in the high LDH group. Nevertheless, it could not independently predict the prognosis of DLBCL in the univariate cox regression model where the effect of the other covariates was ignored.

The level of LDH enzyme has been found to engage in malignant tumor metabolism. A characteristic feature of cancer cells is an abnormal disrupted metabolism, in which tumor cells depend primarily on anaerobic respiration as an energy source. This phenomenon involves the production of lactate substances from glucose and is scientifically known as the Warburg effect (Liberti and Locasale, 2016). Consequently, malignant cells absorb the extra glucose and utilize it to expedite their division. Many tumors can elevate serum LDH levels, and this might be used as a marker for malignancy. However, its wide dispersion might not be decisive of the tumor-specific type (Yadav et al., 2016).

This result was in accordance with previous studies (Wudhikarn et al., 2020) **(**Ting et al., 2020)**.** However, other studies did not report a significant difference between patients with lymphoma and their counterparts, although they suffered lower survival rates (Gandhi et al., 2019).

17.2.2 Independent Predictors of DLBCL Prognosis

Other study variables showed an independent prediction potential in the Multivariate Regression model. These included concurrent over expression of MYC and BCL2 proteins illustrated as Double Expresser Lymphoma DEL (p=0.035) and Ann Arbor staging (p=0.001).

Ann Arbor staging: Upon diagnosis of Diffuse Large B cell Lymphoma, as previously described, further investigations should be carried out to acquire additional information about the extension of the malignant tumor. This process is termed 'staging.' This is crucial for the practical determination of the treatment plan and course. Staging of Diffuse Large B cell Lymphoma is generally done using the Ann Arbor Staging System (later modified to Lugano classification) (Figure IV.17.1). This staging system defines the malignant tumor location and clinical symptoms (Carbone et al., 1971).

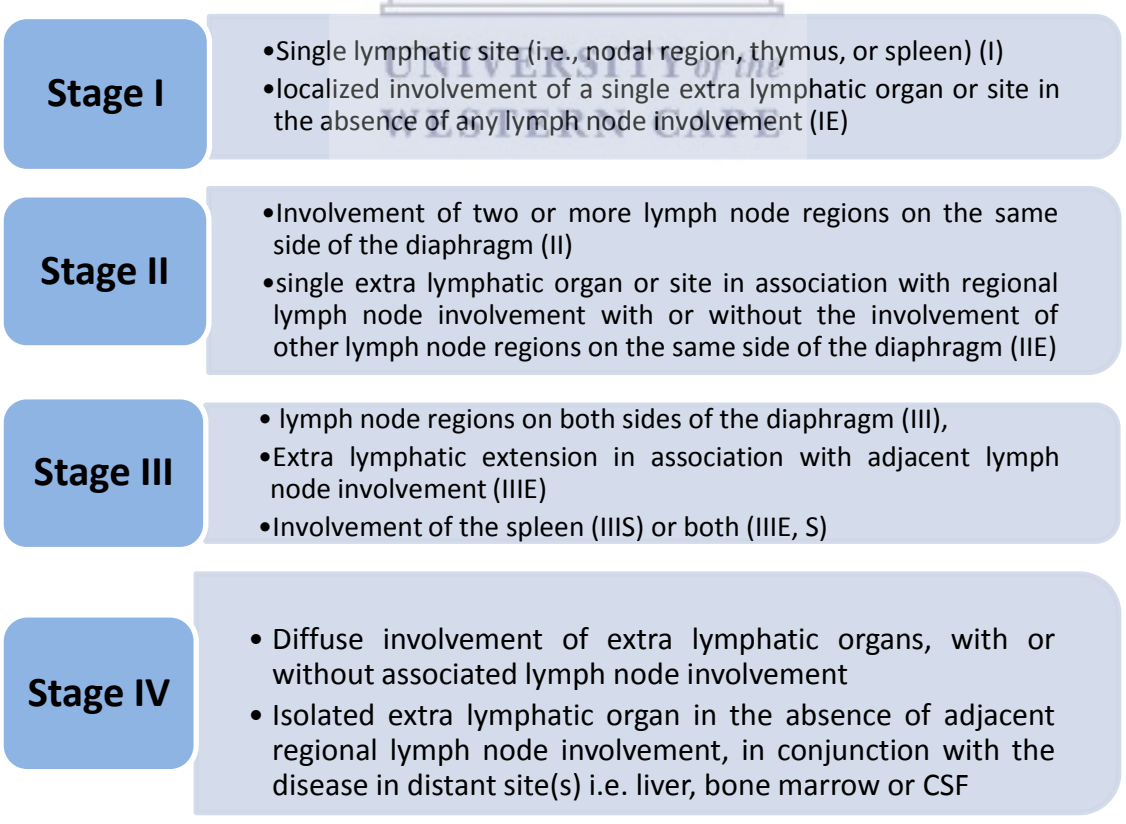


Figure IV.17.1 Ann Arbor (Lugano) staging classification for Diffuse Large B cell Lymphoma (Carbone et al., 1971).

Table IV.1 Description related stages of Ann Arbor staging classification. Adapted from AJCC: (Hodgkin and non-Hodgkin lymphomas/ AJCC Cancer Staging Manual. 7th ed. New York 2010). (CC, 2010)

Description related to each stage	
A	No symptoms.
B	Fever (temperature >38.0ºC), drenching night sweats, unexplained loss of >10% of body weight within the preceding six months.
E	Involvement of a single extranodal site that is contiguous or proximal to the known nodal site
S	Splenic involvement

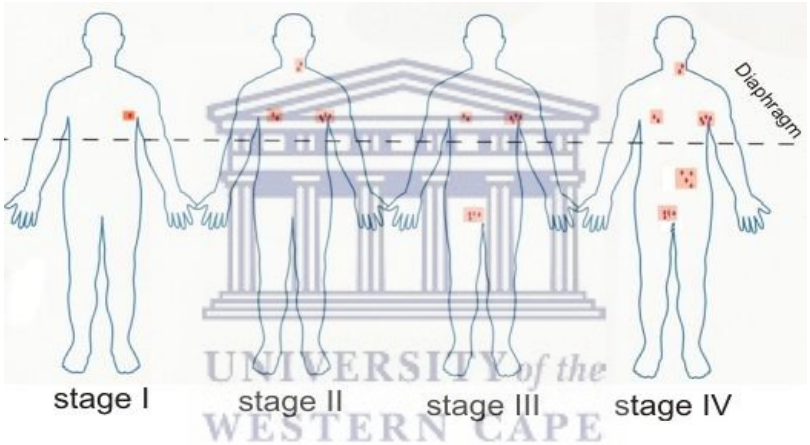


Figure IV.17.2 Schematic illustration of Ann Arbor Staging of Diffuse Large B cell Lymphoma (adapted from Favrille patient education material).

In this study, the tumor stage has shown independent predictive potential for the prognosis of DLBCL in multivariate cox regression analysis. The majority of the study samples presented at late stages of the disease, 46% presented with stage IV, and 16% of study samples were in stage III. Unfortunately, when calculating the hazard of death in relation to the Ann Arbor stage, 87% of stage IV patients died of DLBCL, while 76% of stage III patients died. This result is consistent with various studies that have shown the strong impact of the tumor stage on the survival of cancer patients (Morgan et al., 2019) (Dunyo et al., 2018).

Late presentation of the patients was one of the crucial findings in this study, resulting in diagnosing the patients at stage III and IV of the tumor (16.56% and 46.36%), respectively. This finding is per other studies in various cancer types (Smith et al., 2015a).

This could be attributed to patient-related factors or professional diagnostic delay, or a combination of both. Various confounding factors define the Health-seeking behavior of patients(Ahmed et al., 2019b). The painless nature of DLBCL at the early stages of the tumor could contribute to the late discovery as the size of the lesion and symptomology have been shown to have a direct correlation (Kawashima et al., 2018).

Another factor that could delay diagnosing malignant tumors is the fear, apprehension of malignancy, fear of social and direct, and indirect financial burden to the patients' families (Chen et al., 2012). Low socioeconomic status directly impacted patients' awareness (Thunberg et al., 2009). Numerous patients may not interpret their symptoms correctly, giving vague or misleading symptoms or mimicking their complaints to common prevalent medical conditions (Yi et al., 2013). Moreover, misconceptions related to social stigma about the disease (van de Schans et al., 2014), and to avoid this, patients tend to have widespread beliefs in traditional healers silencing their concerns with natural herbs and unprescribed medicine (Torre et al., 2015).

Accessibility to medical care services is a critical factor that impacts patients' health-seeking behavior (Goodwin et al., 1987). Sudan is a developing low-income country where the vast majority of the population lives in poverty and severe political disturbances (Kravdal, 2013). The medical infrastructure is located mainly in the capital Khartoum. Patients face a scarcity of primary health care centers and trained professional medics in the rural areas and the high travel expenses to and from Khartoum.

In this study, 31% of the study participants resided in Khartoum state. In contrast, 70% had to travel from their residential areas to RICK because of an absolute lack of histopathological diagnostic and treatments centers. Only 17% of the participants were referred to RICK by their private physicians, reflecting the shortage of consultants and the inadequate geographical distribution of medical services in Sudan.

Professional delay is one of the crucial factors contributing to the late diagnosis of cancers (Kravdal, 2013). Provisional misdiagnosis and incorrect referrals by general practitioners and untrained healthcare workers caused many delayed detection cases (Yang et al., 2019). This delay could be attributed to a reduced suspicion index (Sneha et al., 2017), lack of screening experience, and patients' vague signs and symptoms (Zhai et al., 2019).

Double Expresser Lymphoma: Various studies have associated poor survival outcomes of Diffuse Large B cell Lymphoma with the Double Expresser Lymphoma subtype of Diffuse Large B cell Lymphoma (Mehta et al., 2020). In this study, 63% of the DEL-positive patients died, elevating the hazard of death related to the DEL subtype of DLBCL. It has been documented that the presence of positivity when the MYC biomarker enhances and promotes the progression and proliferation of malignant cells (Wierstra and Alves, 2008). Furthermore, BCL2 protein was shown to inhibit apoptosis of tumor cells, prolonging cell cycle survival (Sesques and Johnson, 2017a). Hence, the effect of the coexpression of MYC and BCL2 proteins indicated in previous studies (Kawashima et al., 2018) was in accordance with our findings in this study, as DEL could independently predict the prognosis of Diffuse Large B cell Lymphoma patients.

The patient's medical condition was a solid predictor of cancer survival in various published studies (Bebe et al., 2019). Unfortunately, in our study, we failed to obtain the data related to patients' comorbidities. This is attributed to the lack of digital registration

of medical reports as most of the past medical history records of patients' were lost or incomplete. The lack of a unified medical network has added to the burden furthermore. An additional factor contributing to the impact of advanced ages is the nutritional status of Diffuse Large B cell Lymphoma patients (Park et al., 2014). Most of these patients' complaints ranged from cervical lumps, dysphagia, and anorexia. Subsequently, patients suffer from malnutrition, massive loss of weight, and compromised immunity (Park et al., 2014).

Furthermore, cachexia could occur in advanced malignancies. Consequently, patients experience a state of generalized body weakening that adversely impacts their ability to withstand chemotherapy sessions and thus lowers the survival rates (Sarkozy et al., 2015a). Numerous reports indicated that survival rates of Diffuse Large B cell Lymphoma might be superior in patients of younger age (Yi et al., 2013, van de Schans et al., 2014). On the contrary, in other types of cancer, few studies have shown no difference in survival in patients of the same stage of the tumor being old or young (Chen et al., 2012).

17.3 Molecular Biomarkers as Prognostic Indicators of Diffuse Large B cell Lymphoma

This study hypothesized that high expression levels of MYC and BCL2 biomarkers in the phenotypic subtype of Diffuse Large B cell lymphoma (Double Expresser Lymphoma) predicts poor prognostic outcome in RCHOP treated patients. The results have proved this hypothesis and illustrated that concurrent expression of MYC and BCL2 in Diffuse Large B cell Lymphoma could independently predict the survival of this tumor in the multivariate analysis.

17.3.1 MYC biomolecule

MYC protein is a molecular proto-oncogene encoding for a leucine zipper transcription factor found on the long arm of chromosome 8 (8q24) and controlled by the immunoglobulin (IG) gene (Riedell and Smith, 2018).

The role of MYC biomarker and its related biological signs of progress in tumorigenesis of Diffuse Large B cell Lymphoma (Xia and Zhang, 2020) and other malignant tumors, i.e., medulloblastoma (Roussel and Robinson, 2013) and neuroblastoma (Brodeur, 2003), has been investigated in various scientific studies.

MYC was found to have a wide range of biological activities that lead to activation and promotion of tumor pathogenesis and development via binding to sequence-specific genes and forcing their transcriptions which stimulates the oncogenic potential of numerous cancers (Bisso and Sabò, 2019). Some of which include cellular metabolism, protein synthesis (Meyer and Penn, 2008), genomic amplification and instability (Dang et al., 2006), lymphomagenesis (Klapproth and Wirth, 2010), cellular differentiation and proliferation. MYC was found to encourage neural progenitor cell proliferation and transformation into various cell lines including intermediate precursor cells Figure (IV.17.3) (Wang et al., 2020a).

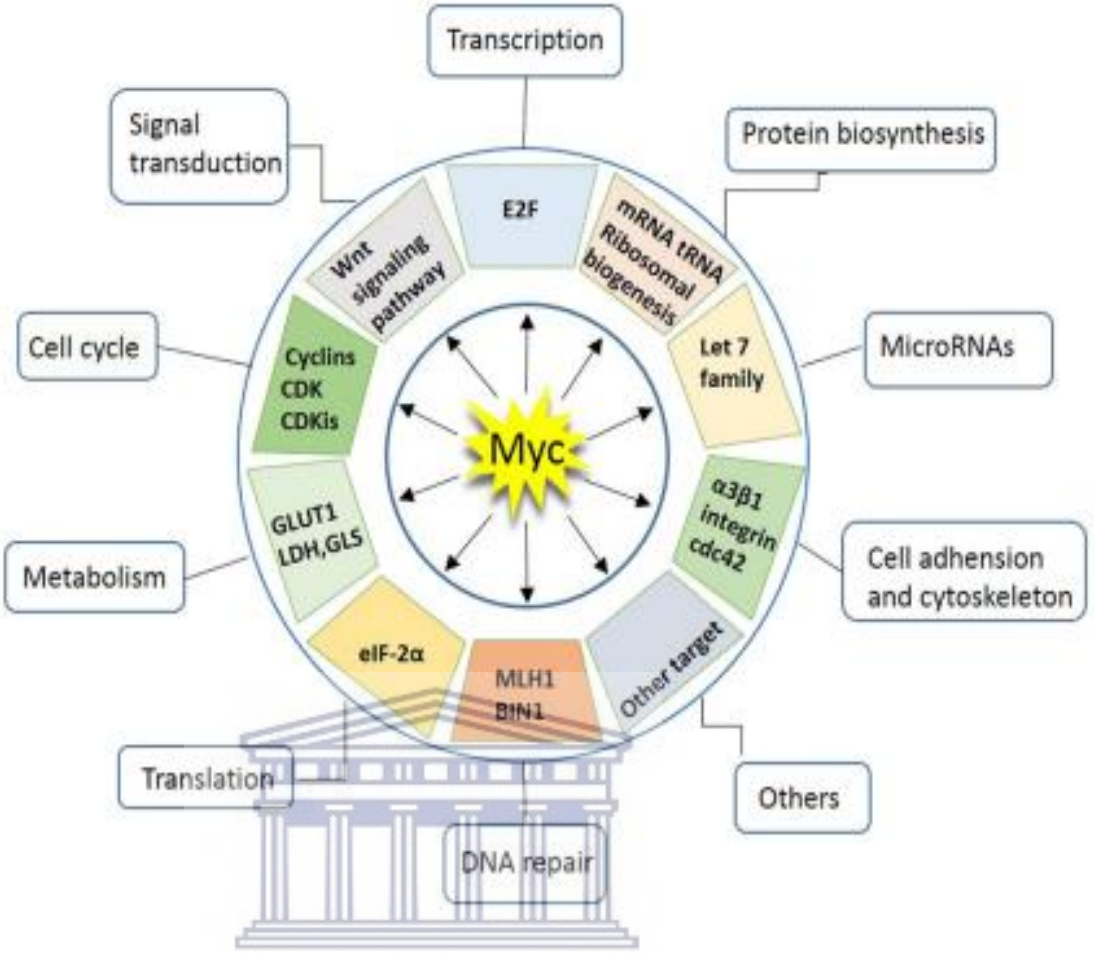


Figure IV.17.3 The spectrum of cellular functions regulated by Myc oncogene, including protein biogenesis, cell adhesion, metabolism, signal transduction, transcription, and translation (Chen et al., 2018a)

The expression of the MYC oncogene is strongly controlled under normal healthy circumstances; however, MYC is often deregulated in malignant tumors. Overexpression of MYC was found consequent to chromosomal molecular translocation/ amplification or mutation of upstream signaling pathways induction tendency of MYC was in the MYC oncogene (Liu et al., 2012). The tendency to induce malignancy coexists with other molecular pathways, i.e., structural alterations of BCL2 biomarker (Sewastianik et al., 2014). The promotion of Myc mediated P53 molecule trans/dedifferentiation in DLBCL, and hepatocellular carcinoma also occurs (Liu et al., 2021).

Among others, the B cell receptor phosphoinositide 3-kinase (PI3K) signaling pathway was found to drive the dysregulation of MYC protein (Wang et al., 2017). GSK-3 (glycogen synthase kinase-3) was also shown to control the tumorigenicity of Myc, as it phosphorylates MYC at Thr58 and it stimulates its degradation through the ubiquitin-proteasome pathway (Huang et al., 2006).

It has been shown that MYC protein was detected in about 40% of new or recurrent Diffuse Large B cell Lymphoma (Sesques and Johnson, 2017b). Overexpression of MYC protein can occur due to numerous mechanisms, including gene mutations, alterations, and derangement, i.e., the translocation of MYC to the immunoglobulin gene (IG) locus (Nguyen et al., 2017).

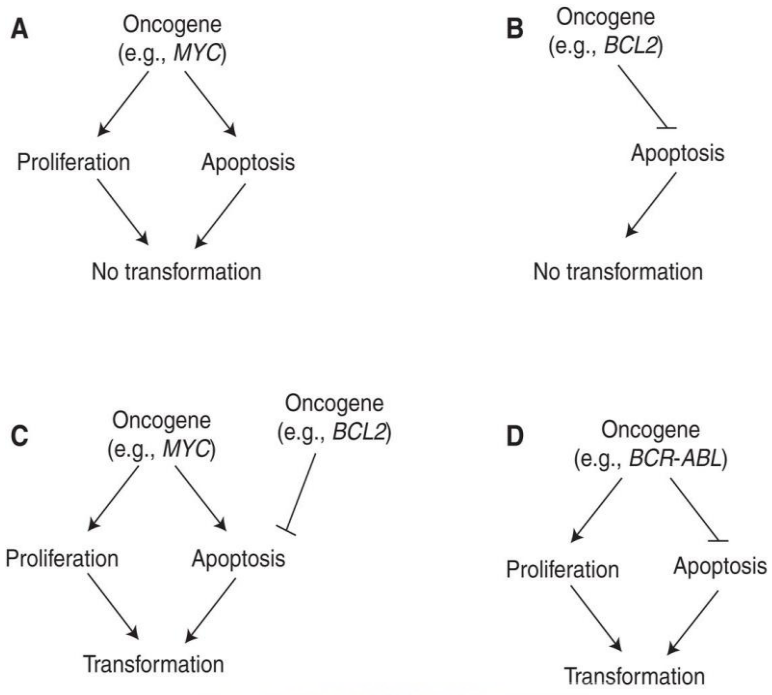


Figure IV.17.4 Cellular transformation through concomitant stimulation of cell proliferation and inhibition of apoptosis (Shortt and Johnstone, 2012)

Cellular transformation endorsed by MYC and BCL2 oncogenes via simultaneous stimulation of cellular proliferation and inhibition of apoptosis is illustrated in figure IV.17.4. Oncogenes such as MYC promote cell proliferation; nevertheless, some intrinsic apoptotic mechanisms such as those activated by the P53 pathway counteract mitosis mediated by MYC and inhibit transformation (Liu et al., 2021).

BCL2 is affiliated to a family of potent suppressors of apoptosis; however, they are poor cell proliferation activators and are insufficient to drive tumorigenesis as a solitary oncogenic incident. The mutual activity of oncogenes such as MYC and BCL2 inhibits apoptosis and induces cellular proliferation resulting in cellular transformation. Specific oncogenes such as BCR-ABL can stimulate signaling pathways that concurrently induce cellular proliferation and inhibit apoptosis, resulting in transformation (Shortt and Johnstone, 2012).

The overexpression of the MYC biomarker is suggested to strongly correlate to the poor prognosis of persons with Diffuse Large B cell Lymphoma (Cook et al., 2014). Nevertheless, the fundamental mechanism for such weakened prognostic outcomes is still being investigated. Some studies suggest an association between MYC overexpression with concurrent genetic alterations and interaction with other proteins, i.e., BCL2, which have been shown to promote and activate the oncogenicity of MYC protein (Miyamoto et al., 2016). Moreover, other findings suggested that the overexpression of MYC oncogene in DLBCL might induce considerable damage to the patient's DNA and result in delayed apoptosis of malignant cells by the assistance of other oncogenic molecules such as P53 (Wight et al., 2018).

The copy number of the MYC gene (the oncogenic dosage) was estimated at 8–20% of DLBCL cases, and it was not necessarily associated with increased MYC protein overexpression (Haws et al., 2016). In this project, the expression level and the oncogenic dosage of MYC in DLBCL Sudanese patients were investigated.

Targeting oncogenic MYC for therapeutic cancer treatment

As previously mentioned, the MYC oncogene has a vital role in nearly every aspect of the tumorgenesis process, coordinating cellular proliferation, apoptotic activity, and differentiation (Cho et al., 2021).

Researchers have focused on MYC inhibition as a potential cancer therapy approach. However, direct targeting of MYC oncogene was found to be a challenge due to the difficulty in pharmacological structuring and hence was considered “undruggable” (Posternak and Cole, 2016). The chemical structure of MYC is deficient for explicit an active site for small molecules linked to MYC-pathway inhibition, resulting in

difficulty in functional inhibition of its activities using pharmacological strategies comparable to those utilized for kinases (Chen et al., 2018a).

Furthermore, MYC is most centrally located in the nucleus; therefore, it is technically not practical to target nuclear MYC with specific monoclonal antibodies. In vivo animal studies have demonstrated that even temporary inactivation of MYC induces marked regression in tumorgenesis, signifying that regulation of MYC oncogene could be connected to the therapeutic potential of malignant tumors (Arvanitis and Felsher, 2006).

To overcome these impediments, pharmacists have broadly explored and experimented with attaining effective anti-tumor products; below are some pharmacological trial approaches for indirect targeting revocation of Myc oncogenic activities:

- **Disruption of MYC binding complexes, i.e., MYC/Max complex:** The MYC/Max network encompasses a cluster of transcription factors whose distinctive interactions are essential in the activation or repression of gene-specific transcription. The MYC–Max complex is necessary for binding MYC to DNA and its consequent activation of target gene transcription. So pharmaceutical companies have developed recombinant MYC/MAX anti-cancer drugs (Walker et al., 2005)**.**
- **Inhibition of MYC transcription and translation:** MYC gene transcription is regulated by the Bromodomain and Extra-terminal (BET) family of proteins. The inhibition of the BET bromodomain showed effective anti-cancer potential in laboratory studies and animal trials in

numerous hematopoietic cancers, which show MYC overexpression (Donati et al., 2018).

- **Destabilization of MYC in addition to the synthetic lethality linked to the overexpression of MYC:** Targeting chemical compounds, i.e., deubiquitinases may lead to destabilization of Myc oncogene and tumor suppression, MYC stability is strongly controlled by the ubiquitin-proteasome system. MYC overexpression increases the cellular sensitivity to apoptosis; hence, targeting a synthetic lethal gene to malignant tumors exhibiting MYC overexpression is supposed to kill merely tumor cells but avoid normal ones (Wang et al., 2021).

17.3.2 B cell Lymphoma 2 (BCL2) Oncogene

BCL2 is a protein originally affiliated to the BCL2 family, composed of multiple proteins that share a sequential biological homology. This family regulates cellular protein expression specifically (apoptosis), a stereotypical programmed cell death vital for abolishing redundant or damaged cells and maintaining cellular viability (Hardwick and Soane, 2013). BCL2 gene is located on chromosome 18q21 and is attached to the outer membrane of mitochondria. Physiologically, this gene functions in sanctioning cellular survival by inhibiting the actions of pro-apoptotic proteins, one of which is the BAX, i.e., BCL-2-like protein, which controls the release of cytochrome C signals in the apoptosis cascade (Aouacheria et al., 2017).

Moreover, the BCL2 gene has been shown to regulate mitochondrial division and fusion, controlling insulin secretion and metabolic pathways in pancreatic cells (Aharoni-Simon et al., 2016). BCL2 is shown to have an antiapoptotic capability and can preserve cellular viability by inhibiting apoptosis activity. The overexpression of the BCL2 oncogene prevents the B lymphocytes cells from going through apoptosis,

elongating those cells' lifespan without an active mitotic division. Moreover, BCL2 could contribute to amendment in cellular membrane permeability essential for commencement of apoptosis (Ola et al., 2011).

Genetic alterations or oxidative stress can suppress BCL2 protein by elevating BH3 proteins expression (Ting et al., 2019). On the contrary, in the tumor cells of lymphomas, overexpression of BCL2 caused by molecular chromosomal translocations and elevated oncogenic dosage fold inhibits the apoptosis and induces the proliferation of malignant lymphoma cells, and subsequently accelerates the MYC-stimulated lymphomagenesis (Riedell and Smith, 2018)

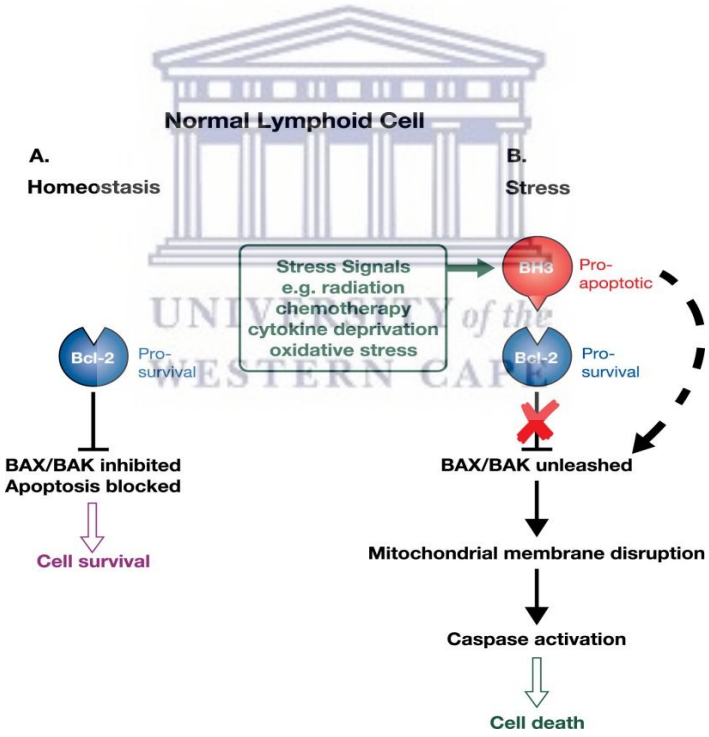


Figure IV.17.5 Control of cellular apoptosis by BCL2 protein (Anderson et al., 2014).

The failure of programmed cell death termed apoptosis underpins the development of tumorigenesis and frequently promotes resistance of malignant cells to cytotoxic therapies. Regarding hematologic malignancies, this disruption of programmed cell

death is regularly caused by overexpression of the pro-survival oncogene BCL2. Since peculiarly high levels of BCL2 maintain these malignancies, targeting BCL2 has been the focus of various pharmaceutical companies as a novel approach to the therapeutic regimens of blood cancers (Manion and Hockenbery, 2003). Till recently, targeting BCL-2 oncogene was considered challenging and was thought to be undruggable until the development of the (NMR) fragment-based nuclear magnetic resonance screening that has altered and widened the potential therapeutic approach for BCL2 (Verdine and Walensky, 2007). Studies have shown that manipulating BCL-2 functions for anti-tumor effects is conceivably the most effective approach of the molecular targets proposed for cancer treatment. One of the novel therapeutics is the development of BH3 mimetic compounds, small size molecules that imitate the BH3-only proteins, which are natural antagonists of pro-survival BCL2 oncogene (Anderson et al., 2014).

17.4 Survival Rates of Diffuse Large B cell Lymphoma

Survival is the aspired outcome that clinicians seek as an assessment tool and a key measure for the efficiency of the provided treatment modalities, consequently reflecting on the quality of the available health care systems.

One of the main objectives of this study was to provide scientific data on the survival rates of DLBCL in Sudanese patients treated at RICK. The survival rates were calculated from the date of the first diagnosis to the date of death or censoring.

The estimated DLBCL survival rates were relatively low despite the currently used treatment regimen and governmental trials for management and therapeutic care progress. In this study, the 1, 2, 3, and 5-year disease-specific survival rates were

98.7%, 80%, 58.9%, and drastically fell to 19%, respectively. Patients had a superior survival rate within the first year of diagnosis; nevertheless, almost half did not survive within the third year.

Several descriptions have been suggested for estimating survival rates of malignant tumors (Shindoh et al., 2016) (Liu et al., 2016). These rates are standardized to specific periods, mainly yearly, i.e., five and ten-year intervals (Zaha et al., 2010). This is done using particular statistical analysis methods that estimate the probability of surviving a specific disease by calculating the number of patients who survived divided by the number of those at risk. These methods frequently consider the number of sample patients whose follow-up was lost or limited for any reason and consider them as "censored observations" (Hedström et al., 2015a).

Survival rates of DLBCL can be calculated via multiple methods; choosing the most suitable mean depends on the tumor type, stage, and parameters (Howlader et al., 2010). These methods include:

Overall Survival

The probability of surviving all possible causes of death, such as the disease or any other unspecified causative factor, is expressed in standardized time intervals. This probability is usually used to estimate survival if the exact cause of death is unknown (Broglio and Berry, 2009).

Net Survival

This method focuses on the direct effect of the disease on the survival rate; it excludes any other death causative factors. It reflects a practical comparative picture among different populations with variant life expectancies, racial/ethnic backgrounds, and

health care systems (Danieli et al., 2012). Net survival is subdivided into assessment methods:

Relative Survival

This is calculated by dividing the disease's overall survival upon diagnosis in a specific population by the survival recorded in a comparable (i.e., age, gender, socioeconomic status, ethnic group, etc.) population that was not diagnosed with the same disease. Ideally, relative survival should have a population life table that matches the target tumor's risk factor. It has the advantage of not relying on the precision of the recorded cause of mortality (Perme et al., 2012).

Disease-Specific Survival

DSS refers to the proportion of individuals or study samples who have survived a specific disease in a preset period that usually starts at the first date of diagnosis and ends at the death time, excluding patients or individuals who have died for other reasons and not from the disease itself (Gschwend et al., 2002). There is no need to match any population in this method, and the primary focus would be on the study group or target. In this study DSS was the parameter of choice.

Non-Hodgkin Lymphoma is a leading cause of death globally, accounting for 248,724 deaths in 2018. Lung cancer has the highest mortality, with close to 1.761.007 deaths worldwide Figure (IV.17.6) (Bray et al., 2018).

Five years survival rates for Diffuse Large B cell Lymphoma demonstrate a noticeable geographical disparity. The American Surveillance Epidemiology and End Results Program (SEER) shows significant improvement in the five years survival rate for Diffuse Large B cell Lymphoma ranging from 40.68% in (1975-1980) to 67.18% in (2012-2017) (Lewis et al., 2017). Moreover, Cancer Research in the United

Kingdom has shown that the five and ten-year survival rate of DLBCL NHLs has improved from 30% to 70% between 1970 and 2011(Mikhaeel et al., 2021). Furthermore, the 2014 Eurocare 5 study presented estimates of population-based cancer survival from 29 European countries during 1999-2007; the mean European age-standardized 5-year relative survival for DLBCL Non-Hodgkin Lymphoma was 48% (De Angelis et al., 2014).

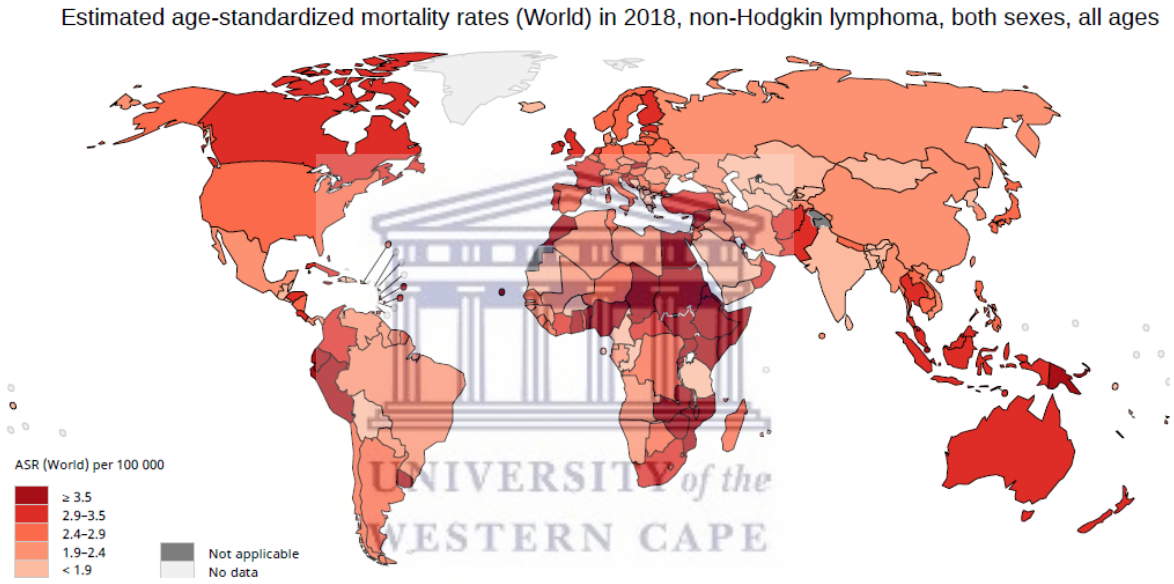


Figure IV.17.6 World map illustrating global age-standardized NHL mortality rate in 2018. Source (GLOBOCAN 2018) (Bray et al., 2018)

In general, mortality and survival rates of Non-Hodgkin Lymphomas illustrate significant global variation (Miranda-Filho et al., 2019). There are marked poorer survival rates in the developing countries that could reach as low as 19% (Lee et al., 2020) compared to developed ones, as the 5-year survival rate for DLBCL is around 64% in the United States(Lewis et al., 2017)**.**

The geographical disparity in survival rates of various types of cancers has been attributed to differences in the quality of cancer registration, accessibility, medical services, and presentation timing.

In China, the overall five-year survival rate of Non-Hodgkins Lymphoma was 37.03% (Chen et al., 2018b), while in Kenya, the survival rate of Non-Hodgkin's Lymphoma was affected by financial restraints like medical insurance coverage (Martijn et al., 2017). On the other hand, the inadequate number of oncology centers to provide medical care for the rising number of African cancer patients has jeopardized the survival rates (Stefan, 2015). The 5-year disease-specific survival rate in our study (19%) was significantly lower than registered rates in the United States, estimated to be from 63.8% between 2010 and 2016 (Li et al., 2019). Furthermore, it has been shown that there is a remarkable variation in the attitude of healthcare-seeking behavior among individuals of different countries (Zhang et al., 2017) (Gupta et al., 2021).

17.5 Factors contributing to the Inferior Five Years Survival Rate of DLBCL

17.5.1 Facility-Related Factors

Patients surviving Diffuse Large B cell Lymphoma is dependent upon various host and tumor parameters, some of which are shown to decrease the odds of good prognosis and were correlated with inferior outcomes (Hedström et al., 2015b).

Various factors may thoroughly explain the inferior five years survival rate of DLBCL detected in our study:

A) The fragile health care system in Sudan resulted in limited access and disorganization in the distribution of the health care centers all over the country. Consequently, patients suffered from a delay in diagnosis and late presentation for treatment that directly affected survival. Moreover, the poverty and difficult financial status of the majority of the population and the complexity to travel from rural areas

to Khartoum, these factors combined with diminished awareness of the public about tumor signs, have pushed the locals to seek traditional healers and herbal treatment and hence resulting in misinterpretation of malignancy symptoms.

B) The scarcity of histo-pathological and referral centers in Sudan has added to the burden of delayed diagnosis and late presentation of patients. While in states as large as Darfur in western Sudan, there are no centers available, and the primary health care centers suffer from a dearth of trained medical personnel. Despite the accumulation of medical centers in Khartoum, there are only two governmental histopathological centers, RICK and STACK. In the central province of Gezira, a single histopathological center NCI is located in Wad Madani city and serves the state and its surrounding villages. Figure VI.17.7 illustrates the density of the population in Sudan that reflects the scarcity of the needed centers compared to the population density.

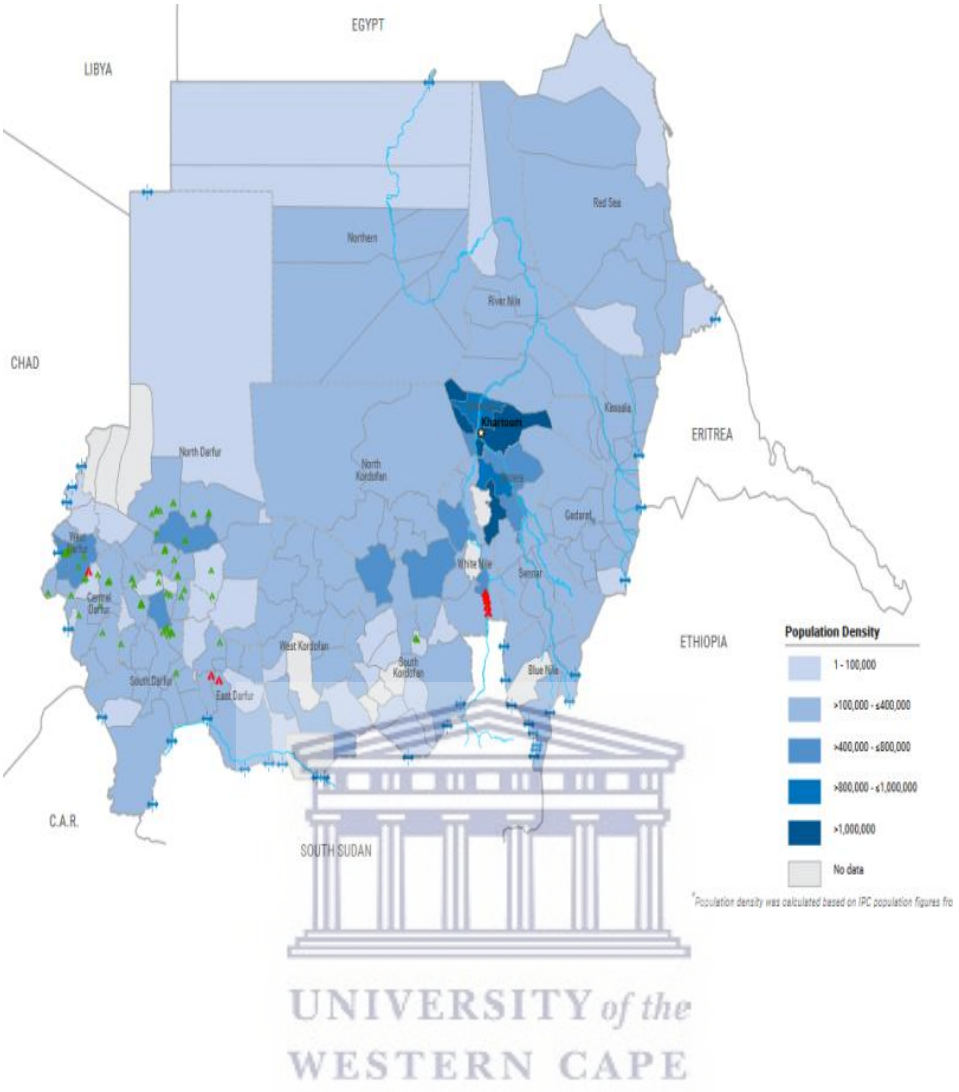


Figure VI.17.7 Population Density per state (Sudan) based on IPC population figures (Lokuruka, 2020) UNOCHA 2020

C) The cost of treatment, including traveling and living expenses, laboratory investigations, radiographs, and hospitalization, is a significant factor that impacts the survival rate of DLBCL patients and the follow-up visits, as patients who were lost

for follow-up resided in rural areas. These expenses create a heavy burden on families who are already of low socioeconomic status.

In addition to the impact of the health care system in Sudan, some factors were related to the tumor and the host itself that directly affected the survival rate of DLBCL.

17.5.2 Tumor Related Factors:

A) Late tumor presentation: Most of the patients in this study samples (62%) presented at stages III and IV of the DLBCL tumor, which has been shown to have an adverse impact on DLBCL survival. The findings of this study confirmed that Ann Arbor staging is an independent survival predictor. This result was in accordance with (Rodriguez et al., 2001) that showed the independent effect of Ann Arbor staging on the survival prediction of Follicular Large Cell Lymphoma, which acts in a close manner as Diffuse Large B cell Lymphoma. Ann Arbor staging has also been shown as part of a nomogram to predict overall survival in Hodgkin Lymphoma (Zhang et al., 2017). In our findings, the one-year survival rate of Diffuse Large B cell Lymphoma was as high as (98.7%). This percentage has drastically deteriorated to 19% for five-year survival rates. It has been shown that delay in presentation and diagnosis resulting from patients presenting at late stages of Diffuse Large B cell Lymphoma showed inferior outcome and poorer prognosis following treatment (Nikonova et al., 2015). Various factors were attributed to delay in diagnosis and treatment, including the rapid, aggressive behavior of DLBCL, misdiagnosis as the tumor presentation could mimic various malignant tumors (Kobayashi et al., 2016).

B) Concurrent overexpression of MYC and BCL2 oncogenes illustrated as Double Expresser Lymphoma: Our project investigated the prognostic values of MYC and BCL2 biomarkers via their expression levels using immunohistochemistry.

It is a widely applied method for screening cancers in developing countries like Sudan (Sengal et al., 2017) (SirAlkhatim, 2012). This is attributed to its cost-effectiveness, rapid processing, easiness, and availability of the required commercial antibodies (Grillo et al., 2017). Furthermore, biologically, proteins function at later stages of cellular interaction; hence, the expression levels of proteins are expected to adequately assess reciprocal genetic activity (Klapper et al., 2012). MYC protein plays a vital pathogenetic effect in the proliferation of B-cell (Quintela et al., 2019). Various studies have shown that overexpression of MYC protein has a substantial role in the pathogenesis of several malignant tumors via numerous molecular pathways, one of which is the endorsement of cellular proliferation resulting from linking protein and serum growth factors like (VEGF) (Bebe et al., 2019). Around 47 to 58% of DLBCL cases exhibited overexpression of BCL2 protein (Park et al., 2014). This protein controls the programmed self-death of normal and malignant cells (Johnson et al., 2012b). Consequently, simultaneous co-expression of both proteins has a considerable role in the progression of DLBCL. This study has confirmed that Double Expresser Lymphoma is an independent survival predictor **(**P value= 0.014**)**. This is in accordance with (Kawashima et al., 2018**),** which stated that Double Expresser Lymphoma showed poorer survival rates in Diffuse Large B cell Lymphoma cases.

17.5.3 Treatment-related factors:

i) Late patients' presentation, in our study (62%), DLBCL patients presented at stages III and IV of the tumor. This delay has jeopardized the possible treatment options that could be offered to the patients.

ii) The insufficiency of the histo-pathological and treatment centers has caused an unfavorable delay in receiving the required medical services starting from diagnostic

investigations, radiographic analysis, and treatment therapies needed, such as chemotherapy and radiotherapy. Radiation and Isotope Center, where this study was conducted, currently has very few machines that are not up to the standard and do not match the accumulating number of cancer patients needing urgent treatment. Delay between the first diagnosis till treatment was recognized as an independent factor in the prognosis of aggressive Non-Hodgkin lymphomas (Olszewski et al., 2018).

iii) The limited financial capability of most patients presenting to RICK has restricted the kind of investigations that oncologists request before the commencement of the selected treatment, e.g., chemotherapy sessions**.** Consequently, it is a real challenge to discover any underlying medical co-morbidity that could affect the treatment decision-making.

iv) Subsequent follow-up of the patients who undergo chemotherapy is compromised in Sudan. This is attributed to a deficiency in the aftercare facilities, including medical services and beds. Patients usually stay for less than a day following their chemotherapy sessions at RICK. This might compromise their nutritional status and general well-being, especially for DLBCL patients who travel for hours back to their residential areas. Very few institutions provide palliative aftercare, one of which is the RICK. Yet, despite the enormous efforts to increase the capacity of these institutions, the need remains vast, and patients suffer from chemotherapy side effects, e.g., disturbances in the blood pressure and electrolytes in addition to dehydration (Gafer and Elhaj, 2014).

v) Compliance of cancer patients directly impacts the survival outcomes. Patients in Sudan face various logistical challenges, traveling from their residential areas to RICK and back forth to have their chemotherapy sessions. Recently, many charity

organizations have tried to cover the need by creating temporary residential accommodations for patients to utilize during their treatment journey. Unfortunately, the gap is still vast, and some patients fail to find financial assistance and prefer to face the worst-case scenario and move back to their hometowns to die around their families.

17.5.4 Host-Related Factors

1) Age in patients who were 60 years and older an increased tumor biological complexity was shown (Smith et al., 2015a), comorbidity (i.e., other tumors, arthritis, diabetes). In addition to their ability to withstand chemotherapy (Janssen-Heijnen et al., 2005) (Varga et al., 2014).

2) Serum Lactate Dehydrogenase enzyme is identified as a malignancy marker, as it imitated the burden of malignant cellular division in various malignancies (Hong et al., 2013). LDH level has shown to have a strong correlation with relapse of Diffuse Large B cell Lymphoma patients. A 1.5-fold increase in the enzyme level resulted in significantly poorer prognostic values. (William et al., 2013).

3) Poor general health condition, few indexes are used to assess the physical and functional ability of DLBCL patients, The Karnofsky's performance status index (KPS) and the Eastern Cooperative Oncology Group Performance Status Scale (ECOG PS) (Figure IV.17.8), the latter more widely used in clinical settings (Buccheri et al., 1996). ECOG performance status score of 2 or greater was shown to have a negative impact on the prognosis value in DLBCL patients.

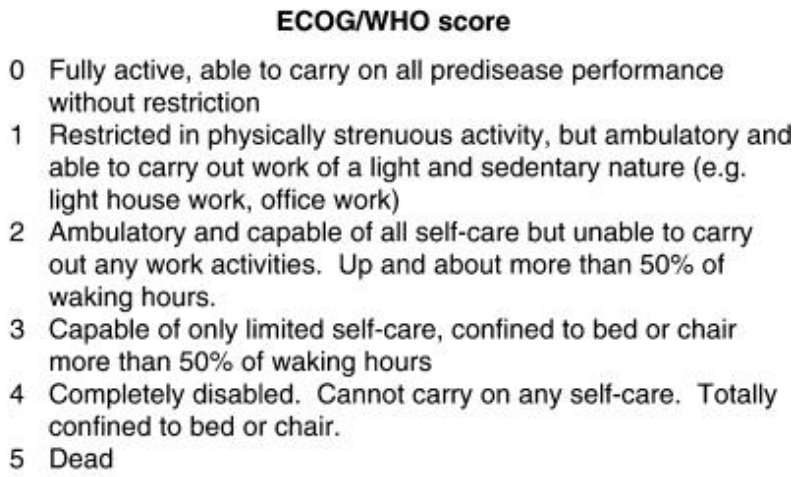


Figure IV.17.8 Criteria of ECOG performance status scale (Smith et al., 2011)

4) More than a single extranodal site affected by Lymphomagenesis, whether the tumor has affected organs outside the lymphatic system crucially affects the disease outcome. One-third of DLBCL patients present an extranodal involvement. The gastrointestinal tract, pancreas, lung, and liver recorded the worst prognostic values (Castillo et al., 2014).

5) Difficulty in Evaluation of treatment response and follow up in DLBCL

For clinicians to evaluate the response of Diffuse Large B cell Lymphoma patients to the recommended treatment, FDG-PET/CT is the current recommended radiographic assessment of post-treatment evaluation (Meignan et al., 2009). Scientists have developed a five-point Visual Deauville scale for assessing the Ann Arbor staging system (Table IV.2). These criteria are based on the tumor complete metabolic response rate. Deauville scores 1, 2 and 3, marks absolute no residual uptake or if it is lower to equivalent to the activity of the liver with/without confirmation of residual mass on the CT radiograph, with and without FDG lesions located in the bone marrow (Hofman and Hicks, 2016). Most patients with a score of 3 illustrate an excellent

prognosis with a standard regimen. Deauville scores 4 and 5 specify residual disease in most cases; hence, it is recommended to take a biopsy. Radiographic evaluation during chemotherapeutic sessions after three to four cycles may be used to exclude progression of malignancy. It is frequently carried out with PET/CT and has been indicated to predict treatment outcomes (Barrington et al., 2014).

Table IV.2 PET 5-point scale (Deauville criteria) (Cheson et al., 2014)

Visual Deauville scale	Tumor complete metabolic response rate
1	No uptake
2	Uptake ≤mediastinum
3	Uptake >mediastinum but ≤liver
4	Moderately increased uptake compared with liver
5	Markedly increased uptake to the liver or new lesions

Patients with Diffuse Large B cell Lymphoma who have been malignancy free two years of malignancy free have a superior overall survival, and they require regular monitoring of the condition. A thorough history and clinical examination and blood count test should be done four times in the first year, following that, twice a year the next two years and lastly once a year, to monitor the appearance of any secondary lesions or long term treatment side effects. High-risk patients with therapeutic alternatives may potentially need more regular evaluation (Petrausch et al., 2010).

Prediction of the survival probability for Diffuse Large B cell Lymphoma patients is based mainly on the International Prognostic Index (IPI), first described in 1993. IPI

system integrates the previously mentioned five clinical parameters solely associated with the survival of DLBCL patients.

This scoring system gives one point for each of the above characteristics, for a total score ranging from zero to five; utilizing these clinical variables, DLBCL can be stratified into three risk groups:

- Low-risk Group: IPI score of 0 or 1 (Three years survival rate is 91%).
- Low to intermediate risk Group: IPI score of 2 (Three-year survival rate is 81%).
- High to intermediate-risk Group: IPI score of 3 (Three years survival rate is 65%).
- High-risk Group: IPI score of 4 or more (Three years survival rate is 59%).

Table IV.3 International Prognostic Index (Smith et al., 2011)

Factor	Adverse Prognosis
Age	More than 60 years
Ann Arbor stage	III or IV
LDH serum level	Above normal
Number of involved extranodal sites	more than or equal to 2
Performance status	ECOG PS more than or equal to 2 *Eastern Cooperative Oncology Group Performance Status

17.6 Quantitative Real-time PCR in relation to the Relative mRNA Expression levels, Oncogenic Dose and Translocations of MYC and BCL2 molecules

Molecular and genetic changes in oncogenic dosage are frequently indicated in various types of human malignancies; these alterations regulate the expression levels of genes (Albertson, 2006). Cells that show malignant potential commonly bear an increasing number of gene alterations, i.e., gains and losses. These cells successively become active and experience uncontrolled growth, conquer cellular constraints, continue proliferation, and invade neighboring tissues, metastasize, and resist treatment caused by such genetic changes (Miao et al., 2019) (Pasqualucci and Dalla-Favera, 2018). Hence, investigating and functional evaluation of oncogenic dosage changes implicated in carcinogenesis is considered crucial for understanding the pathogenesis of the tumor and may lead to enhanced treatment possibilities (Rushton et al., 2020).

Various studies have investigated MYC biomolecule's oncogenic gene copy (dosage) as a prognostic indicator in Diffuse large B-cell lymphoma patients with varying results. (Lu et al., 2015) have shown increased MYC oncogenic fold change exists in 8–20% of DLBCL patients and was not associated with increased MYC protein expression. In our study, the oncogenic dosage fold change was detected in 67.4 % of the samples. It was found to be statistically associated with poor survival as these samples were of dead patients.

Moreover, Disease-specific survival of DLBCL with positive MYC protein overexpression and significant fold change in oncogenic dose were compared to negative samples, and the statistical testing indicated a prognostic significance of MYC oncogenic dosage (P value= 0.0031). This result was contrary to the findings of

(Haws et al., 2016). They found no statistically significant difference in the survival between Diffuse Large B cell Lymphoma patients with MYC increased oncogenic dosage and patients who showed no statistical significance and no MYC abnormality in gene copy numbers (P-value = 0.58).

Regarding quantifying the translocations of BCL2 oncogene in Diffuse Large B cell Lymphoma, results have indicated that within the selected samples, forty were Double Expresser Lymphoma positive patients, 35% of the samples showed noticeable translocations. The displayed results in our study concurred with studies of larger sample sizes. BCL2-IGH translocation within MBR and MCR regions was found in 50% and 5% of patients, respectively, at chromosome 18 in follicular lymphoma patients and Diffuse Large B cell Lymphoma via real-time PCR at a Weinberg et al. 2007 study (Weinberg et al., 2007). Furthermore, Van Dongen et al. reported 67% and 9% had a breakpoint within MBR and MCR, regions of the chromosome (14;18) in follicular and Diffuse Large B cell lymphoma patients (Van Dongen et al., 2003). Since all samples that indicated BCL2 translocations in our study were Double Expresser Lymphoma positive, and DEL was shown as a poor prognostic independent predictor, we can conclude that BCL2 translocations suggest inferior survival Diffuse Large B cell Lymphoma.

17.7 Diagnosis and Availability of Treatment Modalities of DLBCL in Sudan

17.7.1 Diagnosis of DLBCL

The results shown in this project have highlighted that diagnostic delay, late tumor presentation, and a single treatment modality regimen have contributed to the inferior survival of DLBCL in Sudan. Hence, the challenges facing the diagnosis and the availability of the treatment modalities will be discussed in this chapter.

Diagnosing Diffuse Large B cell Lymphoma involves hierarchical investigations that usually start with self-assessment. Patients typically complain of varying degrees of fatigue, fever (Ren et al., 2016), weight loss (O'Brian et al., 2016), itchiness, and night sweats (Shi et al., 2019). The most common symptom that triggers patients' care-seeking behavior is the feeling of swollen or enlarged lymph nodes, a medical condition known as lymphadenopathy (Whooley et al., 2018). Patients may experience additional symptoms depending on the Diffuse Large B cell Lymphoma location include abdominal disturbances, bloody stool (Suresh et al., 2019), respiratory distress symptoms, i.e., cough and breathing difficulties (Jandial et al., 2018). Essential diagnostic investigations that are crucial for definitive diagnosis of Diffuse Large B cell Lymphoma are:

17.7.1.1 Medical History and Clinical Examination: Since the symptoms experienced in DLBCL are familiar among various disease entities, a detailed medical history and a thorough physical examination are mandatory. A medical history may expose potential risk factors that reflect the probability of Diffuse Large B cell Lymphoma; these risk factors are discussed in section II. History should include the patient's demographics, medical comorbidities, currently utilized medications, family

history of malignancies, nature, history of the chief complaint, and accompanying symptoms.

Clinical examination is usually preceded by checking the patient's vital signs, including temperature, pulse rate, respiratory rate, and blood pressure; moreover, the body weight should be recorded for future comparative measures. The focal point of the physical examination is palpating the size, consistency, and mobility of the lymph nodes along with any involved organs. Lymphadenopathy in DLBCL is characterized by its painless nature, firm, rubbery texture, and mobility of the lymphoid tissues. Physical examination should be done thoroughly to include multiple organs as multiple findings suggest DLBCL, i.e., splenomegaly, hepatomegaly, and reddish skin nodules.

17.7.1.2 Hematopathology laboratory investigations:

- Differential Complete Blood Count (D-CBC): this is a laboratory test to assess the proliferative and immunological function of the bone marrow and gives an accurate comparative measurement of the blood components, WBC, RBCs, and platelets. Lower panel findings, i.e., anemia and neutropenia, suggest DLBCL (Shimono et al., 2019).

- A comprehensive metabolic panel test: is a blood test that measures fourteen elements in the patient's blood; it reflects the chemical metabolic balance of the blood. CMP involves Calcium, potassium, carbon dioxide, chloride, Sodium, Glucose, Albumin, liver enzymes, i.e., ALT, ALP, AST, Urea, Creatinine, and Bilirubin (Yamauchi et al., 2015).

- Liver Function test: The prognostic value of liver enzymes, i.e., AST, has been associated with the overall survival in patients treated with R-CHOP regimen in DLBCL (Lu et al., 2019).

- Uric acid: Assessing the level of uric acid in patients diagnosed with DLBCL is of significant value as it indicates the development of Tumor Lysis Syndrome, a condition where the rapid lysis of cancer cells impairs the renal capacity of the patient resulting in severe electrolytes disturbances (Belay et al., 2017)

- Lactate dehydrogenase enzyme (LDH) level: LDH is an enzyme that is located intracellularly; it has been found to be elevated in blood samples of Diffuse Large B cell lymphoma patients and has been linked to hematopoietic cell damage and relapse in the central nervous system (Kim et al., 2016c).

- Hepatitis B and C viruses' tests: HBV was shown to be reactivated due to the R-CHOP regimen of DLBCL (Guo et al., 2018). Both viruses were indicated as established risk factors with poorer outcomes in DLBCL patients (Rong et al., 2019).

- Human Immunodeficiency Virus test: DLBCL related HIV is the most common type of lymphoma related to HIV; it represents a distinct tumor phenotype with specified treatment regimens that consider the patient's active or latent virus state (Re et al., 2019).

17.7.1.3 Radiographic Examination: Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) and contrast Computed Tomography (CT) scan are considered the most significant and widely used imaging modalities throughout the different stages of diagnosis, treatment planning, and follow up of Diffuse Large B cell Lymphoma course (Marcheselli et al., 2019). PET utilizes sugar-based substances traced via a special camera and shows the malignant cells in a brighter shape than their healthy counterparts as cancer cells consume sugar-based materials more rapidly (Bai and Liang, 2017). Magnetic Resonance Imaging (MRI) and ultrasound are frequently used as adjuvant tools for the initial morphological examination of and of the biopsies (Wang et al., 2018) (Liu et al., 2019).

17.7.1.4 Surgical Biopsy: A biopsy is a diagnostic procedure involving surgical removal of suspected cancer tissues for further morphological and molecular investigations (Alzahrani et al., 2016). In Diffuse Large B cell Lymphoma, the biopsy is primarily taken from the lymph nodes of the involved organ; it provides ultimate morphological confirmation of the malignancy in addition to reflecting the current stage of the diagnosed tumor.

Two main types of biopsies are commonly performed for the diagnosis of DLBCL:

- Excisional biopsy: in this procedure, the targeted lymphoid tissue is entirely removed (Kiliçarslan et al., 2017). In DLBCL, the excisional biopsy is usually advisable as the stroma of the lymph node is preserved. Hence, the morphology of the tested tissue reflects the type and stage of the tumor (Liu and Barta, 2019).

- Incisional biopsy: partial excision of the suspected lymph node is performed, jeopardizing the quality and quantity of the cancer tissues that would be later used for confirmatory investigations (Matsue et al., 2019).

- Bone marrow biopsy and aspiration: A procedure typically performed in diagnosing DLBCL when the PET scan results suggest osteogenic malignant involvement. The removal of liquid bone marrow via aspiration and hard bone marrow via biopsy is usually performed simultaneously using two needles that differ in their inserted diameter and hollow.

- Spinal fluid (Lumbar puncture) test: DLBCL that is related to the central nervous system and has neural involvement is common among patients who are older than 65 years of age, at advanced stages of DLBCL, whose laboratory investigations show an elevated level of lactate dehydrogenase enzyme and with the low-performance status scale of two or even less (Peñalver et al., 2017). The cerebrospinal fluid is tested for signs of malignancy via lumbar puncture procedure (Zheng et al., 2017).

- Fine Needle Aspiration and Core needle biopsy: The use of fine-needle aspiration biopsy (FNA) and core needle biopsy in the diagnosis of DLBCL is controversial, as some studies have shown adequate diagnostic values of FNA (Zhang et al., 2014), while these procedures have demonstrated suboptimal diagnostic outcomes in DLBCL, as the tissue suctioned via the creation of a vacuum by a syringe has failed to collect the needed amount and quality of the suspected tissues (Abdulla et al., 2017).

Upon adequate collection of the required tissue, the sample will undergo initial specific tests to evaluate the expression levels of particular tumor-defining proteins.

Immunohistochemistry is the most widely used method for this purpose. A panel of proteins is often partially or entirely assessed to confirm the diagnosis of DLBCL via linking dye-marked antibodies to the surface of the tumor cells (Bellas et al., 2014). CD20, CD10, MYC, BCL2, BCL6, and Ki67 are the most commonly tested biomarkers in DLBCL (Rasheed et al., 2018) (Tang et al., 2017) (Lu et al., 2016).

In the majority of the DLBCL specimens obtained via biopsy procedure, the hematoxylin and eosin (H&E) stained slides illustrate very distinctive morphological characteristics (Zou et al., 2018a), i.e., double-sized B lymphocytes, disjointed malignant cells, and lack of organic cellular structure (Chettiankandy et al., 2016). Nevertheless, DLBCL cytomorphology might resemble other types of malignancy. False resemblance could occasionally be attributed to plentiful artifacts in the H&E slides (Li et al., 2018b), while actual cytological similarity might present as spindle-shaped tumor cells, microvillous pattern, and a cytoplasmic matrix of the fibrillar environment (Devin et al., 2019).

Differential diagnosis of Diffuse Large B cell Lymphoma varies and includes non-neoplastic lesions like Kikuchi Lymphadenitis (Supari and Ananthamurthy, 2014). However, the cells of DLBCL exhibit heterogeneous cells with prominent nuclei in contrast to Burkitt Lymphoma, which shows uniform cellular morphology with 100% Ki-67 expression and sky-star cellular pattern (McGowan et al., 2012).

Plasmablastic Lymphoma shares comparable morphological characteristics with DLBCL, but plasmablastic lymphoma is a lesion of the oral cavity and several anatomical sites in HIV-positive patients that are positive to CD138 and negative to CD20 (Montes-Moreno et al., 2012). At the same time, DLBCL presents in various

body organs occasionally in HIV-positive patients, with negative interaction with CD138 and strongly positive to CD20 (Lopez and Abrisqueta, 2018).

17.7.2 Treatment Modalities of Diffuse Large B cell Lymphoma

The fundamental objective of treating Diffuse Large B cell Lymphoma is to eradicate the tumor, preserve the function of the organs, reduce the side effects of the therapeutic agents, and, lastly, avoid recurrence or metastasis of cancer (Kwak, 2012). Furthermore, the quality of life of DLBCL patients is of enormous importance and should be considered when planning the treatment course of affected patients (Tam et al., 2019) (Maziarz et al., 2020). The solo or combination of Chemotherapy and Radiotherapy is currently the most utilized treatment modality for DLBCL (Kubuschok et al., 2015). However, scientists have introduced innovative patterns that tackle individualized cases by what is referred to as precision-based medicine, i.e., immunotherapy and molecular gene therapeutic modalities (Coccaro et al., 2020).

Currently, in Sudan, all patients with DLBCL undergo a single treatment modality which is the RCHOP chemotherapy regimen. Ann Arbor staging and general epidemiological parameters are utilized to decide on the treatment plan and predict the prognosis of Diffuse Large B cell Lymphoma in Sudan. These parameters are insufficient for patients' stratification and precise treatment.

17.7.2.1 R-CHOP Chemotherapy treatment regimen

Composition and mode of administration

Diffuse Large B cell Lymphoma is a rapid form of cancer with a fast progression rate; typically, it requires urgent treatment (Costa et al., 2017). Chemotherapy involves utilizing cytotoxic medications that have the potential and competence to stop the spread of malignant cells (Dirani et al., 2020). This regimen is usually attained via

administering specified doses of chemotherapeutic agents throughout the cancer patient's bloodstream (Hong et al., 2016).

The most widely used treatment regimen is a combination of chemotherapeutic agents with or without radiotherapy. The combination of chemotherapeutic drugs used in treating Diffuse Large B cell Lymphoma is currently recognized as (R-CHOP) regimen (R=Rituximab, C=Cyclophosphamide, H=Doxorubicin Hydrochloride, O= Vincristine Sulfate Oncovin, and P=Prednisone). Rituximab is a chimeric monoclonal protein that targets the CD20 antibody; it is located on the surface of B lymphocytes. The binding of Rituximab and CD20 triggers the immune system resulting in the death of malignant B cells (Subramanian et al., 2017) (Weiner, 2010).

Cyclophosphamide is an immune suppresser chemotherapeutic agent composed of nitrogen mustard (Ahlmann and Hempel, 2016). Its action is via inhibition of protein synthesis through binding metabolites to DNA resulting in its alkylation; consequently, this prevents cells from copying DNA, promoting cell death (Mills et al., 2019). Hydroxydaunomycin is an anthracycline antitumor antibiotic; it is extracted from Streptomyces peucetius caesius bacterium and has an antineoplastic activity (Rabbani et al., 2005). The molecular action of Hydroxydaunomycin is described as an interference with the transcription and replication of DNA, resulting in the apoptosis of malignant cells (Clozel et al., 2013).

Oncovin or Vincristine is an agent affiliated with the vinca alkaloids organic family, an extract of a plant source known as 'periwinkle' composed of several chemical elements, including hydrogen, nitrogen, oxygen, and carbon (Zhou et al., 2019). The mechanism of action of this drug involves its role in the inhibition of the formation of mini tubules. This action seizes cell division of malignant cells via disrupting cellular

mitotic phases (Martino et al., 2018). Moreover, vincristine interferes with the synthesis of the nucleic acid of the cancer cells' DNA via inhibition of nuclear utilization of glutamic acid (Beaver, 2018).

Prednisone is an anti-inflammatory corticosteroid used in combination with the above-mentioned therapeutic agents in treating Diffuse large B cell lymphoma for its capability and potential to decrease the toxic effects of the first cycles of chemotherapy (Lakshmaiah and Asati, 2018). These side-effects may include episodes of high-grade fever accompanied by neutropenia (Choi and Jeong, 2014) and a decline in the patient's capability of performing daily activities (Bowcock et al., 2012). In specific T-cell-related cancers, Prednisone promoted programmed death of malignant tumor cells (Xing et al., 2015).

Clinical Guidelines for Management of Diffuse Large B cell Lymphoma:

The R-CHOP course of therapy is usually administered in twenty-one-day cycles (once/twenty-one days) with six cycles as an average round of treatment. The mode of administration is usually an infusion of rituximab over some hours on the first day of the treatment regimen, while the other chemotherapeutic CHOP agents are administered the following day (Messmer et al., 2019).

The length and choice of therapeutic agents are subjected to numerous factors (van der Poel et al., 2015):

Patient-Related Factors:

- Demographics (age, occupation, residential area, and socioeconomic status).
- Overall health status, i.e. (medical comorbidities, patient's tolerance to treatment, and compliance).

Tumor Related Factors

- Stage of the tumor: which is determined by the site of the malignant cells, stage I DLBCL involves a single lymph node region, of a single extranodal site, stage II involves two or more lymph nodes on the same side of the diaphragm, with or without localized involvement of an extranodal site, in such cases three to four cycles every fourteen days followed by radiotherapy are generally the treatment of choice.
- The subtype of the DLBCL cancer: occasionally, in some types of DLBCL like HIV-related Diffuse large B cell Lymphoma, a supplementary chemotherapeutic agent known as etoposide, is adjoined to the R-CHOP regimen; hence, the regimen is termed as R-CHOEP, which is given as an uninterrupted infusion over four days of treatment.

- Gene expression profile of DLBCL: researches have shown that the ABC subtype of DLBCL is more receptive to the ibrutinib chemotherapeutic agent than the GCB phenotype (Nowakowski and Czuczman, 2015). This finding is of significant significance considering that the ABC phenotype responds poorly to the typical R-CHOP regimen.
- Individual risk stratification profile: An international prognostic index is a tool that is used for the prediction of survival outcomes in DLBCL; it includes three elements the performance status of the patient, the level of the LDH enzyme, and the Ann Arbor stage (Ruppert et al., 2020).

Globally, various guidelines for treating Diffuse Large B cell Lymphoma have been proposed to help oncologists precisely and thoroughly follow a treatment plan according to each patient's characteristics to reach an optimal survival

outcome (Wong Doo et al., 2019). The National Comprehensive Cancer Network (NCCN) has specified comprehensive guidelines for diagnosing and treating Diffuse Large B cell Lymphoma patients (Pluchino and D'Amico, 2020). The British Society for Haematology has precise, detailed clinical guidelines for managing the various subtypes of Diffuse Large B cell Lymphoma (Chaganti et al., 2016).

Specific considerations should be considered regarding patients who suffer from immunosuppressed conditions, i.e., HIV and Hepatitis B virus. HIV patients should receive the same therapeutic regimens as HIV-negative patients in conjunction with anti-viral therapy (Coutinho et al., 2014). In contrast, HepB patients are at risk of viral reactivation during chemotherapeutic treatment. Hence, prophylactic medications should be administered along with the standard DLBCL treatment, and in case of reactivation of Hepatitis B virus, antiviral drugs should be prescribed (Seto et al., 2014).

Common Subsequent side-effects of R-Chop regimen:

R-CHOP chemotherapeutic regimen has various well-documented adverse undesirable effects in cancer patients, which may vary in intensity according to the dose, duration, and infusion rates of the administered agents. Some of which are:

- *Physical alterations*: i.e., hair loss from the scalp, eyebrows, and other body areas, yet, it is of a temporary nature of failure as it grows back upon completion of the regimen cycles (Paus et al., 2013).
- *Frequent episodes of nausea and vomiting* are characterized by a rapid onset after the second treatment cycle and continue a few days after the

chemotherapy session (Takahashi et al., 2016). In aggressive chemotherapy sessions, the patients might suffer from (CINV). Chemotherapy-induced nausea and vomiting, a condition in which the patient under treatment senses a subjective feeling of the disturbed stomach in the epigastric area joined with a need to expulse the stomach contents orally (Rapoport, 2017). The pathophysiology of CINV involves the aggressive chemotherapeutic agents which generate free particles that stimulate enterochromaffin cells in the GIT, resulting in the release of serotonin which binds to the vagal afferent nerve fibers of the intestine through specific receptors known as 5-HT3, triggering the reflex of vomiting (Janelsins et al., 2013)

- *Gastrointestinal disturbances*: Chemotherapy-induced Diarrhea has been directly linked to high doses of chemotherapy (Verstappen et al., 2003). Patients who receive aggressive forms of chemotherapy and have CAD face enormous adverse effects concerning their treatment course (Shafi and Bresalier, 2010); 60% of these patients have their chemotherapy regimen modified, while the dose of 22% of cancer patients is reduced. Furthermore, the treatment course of 15% of patients is terminated consequent to CAD (Dranitsaris et al., 2005). Chemotherapy-associated Diarrhea in its severe form is associated with a form of cachexia, in which the patients suffer from malnutrition and weight loss (Lanic et al., 2013) (Go et al., 2019). Chemotherapy Induces Constipation is clinically defined as reduced regularity of bowel excretion movement accompanied by harder stool consistency in cancer patients receiving chemotherapy (Izumi, 2014). The prevalence of constipation is estimated to be between 50–87% in patients suffering from malignancies (Abernethy et al., 2009), while 16% of patients treated via

chemotherapy suffer from constipation (Wang et al., 2019). A 2017 study found that 40.8% of patients treated via the R-CHOP regimen suffered constipation (Hayashi et al., 2017). The vincristine chemotherapeutic agent, in addition to opioids prescribed for cancer patients for pain relief, was also found to cause variable degrees of constipation during the treatment course (McQuade et al., 2016).

- *Oral infections*: Several risk factors have been shown to increase the susceptibility to oral infections during cancer treatment. The type of cancer; patients diagnosed with hematological malignancies like Lymphomas have a higher risk than other patients due to their cancer-associated myelosuppression (Kusiak et al., 2020) (Hiyama et al., 2015). One-third of Non-Hodgkins Lymphoma experience oral ulcerative mucositis at some stage in treatment (Mansour et al., 2019). The incidence of oral mucositis amongst younger cancer patients was higher than among elder ones undergoing the same treatment regimen (Miller et al., 2012). Moreover, epithelial growth factors were shown to have a greater quantification in younger cancer patients, which helps them improve faster than their counterparts (Park et al., 2016). The degree of oral care during cancer treatment therapy has been shown to significantly impact the outcome of their oral health.
- Additionally, Xerostomia before and during cancer treatment increases the risk for ulcerative mucositis; oral care protocol is advised before and during chemotherapy (Pai et al., 2019). Oral ulcerative mucositis typically occurs in the non keratinized mucosa of the lips, cheeks, soft palate, and the ventral surface of the tongue one week subsequent to the chemotherapy session. It takes around two weeks to heal, usually with no scar (Loo et al., 2013).

Specific chemotherapeutic agents were found to cause a higher level of stomatotoxicity, one of which is the vincristine part of the R-CHOP regimen (Madsen et al., 2019). Oral candidiasis is the most common oral disease in cancer-treated patients; studies have indicated the incidence of oral candidiasis to vary between 7 to 52% in cancer patients treated with chemotherapy and radiotherapy (Jayachandran et al., 2016). (HSV-1) Herpes simplex virus type 1 is the most common oral viral infection in patients undergoing chemotherapy in the head and neck region that could be primary or a reactivated latent virus infection (Valyi-Nagy et al., 2018). Bacterial infections, altered taste, and halitosis are frequent oral symptoms in chemotherapy-treated cancer patients (PAN et al., 2014).

- *Increased susceptibility to infection*: It has been shown that in 10–20% of lymphoma patients who were treated via R-CHOP regimen, an infection caused a neutropenic fever that affected their morbidity and mortality (Smith et al., 2015b, Nissen et al., 2014). Patients with lower immune response-related cancers, i.e., HIV-related DLBCL, were shown to have more susceptibility to develop infections during chemotherapy (Kanemasa et al., 2016). The suppression effect of the R-CHOP treatment regimen on B-cell lymphoma patients' immune response was shown to continue for more than two years (Ito et al., 2016). Additionally, mild upper and lower respiratory infections were shown to delay scheduled chemotherapeutic sessions among cancer patients. Clinicians are urged to prevent infections like influenza and laryngotracheitis in chemotherapy patients (Taha et al., 2015) (Rusu et al., 2018).

- *Bleeding and Embolism*: Cancer patients have a higher hazard of primary in addition to the recurrent form of venous thromboembolism (VTE) (Houghton et al., 2017). Furthermore, bleeding was a typical manifestation that is intensified by the frequent thrombocytopenia in patients diagnosed with hematologic cancers and treated with rigorous immunosuppressive chemotherapeutic regimens (Al-Samkari and Connors, 2019). It has been suggested that the temporary discontinuation of anticoagulants could reduce the adverse effect during thrombocytopenic episodes (Samuelson Bannow et al., 2017). Yet, a dilemma arises as contradictory; the suitable utilization of antithrombotic drugs can prevent the patient from developing VTE; however, this might considerably put the cancer patient at higher risk of bleeding. Hence this should be calculated thoroughly before the commencement of chemotherapy (Riondino and Ferroni, 2020).

17.7.2.2 Radiation and Immunotherapy

Radiotherapy treatment modalities

Radiation therapy is a well-established treatment modality widely used in oncology (Grass et al., 2019). The base of radiation therapy is based on the targeted use of high-energy X-rays or radioactive materials to stop the growth of malignant cells either via complete cellular damage or disruption of their mitotic activity (Gianfaldoni et al., 2017). Radiation is commonly segregated into two streams; the first line of radiotherapy is limited to the involved lymphatic tissue and is referred to as IFRT (involved-field radiation therapy) (Torka et al., 2020). Moreover, in cases of extranodal involvement, the radiation will target the original tumor site, where cancer

has primarily originated and hence termed EFR (extended field radiation) (Wirth et al., 2019).

Radiotherapy has given a superior treatment outcome in conjunction with the R-CHOP regimen in head and neck Diffuse Large B cell Lymphoma (Jeong et al., 2017). Radiation therapy is administered via a specified rays-producing machine. The beam of rays radiated contains ions, protons, and photons collectively known as external beam radiation (O'Steen et al., 2017). Radiotheraputic sessions are typically administered in a five days cycle. The interval of cycles, the dose, and the radiation target are determined prior to the treatment session by the oncologist according to the patient's status (Furlan et al., 2017).

The International Lymphoma Radiation Oncology Group has established guidelines for the use of radiotherapy in the different types of lymphoma to guide the dose and duration of radiotherapy administered to patients of hematological tumors and hence improve the outcome and quality of life of affected individuals (Mikhaeel et al., 2019).

The guidelines are updated frequently; the latest update was done in May 2020 to assess the risk of the COVID 19 virus on the frequency and administration of radiotherapy for hematological cancer patients (Yahalom et al., 2020). Innovative radiotherapeutic modalities are being developed, including intensity-modulated radiotherapy (Yoder et al., 2019) and dimensional image-guided radiotherapy (Shah et al., 2017). In these techniques, radiotherapy was successfully incorporated into DLBCL patients via contemporarily utilizing localized targeting of lymphoma cells by CT scans and radiotherapeutic modalities (Voltin et al., 2020). The resultant

targeted therapy is expected to significantly reduce the damage of unaffected healthy tissues yet still attain the desired onco-therapy (Goda et al., 2019).

Side effects of Radiotherapy

Radiotherapy-induced side effects unfavorably affect the quality of life of patients undergoing various degrees of onco-therapies. Patients undergoing radiotherapy treatment often suffer from different side effects (Dilalla et al., 2020).

- *Radiotherapy Induced Fatigue*: one of the most reported side effects is radiotherapy-induced fatigue (RIF) (Busson et al., 2019). It is characterized as a persistent, subjective feeling of weakness and over a continuous phase of time. This sense of physical and emotional exhaustion and distress is usually not proportionate to the patient's recent exerted efforts and delays daily personal activities (Hsiao et al., 2016). 80% of cancer patients of multiple tumor types indicated suffering from acute episodes of fatigue upon receiving radiotherapy sessions. In comparison, 30% of patients reported experiencing chronic fatigue for months and sometimes years subsequent to radiotherapy (Network, 2013).
- *Psychological Disorders:* Various studies have shown an increase in the prevalence of a range of psychological manifestations, i.e., anxiety, depression, and distress, in patients undergoing radiotherapy is globally increasing (Dilalla et al., 2020) (Pitman et al., 2018). The challenges facing the mental health of cancer patients are enormous; studies have shown substantial gaps in the services provided to cancer patients during stressful circumstances like highly contagious viruses, i.e., COVID-19 (Wang et al.,

2020b). In the Netherlands, mask anxiety was estimated to be between 16-24% between patients diagnosed with head and neck cancers and treated with radiotherapy (Nixon et al., 2018). Patients with Diffuse Large B cell lymphoma endured depression and anxiety and could not recover with time (Oerlemans et al., 2014). Unmet needs of cancer patients that appear after treatment were shown to have a significant association with the survival outcome and magnify stress and anxiety (Oberoi et al., 2017).

- *Radiotherapy-Induced oral manifestations:* Around 80% of patients diagnosed with cancers in the head and neck region undergo at least a single session of radiotherapy throughout their onco-therapy (Strojan et al., 2017). Radio therapeutic sessions targeted for the lymph nodes of the head and neck regions may result in various manifestations (Pinna et al., 2015). Xerostomia or mouth dryness usually presents in varying degrees according to the dose and duration of the radiotherapy; partial or complete damage of the salivary acini causes a marked decrease in the salivary flow below the normal range (Salum and Medella-Junior, 2018). Hyposalivation can be compensated via artificial salivary substitutes or by stimulating the dysfunctional gland by administering cholinergic drugs, i.e., pilocarpine (Ma et al., 2019). Alteration of taste was shown to occur in 70% of patients undergoing radiotherapy (Baharvand et al., 2013); it might arise as a partial or complete dysfunction; patients report this condition four weeks following radiotherapy and usually recovers within a month after the treatment is discontinued (Silva et al., 2016b).

  The hazard of dysphagia in patients being treated with high-dose radio therapeutic modalities is high, especially among those diagnosed with malignant tumors involving lymph nodes of the head and neck region (King et

al., 2016). The pathophysiology of radiotherapy-induced dysphagia involves the fibrosis of the muscles responsible for the swallowing action due to the exposure to the high radioactive x-rays, decreasing the nutritional intake of the cancer patient. If not compensated, this might lead to malnutrition or dehydration (Andrade et al., 2018) (Straub et al., 2015). The treatment of this condition is of a behavioral rehabilitation basis through speech-language muscular exercises (Greco et al., 2018) (Guillen-Sola et al., 2019).

- *GIT disturbances:* Around 50 to 80% percent of cancer patients under radio treatment sessions experience symptoms of nausea and vomiting during their treatment course (Feyer et al., 2014). The incidence of RINV depends on the dose, duration, and site of the radiotherapeutic x-rays; the prevalence ranged from 28 to 39% (Enblom et al., 2009) (Maranzano et al., 2010). It could interrupt the treatment course causing delay or complete discontinuation of the radiotherapy (Feyer et al., 2015). Since the quality of life of cancer patients under radiotherapy is enormously affected by induced nausea and vomiting (Kang et al., 2018), multiple international associations, i.e., European Society of Clinical Oncology (ESMO), Multinational Association for Supportive Care in Cancer (MASCC), and the American Society of Clinical Oncology (ASCO), have stated specific guidelines for the use of antiemetic medications and these guidelines are updates periodically upon introduction of new determinants (McKenzie et al., 2019).

Consequently, to improve the quality of life of DLBCL patients, it is of crucial importance to perform screening and risk assessment measures prior to the introduction of radio therapeutic modalities. Family physicians or general

practitioners can help oncologists determine patients' medical co-morbidities and urge patients to endorse healthy lifestyles.

Immunotherapy

Immunotherapy, referred to as immune-oncology, is a typical therapeutic regimen of lymphoma that explicitly targets, attaches, and destroys the antigens located at the surfaces of malignant cells, activating the body's immune system to kill fast-dividing cancer cells (Zhang et al., 2018). The most frequently utilized immunotherapeutic agents in the treatment of Diffuse Large B cell Lymphoma are monoclonal antibodies. These biological drugs are genetically modified via tissue engineering methods against malignant lymphoma cells (Singh et al., 2018).

Rituximab is the monoclonal antibody used in the immunotherapy of Diffuse Large B cell Lymphoma (Ayyappan and Maddocks, 2019). It has a human as well as a murine origin (Pierpont et al., 2018). It attaches to CD20 of malignant cells, and its primary function is to mark the cancer cells and might additionally have an adjuvant role in destroying these cells (Stewart et al., 2020) (Figure II.11.1).

Four antibody-dependent pathways were shown to describe the pathophysiological via which Rituximab binding to CD20 kills malignant cells (Abulayha et al., 2014). Such courses include cellular cytotoxicity, phagocytosis, complement-dependent cytotoxicity, and direct adverse effects on the tumor cells resulting in cellular apoptosis (Figure IV.17.9) (Gil'deeva et al., 2015). Rituximab is usually administered subcutaneously; its dose is determined based on the patient's histological characteristics, Ann Arbor staging, age, and co-morbidities (Salles et al., 2017).

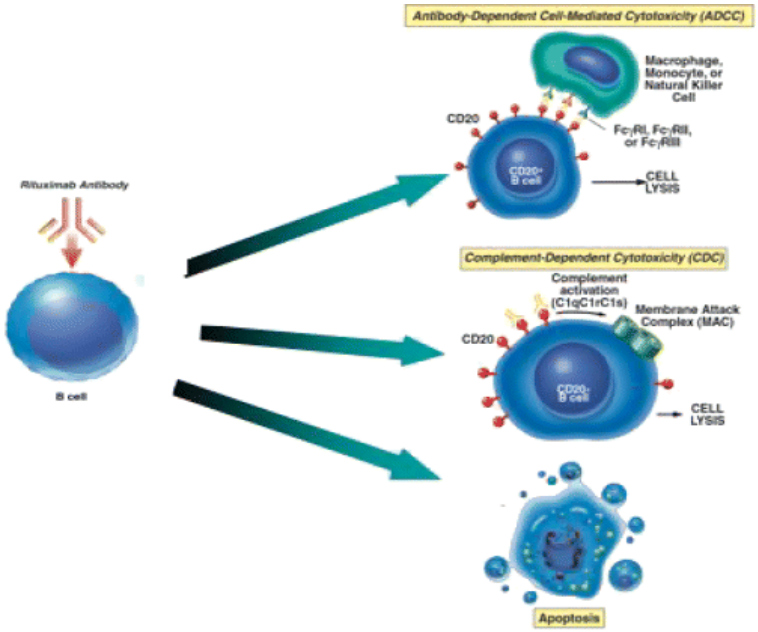


Figure IV.17.9 Mechanism of Action of Rituximab, Anti-CD20 Monoclonal Antibody (Pescovitz, 2006)

Monoclonal antibody immunotherapy has various side effects: allergic reactions, respiratory infections, GIT disturbances, i.e., nausea, vomiting, diarrhea, and fatigue (Matucci et al., 2016) (Castelli et al., 2019).

Furthermore, immunotherapy involves an additional regimen for treating Diffuse Large B cell Lymphoma termed immunomodulating drugs (Ayyappan and Maddocks, 2019). These drugs stimulate the cancer patient's immune system to prevent the mitotic activity of the tumor cells. Lenalidomide is a drug classified as an angiogenesis inhibitor and has shown to have a significant adjuvant effect on treating Diffuse Large B cell Lymphoma (Ma and Su, 2018).

17.7.2.3 Novel emerging therapeutics for Diffuse Large B cell Lymphoma

Stem Cell transplant

Stem cell transplantation in hematological cancer therapy replaces malignant cells and cells damaged as treatment sequelae with healthy ones (Zagozdzon and Golab, 2015). In regenerative cancer therapy, stem cells have distinctive biological behaviors; these include controlled self-renewal, targeted migratory potential, and the ability to differentiate into different cell lineage (Chu et al., 2020).

Regarding Diffuse Large B cell Lymphoma, the relapsed form of the tumor is recently being treated via Stem cell therapy; in this approach, the transplanted stem cells are directed to activate the production of healthy hematopoietic cells (Kondo, 2016). Prior to stem cell transplantation, DLBCL patients undergo heavy doses of chemotherapy and occasionally radiotherapy sessions; this is crucial to prepare the host tissues to decrease the chances of rejection (Gyurkocza and Sandmaier, 2014). The duration of this process is dependent upon the stage and condition of each individual cancer patient. It is usually performed in a hospital setting as patients end in an immune-compromised state with a higher risk of infection (Ullmann et al., 2016). The most important types of stem cells transplantation therapy are:

- Autologous hematopoietic stem cell transplantation: This type of stem cell transplant utilizes healthy hematopoietic stem cells from the patient's body tissues to replace the malignant and chemotherapy-damaged cells of the bone marrow (Zhang and Zhang, 2016) (Zahid et al., 2017). These cells are harvested, engineered, and infused into the cancer patient's bloodstream (Lekakis and Moskowitz, 2019). The procedure usually involves multiple steps that include taking prescribed medications that stimulate the bone

marrow to produce an additional number of stem cells in the blood to facilitate the collection process (González-Barca et al., 2020). Afterward, a process known as apheresis is performed by collecting the patient's own blood into a machine that filters or segregates the blood components via centrifugation. Consequently, the targeted stem cells are removed. The rest of the blood components are reinfused into the bloodstream, A preserving substance is added to the extracted stem cells, and subsequently, they are frozen to be used for later stages of the cancer therapy (Kiki, 2017). Afterward, patients of planned stem cell transplantation undergo aggressive chemotherapy and or radiotherapy sessions known as the conditioning stage; this step aims at killing malignant cells before receiving the stem cells (Eskian et al., 2018). Lastly, the cancer patient receives the cryopreserved stem cells, which will target the bone marrow and differentiate into new healthy blood cells in a process known as engraftment (Henig and Zuckerman, 2014).

- Allogeneic stem cell transplantation (allo-HSCT): Stem cells are harvested from a matching donor in this type of transplantation. The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) has specified multiple recommendations for matching criteria for selecting the graft donor prior to planning stem cell transplantation (Howard et al., 2015). All steps thoroughly explained in the autologous transplantation are followed in allogeneic transplantation, except for the stem cell source. One of the most common complications of this kind of transplantation is the susceptibility for rejection of the donated cells before they reach the bone marrow. The host immune system may attack newly infused stem cells resulting in their destruction (Masouridi-Levrat et al., 2016). Furthermore, a condition termed graft versus

host disease (GVHD) can result as sequelae of this specific kind of transplantation; in which defense cells from the graft tissues may destroy the healthy host cells, the skin, eyes, muscles, and liver are the most affected body organs (Kuba and Raida, 2018).

- Reduced-intensity transplantation: In this type, the chemotherapy regimen used for the conditioning preparatory step is usually less toxic and administered in lower doses than the dosages used in allogeneic transplantation (Klyuchnikov et al., 2015). It is the most suitable stem cell transplant for the elderly, immunocompromised, and medically co-morbid patients (Kanate et al., 2016).

CAR T-Cell Therapy

Chimeric antigen receptor (CAR) T-cell therapy is a new therapeutic pattern that targets CD19 and is directed towards managing lymphomas with poor outcomes. CD19 is a transmembrane glycoprotein expressed throughout the malignant alteration stages of B lymphocytes, and it acts as a regulator of their activation via the antigen-receptor-dependent method (Chavez and Locke, 2018). T-cells from the cancer patient are segregated from the other components of the blood; the gene is added to their surfaces, and they are reinfused into the bloodstream (El-Galaly et al., 2020). CART-Cell therapy is currently considered a costly method with limited products available for commercial utilization; future approaches are currently under investigation to widen the scope of the reactivity of CART therapy to additional cancer antigens to conquer any possible resistance to the therapy (Lin et al., 2019).

It is noteworthy that these innovative modalities are not available in Sudan, and DLBCL patients are all treated under the same chemotherapy regimen protocol with no personalized, targeted treatment options available.

CHAPTER 18: Cancer Registry in Sudan

18.1 Overview

The cancer registry is defined as a tool that facilitates the collection, stratification, storage, and analysis of the data of cancer patients (Parkin, 2008). Initially, registries were only utilized for the illustration of tumors patterns. This information allocation is the fundamental role of a population-based cancer registry that records the new cancer cases in a specific population.

Afterward, this limited function of the population-based cancer registry further advanced to provide epidemiological data for cancer research, including the etiology, subtypes, follow-up, and survival data (White et al., 2017). Cancer registries gather patients; information from several sources. The primary sources are archival records at hospitals, histopathology centers, and pathology departments at medical colleges (Curado, 2019).

Ideally, an individualized record should be linked throughout the health care facilities; this is crucial to avoid duplication of data registration. National identification numbers could have ideally been used in registries to attain the target of individualized records; nevertheless, in reality, various developing countries utilize patients' personal data, i.e., name, age, gender, or residence, for cancer registries (Gakunga and Parkin, 2015).

One of the significant challenges that impact the reliability of cancer registries is the quality of the data, which in return relies on various factors that include the collection methods, storage capacities, digitalization completeness, accuracy, and frequency of

the data entry, within the health care facilities (Parkin et al., 2001). Sufficient financial funds and adequately trained staff enable cancer registries to utilize patients' data suitably as cancer registries function with high economic costs (Tangka et al., 2016).

In Sudan, the pioneer national cancer registry was created in 1967 by the "International Union against Cancer" (Saeed et al., 2014). Cancer patients' data was collected via the Pathology Department at the Faculty of Medicine, University of Khartoum, and the central medical laboratory at that time, "Stack." Unfortunately, shortage or deficiency in funds led to registry stop in the early eighties (Elamin et al., 2015). In 2009, the Sudanese Ministry of Health established a population-based cancer registry project in Khartoum (Awadelkarim et al., 2012). This attempt started by using CANREG5 software, with specific variables for collection, including basic demographics of the cancer patient, i.e., name, gender, residential area, occupation, in addition to tumor-related parameters, i.e., the first date of diagnosis, cancer site, and histology. The registry utilized active and passive cases discovery from public governmental and private centers (Abuidri et al., 2009).

Unfortunately, with the emerging economic and political disturbances Sudan has been facing in the last few years, the project has failed to reflect the needed methodological objectives. The intermittent high-cost internet services and the shortage of trained personnel to run the program are some of the challenges that face such projects in Sudan (Elamin et al., 2015).

Consequently, the country still lacks the unified data collection method, and reports were randomly collected from histo-pathological centers and hospitals in Khartoum.

The lack of integrated digital entry software with global disease classification codes and patients' personal information stratification has affected the reliability of the collected data. Most hospitals and histo-pathological centers rely on manual data entry and storage; lack of residential postal system, and non-availability of death certificates, all these factors jeopardize cancer patients' records' quality, completeness, and accuracy**.**

Furthermore, the centralization of the health care facilities in the capital Khartoum has affected the number of detected cancer patients. Most preventive initiatives established by non-governmental organizations and the local community are situated inside Khartoum, disregarding the rest of the country (Fadlelmola, 2016).

The Radiation and Isotope Center in Khartoum, the center where we conducted this study, receives almost one thousand cases per month, according to the 2016 census. This number is rising, yet cancer care facilities are still unsatisfactory and underdeveloped (Saeed et al., 2016). Histopathology centers are few, with old and out-of-date equipment. The governmental hospitals fail to provide the needed diagnostic and therapeutic requirements of cancer patients. Furthermore, there are long waiting lists and inadequate high-quality radiographic scans (Suliman et al., 2015).

18.2 Confidentiality

Confidentiality is a crucial factor that we will adequately control at a considerable level of care. Laryea et al.(Laryea and Awittor, 2017) have described updated collective methods via which confidentiality of cancer patients could be ensured with particular regards to registering their personal and medical data. Hence, we stated

specific terms and conditions that shall be signed upon in each facility where the software will be installed:

- The staff of the registry unit should sign the confidentiality agreement that follows the guidelines of the international association of cancer registries.

- The agreement should state the terms and conditions for a third party's involvement in the data release.

- The agreement should clearly state the consequences of violation of any of the articles.

- The registrars should have no contact with patients during the registration process.

- Forms, computers, and patient data should be electronically guarded via passwords, and access should be restricted to registrars and unit directors.

18.3 Data Reliability

It's critical to assess the quality of cancer registry data because it can be used for various applications. Several researchers have looked into the extent to which registries can ascertain the completeness of data sets (Sirirungreung et al., 2018). The validity of cancer registry data has rarely been investigated.

To ensure the reliability of the data collected via the software we developed in this project, we suggest using the re-abstracting method, widely used for reliability check purposes in scientific research (Suwanrungruang et al., 2011) (Peres et al., 2016). This

method involves the selective retrieval of medical records using the probability proportional to size method. Then the records are usually stratified by specific elements, e.g., gender and registered year, and then matched with registry data. Subsequently, the match rate will be statistically computed and calculated to signify the actual data reliability (Chiang et al., 2015).

To conclude, we expect that this registry software will enable researchers in Sudan to obtain accurate and complete cancer data that may be utilized for cancer control and epidemiological research and public health program planning and patient care enhancement. All of these actions, in the end, shall help to minimize the burden of cancer in Sudan.

CHAPTER 19: Limitations of the study

Although this project was confined to Diffuse Large B cell Lymphoma patients who attended Radiation and Isotope Center in Khartoum, this institution is the principal tumor and histo-pathological center in Sudan. RICK receives patients from all over Sudan; this was reflected in the broad range of the study participants' demographics. Therefore, despite the fact the study was conducted in RICK solely, the observations are reasonably representative of Diffuse Large B cell Lymphoma patients across the country.

While this study was a pioneer project to investigate the diagnostic and the prognostic values of specific biomarkers on the survival rates of DLBCL Sudanese patients; it is vital to acknowledge the probable effect of lack of population life-tables and death record, in addition to the massive impact of the absence of a reliable national tumor registry.

Numerous challenges were faced during the data collection phase. As a result of the inadequacy of the patients' records which were stored manually at the RICK archival room, most of the retrospective data concerned with patients' co-morbidities were unavailable or lost. Moreover, the inferior health care system dynamics hindered the accurate acquisition of the follow-up information of Diffuse Large B cell lymphoma patients. There was no linked network between local primary health care centers and central histo-pathological centers.

To conquer these challenges, we had to personally call the patients and their families to obtain the information of lost patients to follow up and confirm the cause and date of death. One of the significant aspects missing in the collected data sets was the socioeconomic status of DLBCL patients. This information was missing since Sudan

lacks a solid socioeconomic stratification system; nevertheless, RICK provides minimal to free medical care, and therefore its attendees are generally from the lower socioeconomic population. Another aspect that could have probably provided us with some interesting data is the nutritional status of DLBCL patients; unfortunately, it was merely unavailable.

With regard to the molecular analysis of the biomarkers, the oncogenic dosage was done for one of the oncogenes (MYC), while quantification of translocation was done for the other (BCL2); this is due to constraints with regards to finances.

Lastly, the global pandemic of COVID 19 has drastically hit the health care sector in Sudan, with a marked high case fatality rate (Altayb et al., 2020). Sudan depends mainly on imported drugs; hence, cancer patients have suffered a tremendous delay in chemotherapeutic cycles in addition to massive shortages in laboratory investigations, as most laboratories experienced deficiency in imported chemical reagents (Lucero-Prisno III et al., 2020). The high infection rate of Covid within health workers, including doctors, nurses, and laboratory technicians with significant insufficiency in personal protective equipment (PPE) and hygiene measures, has led to a massive strike among health workers (Elamin et al., 2020). Furthermore, the nationwide lockdown has tremendously affected the ability of the patients to reach RICK as gatherings and public transport was halted. Consequently, this has affected the data collection, selection, and utilization of molecular techniques, i.e., most Next Generation Sequencing laboratories were closed consequent to the lockdown.

SECTION V: Conclusions and Recommendations

CHAPTER 20

CHAPTER 21

Preamble

This section includes Chapters 20 and 21. Information portrayed in these two chapters is concluding remarks and propose suggestions for further investigations. Moreover, the future publications are listed in Chapter 22.

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21.6 Encouragement of the use of immunohistochemical and molecular techniques for detection of MYC and BCL2 molecules in DLBCL patients

21.7 Future work and upcoming potential research

21.8 Implementation and prioritizing preparedness over response

21.9 Dissemination of the study findings via various scientific platforms

CHAPTER 20: Conclusions

20.1 Conclusions Based on the study findings

The author concludes that the use of specific biomarkers MYC and BCL2 as diagnostics and prognostic determinants of Diffuse Large B cell Lymphoma in Sudan represented in patients attending RICK over a period from 2013-2018. Pragmatic steps are also suggested to utilize opportunities to improve the diagnostic potential and outcomes for patients diagnosed with DLBCL.

The key findings of this project can be summarized as follows:

- This study covered five years from 2013 till 2018 retrospectively. Hundred and fifty-one samples had complete clinical and archival files in the Radiation and Isotope Center in Khartoum. Only one-third of the participants were habitants of Khartoum (31.13%), while almost two-thirds were from rural areas seeking health care services at RICK. The mean age of patients at first presentation was 57 years. Males had a higher prevalence of Diffuse Large B cell Lymphoma with a percentage of 56.95%. The most common lesion sites were cervical lymph nodes and submandibular lymph nodes (31.13%, 13.25%), respectively.

- This study highlighted the substantial role of MYC and BCL2 biomarkers in the diagnosis and prognosis of Diffuse Large B cell Lymphoma in Sudan; nevertheless, the impact of other factors involved cannot be overlooked. Therefore, further research projects are required to explore the impact of other elements such as viruses (i.e., HIV), diet, and the surrounding environment in the etiology and prognosis of DLBCL.

- Surprisingly, the self-referral pathway dominated this study, with over 50 percent of the patients approaching RICK by themselves. The governmental section contributed only to almost 30 percent of the referral procedure (31.79%). Whether the dental sector has contributed to these referral pathways is to be investigated in upcoming studies.

- A finding of note in this project was that the majority of the study participants presented at late stages of the tumor, stage III (16.56%) and stage IV (46.36%). Moreover, Double Expresser Lymphoma DEL with simultaneous high co-expression of MYC and BCL2 biomarkers was detected in (83.44%) of the study samples, with (63.58%) of the participants indicating high levels of Lactose dehydrogenase enzyme.

- The significant diagnostic delay in seeking treatment encountered by DLBCL patients, which is late mainly driven by patient delay and to a lesser extent by professional delay, can be related to this exceedingly late presentation. The three main reasons for the patient delay were lack of awareness, limited financial resources, and long distances from the capital Khartoum. Due to a shortage of understanding of Diffuse Large B cell Lymphoma, many patients misread their symptoms. Many patients felt they were suffering from a mild illness that would not significantly affect their daily activities and tried to self-treat themselves via herbs, over-the-counter antibiotics, and painkillers without being advised by proper physicians.

- It is critical to increase public knowledge and awareness about Diffuse Large B cell Lymphoma and emphasize the necessity of early detection to enhance the treatment and survival of the disease. Patients have to bear all financial costs related to the disease diagnosis and treatment as currently, not even the Radiation and Isotope Center provides complete free health care coverage. Several patients opt out of therapy since they face challenging circumstances in which their families are forced into poverty due to health care requirements.

- The distance from the capital Khartoum influences healthcare utilization; the geographical distribution of the population is affected by political instabilities, poverty, and unfavorable weather circumstances, e.g., drought. Hence, two-thirds of Sudanese reside in rural areas where the health care facilities and the number of trained personnel are suboptimal. Thus, families have to burden additional accommodation costs when seeking help in distant Khartoum.

- Moreover, in this study, some of the diagnostic obstacles that resulted in a considerable diagnostic delay of Diffuse Large B cell Lymphoma were highlighted. In Sudan, most primary health care centers in rural areas depend mainly on new graduates who have minimal experience in diagnosing complex malignancies like DLBCL. Consequently, patients lose a substantial amount of time following the correct path for diagnosis and treatment, especially in the absence of an established referral system.

- Given the late stages of malignancy at the time of patient presentation indicated in this study, it is unsurprising that the survival rates among Sudanese Diffuse Large B cell patients were alarmingly low. The overall 5-

year survival rate dropped from 98.7% in the first year of the diagnosis to 19% in the fifth year.

- Various elements investigated in the study negatively impacted the disease-specific survival of Diffuse Large B cell Lymphoma; in this study, 84 out of 151 samples died within the course of the disease (55.63%); The advanced stage of the malignancy had a negative impact on the disease-specific survival as 87.14% of stage IV patients were dead at the time of the study. Double Expresser Lymphoma, in which patients experienced high dual expression of MYC and BCL2 molecules, exhibited a 63.49% death rate within the study samples which showed positive DEL diagnosis. Cervical lymph nodes were the site lesion that experienced most dead patients as out of 47 samples; cervical lymph nodes were the lesion site, 28 were dead, constituting 59.57%. A high level of Lactose dehydrogenase enzyme negatively affected the survival of DLBCL as 76 out of 96 'High' LDH patients died, constituting 79.17%.

- Evaluating the expression levels of MYC and BCL2 biomarkers using immunohistochemistry and quantitative PCR techniques has provided insight into their biological interactions and assisted in studying their impact on the disease-specific survival and various clinicopathological factors of Diffuse Large B cell lymphoma. In this project, simultaneous detection of high co-expression of MYC and BCL2 biomarkers illustrated as positive Double Expresser Lymphoma subtype of DLBCL independently predicted an inferior disease-specific survival. This result suggests that detecting MYC and BCL2 biomarkers in different tumor compartments could serve as the foundation for creating a valuable tool for patient classification and target-based treatment.

- A comprehensive molecular analysis of MYC and BCL2 oncogenes rearrangements is shown to have a beneficial impact on the diagnosis of Diffuse Large B cell Lymphoma and should be performed to recognize phenotypic populations of DLBCL, in addition to paving the path to an enhanced understanding of the pathogenesis and target treatment of DLBCL.

20.2 Future Publications

1. Clinicopathological features and concurrent expression of MYC and BCL2 as diagnostic and prognostic keys in Diffuse Large B cell Lymphoma in a cohort of Sudanese patients.
2. Double Expresser Lymphoma in Sudan, clinicopathological characteristics, survival rates, and determinants of prognosis.
3. Correlation between the relative expression level of messenger RNA and protein expression level of MYC and BCL2 oncogenes in Diffuse Large B cell Lymphoma.
4. Detection of Chromosomal Translocations in BCL2 oncogene in Diffuse Large B cell Lymphoma via Quantitative Real-Time PCR in Sudanese Patients.
5. Concomitant overexpression of MYC and BCL-2 oncogenes predicts inferior prognosis and lower survival rates of Diffuse Large B-Cell Lymphoma in Sudan.
6. Disease-Specific Survival among Diffuse Large B cell Lymphoma patients in Sudan.
7. Validation of MYC Gene Dosage Quantification in Diffuse Large B cell Lymphoma in Sudan.

8. Specification of Tumor Registry Software as a foundation to establishment of population-based registry in Sudan

CHAPTER 21: Recommendations

In light of the findings of this project, our suggested recommendations are divided into several categories:

i. Raising awareness about Diffuse Large B cell Lymphoma.

ii. Enhancing access to healthcare and establishing a more medical-oriented referral pathway.

iii. Digitalizing the storage of patients' files and medical records

iv. Developing relationships with stakeholders, policymakers, and strategic medical planners.

v. Resolving unanswered questions via research,

vi. Promoting strategies that ensure preparedness over response.

21.1 Increasing Public and Professional Awareness about Diffuse Large B cell Lymphoma

In order to raise public and professional knowledge and awareness of Diffuse Large B cell Lymphoma, an intense concerted effort is needed at both the regional and national levels. The objectives of nationwide public health campaigns should emphasize the following: (i) the importance of early detection in lowering morbidity and death; (ii) enhancing the health-seeking behavior of the lay population in terms of proper medical channels to reach, correct misconceptions, decrease social stigma and direct the public for the proper medical care path.

At the public level, the following recommendations are suggested:

A) Early indications and symptoms of Diffuse Large B cell Lymphoma and the benefits of early detection must be emphasized in public health education programs. It's critical to highlight the link between DLBCL and known risk factors. Messages to the rural community should be delivered via social centers, mosques, schools, and local radio stations. Native community leaders, mosque imams, and celebrities endorsing the campaign would provide extra weight to the campaign's vital messages. Moreover, the value of a yearly oral health checkup as an opportunistic screening tool for Diffuse Large B cell Lymphoma must be made known to the public.

B) To improve the health-seeking behavior of the targeted population, the help of traditional healers and alternative medicine practitioners should be utilized as an integral role in promoting awareness at the community level, as patients frequently seek their counsel first. They must be formally educated and certified on recognizing malignant lesions and refer patients in a timely and effective manner. This could be achieved by providing these healers with incentives to encourage them to participate in the early detection process.

C) Th author advocates establishing early detection and screening efforts to identify people at high risk of DLBCL and raise their awareness of the disease. These initiatives should be carried out in coordination with the Ministry of Health and with the help of medical and dental students at governmental and private sectors, who frequently organize field tours and programs to remote parts of Sudan to deliver free medical and dental services.

At the professional level, the following suggestions are proposed:

A) Integrating the roles of The Ministry of Health, The Sudanese Medical Specialties Board, and the medical and dental colleges in adding the diagnosis of malignancies, i.e., DLBCL, to undergraduate and postgraduate programs' curriculum in theory and practice. Dentists should be trained to examine patients extensively, especially lymph nodes examinations, and regularly assess any changes in size, shape, or mobility. They should seek advanced help from their seniors for further definitive diagnosis.

B) Furthermore, relevant professional organizations such as the Federal Ministry of Health, Oral Health associations, and the Sudanese Dental Association could significantly impact recognizing and supporting dentists' and physicians' roles in early detection of DLBCL. They can also urge more dentists to get involved in community health by cooperating with local non-governmental organizations such as Basamat Organization to support cancer patients.

21.2 Expanding Access to Health Care and establishing a comprehensible Referral Pathway

The substantial diagnosis delay observed in this study was attributed primarily to patient-related reasons, such as a lack of understanding of the signs and symptoms of DLBCL, financial constraints, and distance from healthcare centers in the capital. The failure of the healthcare professional to detect the true nature of the lesion and coordinate a timely and suitable referral caused the professional delay.

Healthcare availability varies greatly depending on geographic population distribution and socioeconomic variations. Policymakers need to address these disparities and strategically plan toward equity within the health care system.

We encourage the creation of a straightforward, concise, and accelerated referral system for cancer cases. A key issue is the lack of an organized referral channel, which leads to indirect referral and significant delays. Hence, As a result, we advocate forming the DLBCL network and screening clinics throughout the country's primary care centers.

Front-line medical employees, such as dentists, hygienists, assistants, or medical interns, should be adequately trained in screening patients with suspicious lesions in these centers. Afterward, they should refer suspected patients to Radiation and Isotope Center in Khartoum or the closest tertiary care center for specialists' comprehensive diagnostic and treatment plans.

21.3 Development of a National Cancer Registry

Throughout the course of this project, the researcher experienced tremendous difficulty in obtaining complete clinical and epidemiological data of DLBCL patients; this is attributed to the manual storage and archival of patients' files, which resulted in significant loss of essential elements of many patients' files and hence had to be excluded from the study. Customized software was developed for cancer cases registration, and this software included elements that data collectors easily identified.

Under the auspices of the Ministry of Health, it is recommended the software is distributed and implemented throughout hospitals and histopathological centers in

addition to private clinics. The widespread of the software will enable consistent time-framed data standardized collection of DLBCL and other malignancies, consequently enabling well stored epidemiological and clinical data which will be digitally entered, stratified, stored, and utilized.

21.4 Utilizing an Integrated Multi-faceted Approach to the management of Diffuse Large B cell Lymphoma

DLBCL has received minimal attention from Sudan's government and health stakeholders, and there are only two centers in the country that diagnose and treat such malignancy throughout Sudan.

Consequently, comprehensive centers with professional medical, social and psychological support teams for the overall management of DLBCL are urgently needed. Such centers would allow DLBCL patients to have coordinated management that covers the various aspect of treatment, i.e., surgical, radiological, psychological, and palliative care. If provided concurrently or at different stages throughout the treatment course, these services would probably result in a more effective, cost-effective therapy.

Furthermore, it's critical to collaborate with the Sudan Medical Specialization Board and dental colleges to incorporate clinical training programs in diagnostic and treatment procedures and palliative care for incoming postgraduate dental trainees. It is also recommended that medical laboratory and nursing staff receive intensive training as a crucial part of the DLBCL management team, as the diagnosis of DLBCL requires previously elaborated sensitive procedures like biopsy sample collection, IHC staining, and advanced molecular techniques.

Patients in RICK must currently wait for treatment sessions for prolonged periods, owing to the lack of sufficient radiation therapy units. To achieve the optimum outcome of radiation therapy, sessions should be administered in pre-scheduled time, and treatment providers should follow the updated guidelines. This would give the patients ample time to plan for their treatment journey and increase their compliance drastically. Therefore, it is recommended to raise funds via governmental, non-governmental, and private sectors to aid DLBCL patients gain the aspired treatment outcome via providing up-to-date three and four-dimensional radiotherapy and medical equipment.

The impact of the easing of the sanctions set by the United States of America on Sudan's healthcare sector has yet to be seen. Because Sudan is still on the embargo list, most foreign corporations have strict prohibitions against selling to Sudan; these sanctions are in the process of lifting. Nevertheless, more time is needed to substantially affect the availability of modern, up-to-date medical equipment in hospitals and histopathological centers all over the country.

Diffuse Large B cell Lymphoma prognosis was suboptimal and considered inferior, even with patients being treated with chemotherapeutic agents. It is noteworthy that patients who undergo chemotherapy sessions at RICK are being discharged promptly after their scheduled sessions, without consequent monitoring for subsequent side effects, with a growing probability of facing post-therapeutic side effects outside the hospital setting.

Consequently, the establishment of specific guidelines for assessing DLBCL patients' wellbeing and conditions is urged, such as (vital signs and physical examination) prior

to and throughout the chemotherapy sessions. Any post-therapy complications should be dealt with in a professional, prompt manner.

21.5 Development of Connections with Legislators, Stakeholders, and Policy-makers in Sudan

Developing effective, well-established, and mutually beneficial connections with legislators, stakeholders, and policy-makers are recommended to improve DLBCL prevention's future. This is suggested via:

- The development of a well-structured tumor registry at the national level via the implementation of digital data collection throughout the country's states. This study developed an updated customized software that considers specific elements unique for the Sudanese population.
- To attract attention to early detection programs and routine screening initiatives, especially among high-risk populations, and direct legislators to prioritize efforts for such programs. Moreover, the adoption and equal distribution of low-cost non-invasive traditional visual and digital examination and screening technologies are highly recommended throughout Sudan's primary health care centers.
- Furthermore, to encourage the government and health care sector to devote more resources to raising awareness about DLBCL and its hazardous risk factors.

21.6 Encouragement of the Use of Immunohistochemical and Molecular Techniques for Detection of MYC and BCL2 molecules in DLBCL Patients

Combining immunohistochemical and molecular methods is used to detect the expression rates of MYC and BCL2 biomolecules and the subsequent subclassification of Diffuse Large B cell Lymphoma cases into Double Expresser Lymphoma subtype did give an insight into their co-heterogeneity and their impact on the survival of Diffuse Large B cell Lymphoma.

The double detection of these specific biomarkers will have a considerable benefit and cost-effectiveness for the patients. It would classify the highest-risk subset of patients who may experience a relapse subsequent to conventional treatment. Such patients may take advantage of intensified chemotherapy regimens or may at least be enrolled in clinical trials exploring novel regimens, which will be reflected in the outcome of the disease (Sesques and Johnson, 2017b). Moreover, it is recommended to enrich the scientific research via advanced molecular diagnostic techniques like Next Generation Sequencing to explore further the genetic pathogenic determinants of Diffuse Large B cell Lymphoma in Sudan.

Hence, multiple pharmacological approaches to target MYC oncogene at various levels indirectly are suggested, such as activation, transcription, translation, and stabilization. These methods should be translated into a strategy for future patient care, as patients with MYC deregulation are likely to respond well to targeted therapy.

21.7 Future Work and Upcoming Potential Research

To facilitate epidemiological studies about Diffuse Large B cell Lymphoma in Sudan, establishing a DLBCL research center is considered crucial and highly recommended. This center should have direct uninterrupted reciprocal collaboration channels with

histopathological and treatment facilities. DLBCL patients' epidemiological, clinical, therapeutic, and follow-up data should be transferred into the research center under standardized protocols.

Throughout our project, several research areas raised potential questions that need to be further investigated; hence, proposed upcoming future studies are:

- A national cross-sectional study throughout the eighteen states of Sudan and the hundred and eighty-five localities to determine the exact incidence and prevalence of Diffuse Large B cell Lymphoma.
- Investigating the risk factors involved in the etiology of Diffuse Large B Cell Lymphoma, i.e., viruses (HIV, HPV), nutritional status and dietary components, agriculture-related substances like fertilizers, and chemical materials.
- Prospective long-term follow-up survival studies to assess ten years survival rates in addition to considering recurrence rates and any related contributing factors.
- Establishing a protocol for thorough clinical evaluation and assessing patients' conditions before and after chemotherapeutic sessions. Moreover, comprehensive studies are needed to evaluate the effectiveness of the currently available chemotherapy agents in Sudan.

21.8 Implementation and Prioritizing Preparedness over Response

The quality of community response to a particular emergency, i.e., epidemics, depends significantly on its level of preparedness. In Sudan, preparedness relates to the preparations taken to enable the health care sector to effectively respond in case

of a disaster event, including continuity of treatment sessions and minimizing the damage on vulnerable cancer patients. The current COVID-19 pandemic illustrated the crucial need for communities to be ready for unexpected situations such as a sudden medical surge or an unexpected lockdown.

Amplifying preparedness of the health care sector via developing disaster plans is strongly recommended, identifying potential response teams, and creating strategic partnerships in order to speed up life-saving assistance necessary in the first stages of an emergency, with particular regard to cancer patients as the timing of diagnosis and treatment has proved to play an essential role in the outcome of the treatment.

Furthermore, the Ministry of Health is encouraged to promote institutional knowledge sharing capacities to impact the quality of response to large-scale emergencies significantly, including COVID-19. This could be achieved via:

A) Strengthening the capacity of community healthcare workers and healthcare providers.

B) Supporting the timely response to disease outbreaks and ensuring adequate mental and psychosocial support, especially for cancer patients.

C) Encouraging the government to make funding commitments towards cancer treatment to avoid complex impacts of limited finances and prioritize preparedness over the response as examining lockdown consequences worldwide, prevention is undoubtedly better than cure.

21.9 Dissemination of the Study Findings via Various Scientific Platforms

The author plans to disseminate, distribute and publicize the findings of this scientific product via diverse scientific gatherings and platforms, i.e., national and international

congresses, articles, Congress of Africa, and publications in well reputed high-impact factors ISI scientific journals.

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SECTION VII: Appendices

Appendix 1: Ethical approval from the Ministry of Health- Sudan

Appendix 2: Ethical Approval from the University of the Western Cape (2019- 2020)

Appendix 3: Ethical Approval from the University of the Western Cape (2019- 2023)

Appendix 4: Ethical Approval from the University of the Western Cape (2021- 2024)

Appendix 5: Radiation and Isotope Center Consent

Appendix 6: Data Entry Sheet

Appendix 7: Consent for Publication

Appendix 8: Information Sheet

Appendix 9: Institution Consent

Appendix 10: Data Management Plan (UWC)

Appendix 1: Ethical approval from the Ministry of Health- Sudan

Republic of Sudan
Federal Ministry of Health

HEALTH RESEARCH COUNCIL

NATIONAL RESEARCH ETHICS REVIEW COMMITTEE

Date: 7/11/2018

Ethical Clearance Certificate

This is to certify that the proposal No (5-9-18) entitled (Diffuse Large B-Cell- Non Hodgkins Lymphomas in Sudan, an exploratory insight at the histological phenotypic patterns (DEL and DHL) using coexpression and molecular translocations of c-MYC and BCL2 proteins) submitted by Dr. Salma Abubaker Abbas Ali from University of the Western Cape, South Africa has been approved by the National Health Research Ethics Committee, Federal Ministry of Health to be conducted in Sudan.

NB

The principal investigator is requested to submit the final report to the Research Directorate- Federal Ministry of Health.

Dr. Nalema Abdalla Wagialla
Rapporteur of the
National Research Ethics Review Committee

Appendix 2: Ethical Approval from the University of the Western Cape (2019-2020)

UNIVERSITY of the WESTERN CAPE

OFFICE OF THE DIRECTOR: RESEARCH
RESEARCH AND INNOVATION DIVISION

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25 March 2019

Dr S Ali
Faculty of Dentistry

Ethics Reference Number: BM19/1/30

Project Title: The use of immunophenotypic markers and next generation sequencing as diagnostic and prognostic indicators of high-grade B-cell lymphoma in Sudan.

Approval Period: 20 March 2019 – 20 March 2020

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report in good time for annual renewal.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

Appendix 3: Ethical Approval from the University of the Western Cape (2020-2023)

UNIVERSITY of the WESTERN CAPE

60 YEARS of hope, action & knowledge

26 May 2020

Dr SAA Ali

Faculty of Dentistry

Ethics Reference Number: BM19/1/30

Project Title: The use of immunophenotypic markers and next generation sequencing as diagnostic and prognostic indicators of high-grade B-cell lymphoma in Sudan

Approval Period: 15 May 2020 – 15 May 2023

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report annually by 30 November for the duration of the project.

Permission to conduct the study must be submitted to BMREC for record-keeping.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

Director: Research Development
University of the Western Cape
Private Bag X 17
Bellville 7535
Republic of South Africa
Tel: +27 21 959 4111
Email: research-ethics@uwc.ac.za

NHREC Registration Number: BMREC-130416-050

Appendix 4: Ethical Approval from the University of the Western Cape (2021-2024)

UNIVERSITY of the WESTERN CAPE

60 YEARS of hope, action & knowledge

2 November 2021

Dr SAA Ali
Oral Medicine and Periodontology
Faculty of Dentistry

Ethics Reference Number: BM19/1/30

Project Title: The use of immunophenotypic biomarkers and qualitative polymerase chain reaction as diagnostic and prognostic indicators of diffuse large B-cell non hodgkins lymphoma in Sudan

Approval Period: 2 November 2021 – 2 November 2024

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project and the requested amendment to the project.

Any further amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report annually by 30 November for the duration of the project.

For permission to conduct research using student and/or staff data or to distribute research surveys/questionnaires please apply via:
https://sites.google.com/uwc.ac.za/permissionresearch/home

The permission letter must then be submitted to BMREC for record keeping purposes.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

Appendix 5: Radiation and Isotope Center Consent

وزارة الصحة ولاية الخرطوم

مستشفي الخرطوم لعلاج الاورام

قسم الانسجة المرضية و الاصبغ المناعية و الخلايا

Khartoum Oncology Hospital-Histopathology Department

Radiation and Isotope Centre

Title of study: **The Use of Immunophenotypic Biomarkers and Qualitative Polymerase Chain Reaction as Diagnostic and Prognostic Indicators of Diffuse Large B-cell Non Hodgkins lymphoma in Sudan**

Principal investigator: Dr.Salma Abubaker Abbas Ali

Name of supervisors: Professor Manogari Chetty, Professor Alan Christoffels, University of The Western Cape- South Africa.

Access to Medical Records and Patients Information

We hereby authorize the aforementioned entities participating in the aforementioned study, to access patient files at the Radiation and Isotope Center- Khartoum and to obtain all the relevant and needed information for the completion of the aforementioned study and to utilize this data for this research and for future research studies.

By providing this access we fully acknowledge that:

RICK will not be enlisted as part of the research yet will be acknowledged.

RICK will not incur any financial costs.

RICK will not be informed of the results/outcomes of specific patients.

Upon accessing the required information, the principal investigator must ensure that: (i) the data will remain confidential, and will only be accessed by the investigators working on the study.

(ii) The personal data of the patients will remain confidential and will not be published or exposed during any stage of the research process and in this study patients data will be grouped based on variables affecting their outcome, not on specific unit/surgeon under which they received treatment.

By signing this form we The Radiation and Isotope Center, consent to providing the information as stated above.

We herewith concur that if we do not object to this consent and communicate our refusal to this request within a period of 15 days the principal investigator may proceed with the process of data collection with our consent.

Name:

Institute:

Signature:

Date:

Appendix 6: Data Entry Sheet

Data Entry Sheet

Code

1. Sample

2. Age

3. Gender ☐ Female ☐ Male

4. Marital Status Single ☐ Married ☐ Divorced ☐ Widowed ☐

5. Occupation Student ☐ Labor ☐ Employee ☐ Retired ☐ Housewife ☐

6. Residence
- North Sudan ☐
- Khartoum ☐
- Eastern Sudan ☐
- Western Sudan ☐
- Eastern Sudan ☐
- South Sudan ☐

7. Referral Mode
- Self Referral ☐
- General Practioner (Governemnet) ☐
- Private Physician ☐

8. Comorbidity

- Myocardial Infarction ☐
- Congestive Heart Disease ☐
- Peripheral vascular disease ☐
- Chronic obstructive pulmonary disease ☐
- Dementia ☐
- Connective tissue disease ☐
- Gastroenteritis disease (Peptic ulcer) ☐
- Irritable Bowel Syndrome ☐
- Hypertension ☐
- Diabetes ☐
- Liver disease ☐
- Renal disease ☐
- Other tumor ☐
- Other ☐

Appendix 6: Data Entry Sheet (Cont'd)

9. Smoking Yes (Current) ☐ Yes(Former) ☐ No ☐

10. Specimen site **Specimen Site**:

C83.30 ..... unspecified site
C83.31 ..... lymph nodes of head, face, and neck
C83.32 ..... intrathoracic lymph nodes
C83.33 ..... intra-abdominal lymph nodes
C83.34 ..... lymph nodes of axilla and upper limb
C83.35 ..... lymph nodes of inguinal region and lower limb
C83.36 ..... intrapelvic lymph nodes
C83.37 ..... spleen
C83.38 ..... lymph nodes of multiple sites
C83.39 ..... extranodal and solid organ sites

Cervical Lymph nodes C ☐
Submandibular Lymph nodes SM ☐
Submental Lymph node SE ☐
Thyroid mass TY ☐
Parotid mass P ☐
Neck swelling NC ☐
Axillary mass AX ☐
Trephine Specimen TE ☐
Inguinal mass IN ☐
Supraclavicular Lymph nodes SC ☐
Abdominal mass AB ☐
Intestinal mass IS ☐
Groin mass GR ☐
Oropharyngeal mass OP ☐
Brain biopsy ☐
Scrotal SR ☐
Tonsills TO ☐
Ovarian mass OV ☐
Skin SK ☐
Other ☐

11. LDH level at Dx High ☐ Low ☐

Appendix 6: Data Entry Sheet (Cont'd)

12. Stage I ☐ II ☐ III ☐ IV ☐

13. Date of first DX ☐

14. Date of last Follow up ☐

15. MYC Expression level >40% ☐ <40% ☐

16. BCL2 Expression level >30% ☐ < 30% ☐

17. Double Expresser Lymphoma ☐ ☐

18.Treatement ☐

19. Current status ☐ ☐ ☐

Appendix 7: Consent for Publication

UNIVERSITY of the WESTERN CAPE

Faculty of Dentistry & WHO Oral Health Collaborating Centre
Diagnostics Cluster
Private Bag X17 Bellville 7535 South Africa
Telephone: +27 21 937 3112

World Health Organization

CONSENT FORM: Publications

Research Project Title: ***The Use of Immunophenotypic Biomarkers and Qualitative Polymerase Chain Reaction as Diagnostic and Prognostic Indicators of Diffuse Large B-cell Non Hodgkins lymphoma in Sudan***

TO WHOM IT MAY CONCERN

I, the undersigned.....consent that data from this study can be used for approved research purposes and publications.

- I understand that all efforts will be made to conceal my identity but that full confidentiality cannot be guaranteed
- I understand that my consent or refusal will in no way affect my dental care

Patient Name (print).....

Patient signature: .....

Principal Investigator print name: .....

Principal Investigator signature: .....

Date: .....

Appendix 8: Information Sheet

UNIVERSITY of the WESTERN CAPE

Faculty of Dentistry & WHO Oral Health Collaborating Centre
Diagnostics Cluster
Private Bag X17 Bellville 7535 South Africa
Telephone: +27 21 937 3112

INFORMATION SHEET

Principal investigator's name: Dr Salma Ali

Contact details of principal investigator: email: 3879325@myuwc.ac.za

Cell No. 0797574839

What is this study about?
This is a research project being conducted by Dr Salma Ali at the University of the Western Cape. We are inviting you to participate in this research project because you are diagnosed with Diffuse large B cell Lymphoma and we are attempting to identify the prognostic and diagnostic value of specific biomarkers (BCL2 and MYC) This will allow us to subclassify this type of Non Hodgkins lymphoma and later precise its treatment for a better outcome.

What will I be asked to do if I agree to participate?
Obtain a detailed record from your folder. Use your diagnosed tissue block in laboratory investigation.

Would my participation in this study be kept confidential?
The researchers undertake to protect your identity and the nature of your contribution. Written informed consent will be obtained from all participants on standardized. Forms which will be available in Arabic and English . All information will be stored in password protected computers which will be stored in a locked office. All personal identifiers will be changed when the data are published.

If we write a report or article about this research project, your identity will be protected.

What are the risks of this research?
There are no, if any risk at all to you as a research participant. This study necessitates only the use of your tissue block stored at the lab. There is no risk of physical, psychological, social or economic harm to the participant or her family during this study.
All human interactions and talking about self or others carry some amount of risks. We will nevertheless minimise such risks and act promptly to assist you if you experience any discomfort, psychological or otherwise during the process of your participation in this study. Where necessary, an appropriate referral will be made to a suitable professional for further assistance or intervention.

What are the benefits of this research?
The findings of this study will contribute to the subclassification and verification of the prognostic value of the chosen biomarkers, along with exploration of the genomic mutations of this disease, this will help us identify the genes responsible for the disease and hence personalize the treatment. In this way, this potentially life threating disease may be managed more effectively.
Your participation in this research is completely voluntary. You may choose not to take part at all. If you decide to participate in this research, you may stop participating at any time. If you decide not to participate in this study or if you stop participating at any time, you will not be penalized or lose any benefits to which you otherwise qualify

What if I have questions?
This research is being conducted by Dr Ali at the University of the Western Cape and the Radiation and Isotope Center, Khartoum- Sudan. If you have any questions about the research study itself, please contact Dr Ali using the details provided on page 1.
Should you have any questions regarding this study and your rights as a research participant or if you wish to report any problems you have experienced related to the study, please contact:

Dr Salma Ali
Principle Investigator
University of the Western Cape
3879325@myuwc.ac.za
Tel No: 0797574839

Prof Manogari Chetty
Supervisor
University of the Western Cape
mchetty@uwc.ac.za
0814472284

This research has been approved by the University of the Western Cape's Biomedical Research Ethics Committee/Humanities and Social Sciences Research Ethics Committee
REFERENCE NUMBER:

Appendix 9: Institution Consent

Faculty of Dentistry & WHO Oral Health Collaborating Centre

Diagnostics Cluster

Private Bag X17 Bellville 7535 South Africa

UNIVERSITY of the WESTERN CAPE

World Health Organization

Institution Consent

Title of the project: **The Use of Immunophenotypic Biomarkers and Qualitative Polymerase Chain Reaction as Diagnostic and Prognostic Indicators of Diffuse Large B-cell Non Hodgkins lymphoma in Sudan**

Principal Investigator: Salma Abubaker Abbas Ali

Name of Supervisors: Professor, Manogari Chetty, Professor Alan Christoffels

Access to medical records and patient information

I hereby authorize the aforementioned entities participating in this project to access files at Radiation and Isotope Center in Khartoum-Sudan and to obtain all the relevant and needed information for the completion of this study and to utilize this data for this research and for future studies.

By providing this access I acknowledge the following:

1. I will not be enlisted as part of this project
2. I will not endure or receive any financial compensation
3. I will not be informed with the results or specific patients under my care

Upon accessing the required information the principal investigator endures confidentiality of the personal data and to ensure that it will not be exposed or published during any part of this project.

By signing this consent I , agree to providing information as stated above, I concur that if I do not refuse this request within 15 days the principal investigator my proceed with the data collection process with my consent.

Name:

Signature:

Date:

Appendix 10: Data Management Plan (UWC)

University of the Western Cape
Data Management Plan template
The template may be refined as not all elements are applicable to all projects.
The DMP should be uploaded to the data repository along with any associated project data and other project documents.
Further details are available on the Guide to Research Data Management
Contact: rdm-support@uwc.ac.za, Mark Snyders (mpsnyders@uwc.ac.za) or Sarah Schäfer(sschafer@uwc.ac.za)
University of the Western Cape
Data Management Plan
Faculty
Department
Administrative Data
Project title
Registration details (registration number)
Funder
Grant number
Abstract - project description (include the research questions)
Principle Investigator (PI)
ORCID (PI)
Contact details of the PI
The timeframe of the research project
Date the DMP was created / submitted
Date /s the DMP was revised
Data
What will be collected? Describe the data and formats (raw and refined/cleaned data).
When describing formats, please identify storage requirements by (expected file sizes and quantities).
Is your data original or will you reuse existing data (or a combination)?
How will the data be collected? (e.g. interview; questionnaire; observation)
Which software and version will be used?
Which operating system is used at the time of collecting the data?

Appendix 10: Data Management Plan (UWC) (Cont'd)

Documentation (legislation, policies and guidelines)
Applicable legislation for legal compliance (e.g. Protection of Personal Information Act - POPIA)
Institutional and funder policies
Metadata schema and version used (e.g. Dublin Core)
Desciptive document (How the data was analysied and how it is used. Uplod this document with the data onto the repository)
Applicable Memorandum of Understanding (MOU) that defines roles and responsibilities for data collection, administration and sharing.
Ethical compliance and approval
Have you received ethical approval (attached letter)
How will you obtain consent?
How will you handle intellectual property issues?
How will you manage copyright concerns?
How will you manage confidentiality concerns?
Secure Storage and Backup
How will the primary (raw) data be securely stored?
Where will the refined data be stored?
How will you share public data?
How will you address security and backup?
Data Sharing
Are the any funder or institutional restrictions on sharing the data?
How will the data be shared?
How will data be securely shared?
Data Selection, Preservation (Archiving) and Retention
Which data will be shared?
What is the long term storage plan?
How long is the data expected to be stored?
END