T.C.

KIRSEHIR AHI EVRAN UNIVERSITY

HEALTH SCIENCES INSTITUTE

DEPARTMENT OF MOLECULAR MEDICINE

ASSESSMENT OF EPSTEIN BARR VIRUS MOLECULAR POLYMORPHISM AND TUMOR BIOMARKERS ASSOCIATED WITH NON-HODGKIN'S LYMPHOMA IN IRAQI PATIENTS

Wiqar Adnan Azeez ABOOMEIMA

MASTER THESIS

KIRŞEHİR / 2022

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THESIS STATEMENT

All the information in the thesis is obtained within the framework of ethical behavior and academic rules.

In this study, which was prepared in accordance with the thesis writing rules, I declare that all kinds of statements and information that do not belong to the source are fully cited.

Wiqar Adnan Azeez ABOOMEIMA

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviations	**Explanations**
NHL	**:**Non-Hodgkin's lymphoma
IRR	**:**Incidence rate ratio
IARC	**:**International Association for Cancer Research
ASIRs	**:**Age-specific incidence rates
LH	**:**Hodgking Lymphomas
HTLV-1	**:**Human T-cell leukemia virus type 1
HCV	**:**Hepatitis C virüs
MALT	**:**Mucosa-Associated lymphoid tissue
AIDS	**:**Acquired immunodeficiency syndrome
WF	**:**Working Form
ILSG	**:**International Lymphoma Study Group
WHO	**:**World Health Organization
FLIPI	**:**Follicular Lymphoma International Prognostic Index
BL	**:**Burkitt' lymphoma
FL	**:**Follicular lymphomas
EFS	**:**Event-free survival
DLBCL	**:**Diffuse large B-cell lymphoma
PEL	**:**Primary effusion lymphoma
NPC	**:**Nasapharyngeal carcinoma
MHC	**:**Major-histocompatibility-complex
PTLD	**:**Post transplantation lymphoproliferative disease
AITL	**:**Angioimmunoblastic T-cell Lymphomas
NK	**:**Naturel killer
HTLV-1	**:**Human T-lymphotropic virus 1
LMP1	**:**Latent membrane protein 1
EBNA2	**:**Especially EBV nuclear antigen
EBNA1	**:**EBV nu-clear antigen 1
TACTP	**:**Autologous Peripheral Stem Cell Transplantation
PET-CT	**:**Positron Emission Comptuted Tomography

ELISA **:**Enzyme-linked immunosorbent assay
CO **:**Cut-off Control
DAB **:**Diaminobenzidine
TMA **:**Tissue microarray
EDTA **:**Ethylenediaminetetraacetic acid
PBS **:**Phosphate buffered saline
FLIPI **:**Follicular Lymphoma International Prognostic Index
ADNP **:**Assessment according to the tumor syndrome
Cx **:**External cortex
CL **:**Lymphoma cells
Ch **:**Chorion
SM **:**Submucosa
GP **:**Small parasympathetic nodes
MALT **:**Mucusa- associataed lymphoid tissue
TLP **:**Tumor progression free time
CLL/SLL **:**Mantle cell lymphoma and chronic lymphoctic leukemia/small lymphocytic lymphoma

ABSTRACT

MASTER THESIS

ASSESSMENT OF EPSTEIN BARR VIRUS MOLECULAR POLYMORPHISM AND TUMOR BIOMARKERS ASSOCIATED WITH NON-HODGKIN'S LYMPHOMA IN IRAQI PATIENTS

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The current study was conducted to evaluate the Epstein-Barr virus molecular polymorphism and tumor biomarkers associated with non-Hodgkin's lymphoma in Iraqi patients.

Our study concerned 50 cases pooled together in the hematology service of the Department of Pathology at Hawler Medical University, Erbil (Hawler) in the Kurdistan Region of Iraq from January 2020 through March 2021. Male patients were 56.1 years and female patients were 56.4 years old without any difference statistically. In our study, we identified 4 age groups where the average age of our patients is 56.5 years. Maximum 18 years old up to 79 years old. Almost half of our patients belong to the age group above 60 years, or 42% of cases, on the other hand, the group of patients under 20 years of age makes up only 8% of cases.

The distribution of patients by sex showed an increase statistical of men, i.e. 60% and only 40% of women, most patients came from different parts of Iraq, which capital region

(Baghdad) with a frequency of 30%, followed by the region of Erbil with a frequency of 20% and the rest distributed among the different regions of the Diyala: 15%, Wasit: 15%, Thi Qar: 10%, and Al-Najaf: 10%. The frequency of anti-EBNA-l antibody in the infected group was higher than in the control group, however, the frequency of anti-EBNA- l IgG Ab in the case group was higher than anti-EBNA- l IgM Ab may be due to latent re-infection, so the high-frequency distribution of anti-EBNA- l IgG Ab were data 19 cases (47.5%) in age group (60-74)years, followed by 8cases (20.2%), in the age group 45-59 and 5 cases (12.5%) recorded in age group >75 years and 30-44 years respectively while the lowest level 3(7.5%) show in the age group 15-29 years. The level of anti-EBNA- l IgG Ab showed 23 cases (57.5%) in males was higher than anti-EBNA- l IgM Ab in 16 cases (76.1) while in females IgG was 17 cases (42.5%) and for IgM 5 cases (23.8).

Evaluation according to the antecedents, the majority of patients (64%) present with ATCD or we notice that heart disease presents the large percentage either 30% followed by type 2 diabetes or 24% while hypertension and tuberculosis have only been presented. By 8% and 2% for each and only 36% of patients did not present with DCDA. Assessment according to general signs Almost all of our patients (74%) presented general signs of fever, night sweats, and weight loss. And only 26% of patients presented no symptoms. The presence of general signs or signs commonly called B symptoms such as fever, weight loss, and sweating were present in the majority of our patients with 74%. Evaluation according to the clinical stage we have observed that a large number of our patients have stage 4 NHL (i.e. 50%). Evaluation according to the phenotype: In our series, the majority of patients are type B NHL with a percentage of 86% and only 14% phenotype T. Evaluation according to the secondary location the major localization is in the bone marrow (40%) followed by the liver (36%) while the spleen represents the least affected organ (6%). 6% of our patients do not present an extranodal extension. Assessment according to the subtypes of NHL almost half of these patients present a large cell NHL (40%) followed by small B cell NHL (29%) while the other subtypes are present with small percentages (20.1%). Assessment according to the tumor syndrome (ADNP) the existence of ANDP in 82 % of cases. Cervical involvement is the most common, estimated at (50%), while axillary involvement is only present in 32% of cases, while only 18% of cases do not present with DNAP.

In the histological study, the histological appearance of the ganglion affected by diffuse large B-cell non-Hodgkin lymphoma, where it shows that the structure of the ganglion

parenchyma is disorganized and erased by tumor cells as well as lymphoma cells (**CL**) are large in size. Gastric histology shows that the chorionic is largely ascended; at high magnification, we note the presence of a polymorphic lymphoid population with a decrease in glandular mass and the presence of foci of a cryptic abscess as well as sectors of intestinal metaplasia sometimes in dysplasia. And shows a medullary space, the whole of which is infiltrated by a diffuse and dense lymphomatous proliferation made up of a rounded lymphomatous cell population of varying sizes.

In Molecular study recorded the Level of L1PA2 Gene Expression significant increase in lymphoma patients' level of L1PA2 gene expression in the blood tissue. And MicroRNA-532-5p gene recorded a significant decrease in the level of lymphoma patients of patients with non-Hodgkin's lymphoma compared with the control group.

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Key words: Epstein-barr virus, L1PA2, microRNA-532-5p gene, non-Hodgkin's lymphoma

ÖZET

YÜKSEK LİSANS TEZİ

IRAKLI HASTALARDA EPSTEIN BARR VİRÜS MOLEKÜLER POLİMORFİZM VE HODGKİN OLMAYAN LENFOMA İLE İLİŞKİLİ TÜMÖR BİYOMARKERLERİNİN DEĞERLENDİRİLMESİ

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II. Danışman: Dr. Mohammed A. AL-Askeri

Bu çalışma, Iraklı hastalarda Epstein-Barr virüsü moleküler polimorfizmi ve Hodgkin dışı lenfoma ile ilişkili tümör biyobelirteçlerini değerlendirmek için yapılmıştır.

Çalışmamız, Ocak 2020'den Mart 2021'e kadar Irak'ın Kürdistan Bölgesi'ndeki Erbil (Hawler) Hawler Tıp Üniversitesi Patoloji Anabilim Dalı'nın hematoloji servisinde bir araya toplanmış 50 vakayı kapsıyordu. Erkek hastalar ortalama 56,1 yaşında ve kadın hastalar ise ortalama 56,4 yaşındaydı. İstatistiksel olarak fark yoktu. Çalışmamızda hastalarımızın yaş ortalamasının 56,5 olduğu 4 yaş grubu belirledik. En fazla 18 yaşından 79 yaşına kadar. Hastalarımızın neredeyse yarısı 60 yaş üstü ya da vakaların %42'sini oluşturmaktadır, buna karşın 20 yaş altı hasta grubu vakaların sadece %8'ini oluşturmaktadır.

Hastaların cinsiyete göre dağılımı erkeklerde istatistiksel olarak bir artış gösterdi, yani kadınların %60'ı ve kadınların sadece %40'ı, hastaların çoğu Irak'ın farklı bölgelerinden geldi, bu da %30 sıklıkta başkent (Bağdat) ve ardından Irak bölgesiydi. %20 sıklıkta Erbil ve geri kalanı Diyala'nın farklı bölgelerine dağılmıştır: %15, Wasit: %15, Thi Qar: %10 ve Necef: %10. Enfekte grupta anti-EBNA-1 antikoru sıklığı kontrol grubuna göre daha yüksekti, ancak vaka grubunda anti-EBNA-l IgG Ab sıklığının anti-EBNA-l IgM Ab'den yüksek olması nedeniyle olabilir. Bu nedenle anti-EBNA-l IgG Ab'nin yüksek frekanslı

dağılımı, yaş grubunda (60-74) 19 vaka (%47,5), ardından yaş grubunda 8 vaka (%20,2) veriydi. 45-59 ve 5 vaka (%12.5) sırasıyla >75 yaş ve 30-44 yaş grubunda kaydedilirken, en düşük seviye 3 (%7.5) 15-29 yaş grubunda görülmektedir. Erkeklerde 23 olguda (%57.5) anti-EBNA- IgG Ab düzeyi 16 olguda (76.1) anti-EBNA- IgM Ab'den yüksek, kadınlarda IgG 17 olguda (%42.5) ve IgM için 5 idi (23.8%) vakalar.

Öncüllere göre değerlendirme, hastaların çoğunluğunda (%64) ATCD mevcut veya kalp hastalığının büyük bir yüzdeyi ya % 30'unu tip 2 diyabetin izlediğini ya da % 24'ünü hipertansiyon ve tüberkülozun sadece sunulduğuna dikkat çekiyoruz. Her biri için % 8 ve %2 hastaların sadece %36'sı DCDA ile gelmemiştir. Genel belirtilere göre değerlendirme hastalarımızın hemen hemen tamamında (%74) genel ateş, gece terlemesi ve kilo kaybı vardı. Ve hastaların sadece %26'sı hiçbir semptom göstermedi. Ateş, kilo kaybı, terleme gibi yaygın olarak B semptomu olarak adlandırılan genel belirti veya bulguların varlığı %74 ile hastalarımızın büyük çoğunluğunda mevcuttu. Klinik evreye göre değerlendirdiğimizde hastalarımızın büyük bir kısmında evre 4 NHL (%50) olduğunu gözlemledik. Fenotipe göre değerlendirme: Bizim serimizde hastaların çoğunluğu %86 ile tip B NHL ve sadece %14 fenotip T'dir. Sekonder yerleşime göre değerlendirmede majör lokalizasyonun kemik iliğinde olduğu (%40) izlenir. Karaciğer tarafından (%36), dalak en az etkilenen organı (%6) temsil etmektedir. Hastalarımızın %6'sında ekstranodal uzanım yoktur. NHL'nin alt tiplerine göre değerlendirme bu hastaların neredeyse yarısında büyük hücreli NHL (%40), ardından küçük B hücreli NHL (%29), diğer alt tipler küçük yüzdelerle (%20.1) mevcuttur. Tümör sendromuna (ADNP) göre değerlendirme, vakaların %82'sinde ANDP varlığıdır. Servikal tutulum en yaygın olanıdır (%50 olarak tahmin edilir), aksiller tutulum vakaların sadece %32'sinde bulunurken, vakaların sadece %18'inde DNAP yoktur.

Histolojik çalışmada, diffüz büyük B hücreli non-Hodgkin lenfomadan etkilenen ganglionun histolojik görünümü, burada ganglion parankiminin yapısının düzensiz olduğunu ve tümör hücreleri tarafından silindiğini ve lenfoma hücrelerinin (CL) boyutunun büyük olduğunu gösterir. Gastrik histoloji, koryonik bölgenin büyük ölçüde yükseldiğini gösterir; yüksek büyütmede, glandüler kütlede azalma ve kriptik apse odaklarının yanı sıra bazen displazide intestinal metaplazi sektörlerinin varlığı ile polimorfik bir lenfoid popülasyonun varlığına dikkat çekiyoruz ve tamamı değişen boyutlarda yuvarlak lenfomatöz hücre popülasyonundan oluşan yaygın ve yoğun bir lenfomatöz proliferasyonla infiltre edilmiş medüller bir boşluk gösterir.

Moleküler çalışmada, L1PA2 Gen Ekspresyonu Düzeyinde lenfoma hastalarının kan dokusunda L1PA2 gen ekspresyonu düzeyinde önemli bir artış kaydedildi ve MicroRNA-532-5p geni, kontrol grubu ile karşılaştırıldığında, Hodgkin dışı lenfoma hastalarının lenfoma hastalarının düzeyinde önemli bir düşüş kaydettik.

Ekim 2022, xviii+85 sayfa

Anahtar Kelimeler: Epstein-barr virüsü, hodgking dışı lenfoma, L1PA2, MicroRNA-532-5p geni.

1. INTRODUCTION

1.1. Non-Hodgkin's Lymphoma" (NHL)

Non-Hodgkin's lymphomas (NHL) are lymphoid neoplasms originating from populations of B, T, or Natural Killer cells, whose clinical behavior and natural history of the disease depend on the lymphoma subtype according to the classification and staging used [1]. Several systems of classification and staging of the disease have been developed in an attempt to better define the behavior and response to chemotherapy. The global prevalence of NHL, as well as the relative frequency of its subtypes, varies according to the geographic region [2]. In Iraq, information about the epidemiology and behavior of non-Hodgkin's lymphomas is scarce [3]. In the United States, it is responsible for 4% of all cancers, being the fifth most commonly diagnosed cancer. Mosul, is the third-largest metropolitan region in Iraq, according to data from the Iraqi Institute of Geography and Statistics, neoplastic diseases were responsible for 19.0% of the causes of death that occurred in hospitals in Mosul in 2005 [4]. In this city, the incidence rate of NHL adjusted for the age, in 2000, was 17.7 cases per 100 thousand inhabitants. The relative frequency of NHL subtypes in Iraq has been poorly studied. The variation in geographic distribution justifies the need for local data for an adequate definition of therapeutic strategies. Non-Hodgkin lymphoma (NHL) appears to be less common than Hodgkin lymphoma (HL) in the Middle East (HL). This is generally expressed as an incidence rate ratio (IRR), with non-age-adjusted NHL: HL IRRs of 2.0 and 1.8 from Iraq's and Jordan's National Cancer Registries, respectively [5]. Globocan's global age-adjusted IRR is 5.2, whereas the Global Burden of Disease IRR is 9.0. The Kurdish governorate of Sulaimaniyah has a well-defined population as well as public cancer facilities with precise patient registration and centrally verified diagnosis. Jordan and Saudi Arabia also have population-based cancer registries that follow the data submission requirements of the International Association for Cancer Research (IARC). The Iraqi Cancer Registry is not affiliated with the IARC, despite the fact that it gathers province data and recently released national estimates that appear to accurately portray the country's cancer risk. The estimates of age-specific incidence rates (ASIRs) and age-adjusted incidence rates (AAIRs) of NHL and HL in Iraq, Jordan, Saudi Arabia, and the US SEER program are compared in this

research. Lymphomas represent 5% of malignant neoplasms of the head and neck and are a group of heterogeneous tumors classified as Hodgkin lymphomas (LH) and non- Hodgkin lymphomas (NHL), depending on the presence or absence of specific cells, called Reed-Sternberg cells. These cells can have two or more nuclei and are found in LH biopsies. Lymphomas can appear in any region that contains lymphatic tissue. Most lymphomas appear in the lymphoid tissue, particularly in the cervical ganglia, and only 24% appear in extraganglionic sites; most frequently in the gastrointestinal tract, skin, bones, and Waldeyer's ring. In the oral cavity, the most frequently affected sites are the gums, hard palate, salivary glands, and tongue.

1.2. Aim of the Study

Assessment of Epstein Barr virus molecular polymorphism and tumor biomarkers associated with non-Hodgkin's lymphoma in Iraqi patients.

2. REVIEW OF LITERATURE

2.1. Etiology of Non-Hodgkin Lymphomas

Infections, environmental factors, immunological weaknesses, and chronic inflammation are only few of the many potential triggers of Non-Hodgkin lymphoma (NHL). Several distinct forms of NHL have been linked to a number of viruses.

Some types of NHL, particularly an endemic form of Burkitt lymphoma, have been related to the DNA virus Epstein-Barr.

In adults, T-cell lymphoma is caused by the human T-cell leukaemia virus type 1 (HTLV-1). A prolonged antigenic stimulation and cytokine dysregulation leads to unchecked B- or T-cell activation and proliferation.

Clonal B-cell expansions are a third consequence of Hepatitis C virus (HCV) infection. Hepatitis C virus may induce two types of NHL: splenic marginal zone lymphoma and diffuse large B cell lymphoma.

Helicobacter pylori infection is related with an increased risk of developing primary gastrointestinal lymphomas of the stomach mucosa-associated lymphoid tissue (MALT).

The use of phenytoin, digoxin, and tumour necrosis factor (TNF) antagonists has also been related to an increased risk of developing non-Hodgkin lymphoma. Additional risk factors for the development of NHL include exposure to organic chemicals, pesticides, phenoxy-herbicides, wood preservatives, dust, hair colour, solvents, chemotherapy, and radiation [6,7].

Increased risk of NHL has been linked to the presence of Wiskott-Aldrich syndrome, SCID, and induced immunodeficiency states caused by immunosuppressive medications. Primary central nervous system lymphoma may occur in AIDS patients (acquired immunodeficiency syndrome).

An increased risk of NHL has been associated to autoimmune illnesses such Sjögren syndrome, rheumatoid arthritis, and Hashimoto thyroiditis. It has been shown that Hashimoto's thyroiditis is associated with an increased risk of developing primary thyroid lymphomas, and that Celiac disease is associated with an increased risk of developing non-Hodgkin lymphomas [8].

2.2. Pathogenicity

In order to combat pathogenic diseases, the immune system relies heavily on B and T cells [9]. Antigen-binding antibodies (Ab) are produced by B cells, whereas antigen-specific T lymphocytes (Th) are the ones responsible for finding and responding to foreign antigens. Activated T cells (especially T helper cells, or CD4+) emit a variety of proteins, or cytokines, that serve to signal and coordinate the local immune response. Considering the crucial role that T cells play in regulating B-cell and overall immune function, it should come as no surprise that the strongest and most well-established risk factors for malignant lymphomas are characterised by dysregulation or suppression of T-cell function (HIV/AIDS, organ transplantation, see below) that permits Epstein-Barr virus (EBV)-driven B-cell proliferation and transformation. Like the development of cancer in general, the neoplastic transformation of T or B cells is a multistep process marked by the growing accumulation of genetic abnormalities that leads to clonal expansion and the creation of a solid or leukemic tumour. Abnormalities in cell growth, signalling pathways, and programmed cell death are all potential mechanisms (apoptosis). Rearrangements in B-cell immunoglobulin or T-cell receptor genes that are too complex to be fixed during normal development and adaptation are indicative of times when these cells are more vulnerable genetically. Naturally occurring DNA double-strand breaks facilitate the aberrant chromosomal translocations that characterise NHL cancers throughout these processes. Up to 90% of NHL patients have been reported to have chromosomal translocations [10, 11]. These translocations may activate oncogenes or inactivate tumour suppressor genes [12], with or without other genetic disorders. In addition to direct carcinogenesis produced by environmental stressors, oncogenic viruses can provide alternative paths for genetic changes. The spatially constant increase in NHL incidence shows a key role for one or more environmental agents in the genesis of NHL, despite the fact that genetic factors play an essential role in lymphoma formation.

2.3. Classification of Non-Hodgkin's Lymphomas

The first description of tumors originating from the lymphatic tissue was made in 1832, by Thomas Hodgkin. In 1865, these tumors came to be called Hodgkin's disease. Subsequently [13], it was noticed that some tumors of the lymphatic tissues had different morphological characteristics from Hodgkin's disease, receiving the name non-Hodgkin's lymphomas

(NHL) to distinguish them from those originally described [14]. Although descriptions of lymphoid diseases occurred in the following decades, attempts to histologically classify NHLs have been a source of frustration for many years [15]. In 1956, Hicks, Rappaport, and Winter described the first modern classification of NHL. This description considered aspects of cytology (undifferentiated, well-differentiated, little differentiated, histiocytic) and the growth pattern (diffuse or nodular) [16]. This system has achieved enormous popularity due to its simplicity and reproducibility. The subsequent recognition of immunological characteristics and cellular markers made it possible for Lukes & Collins, in the United States, in 1974, and Lennert and collaborators, at Kiel University in Europe, to propose classifications that related the lymphocyte lineage to morphology. The availability of several classification systems resulted in difficulties in standardizing the language used, but the recognition of new entities and the improvement of existing categories resulted in improvements in the classification systems of NHL [17]. In order to standardize the classification of NHLs, the National Cancer Institute (National Cancer Institute) proposed the International Working Formulation (WF) in 1982 [18]. Lymphomas were classified into prognostic groups or clinical degrees but did not take into account the aspects of immunophenotypic. Despite efforts, WF did not achieve the standardization it had intended. In 1991, a group of pathologists entitled the International Lymphoma Study Group (ILSG) proposed a new classification that received the name Revised European-American Classification of Lymphoid Neoplasms (REAL) [19]. In the REAL classification, NHLs were classified on a morphological, immunophenotypic, genetic, and clinical basis [20]. One of the biggest advantages of the REAL classification is the initial separation of the NHL into phenotype (B or T) [21]. The REAL classification represented an evolution in terms of clinical relevance and reproducibility. The distribution of NHL cases according to the subtypes defined in the REAL classification, in a cohort of 1403 cases from eight centers located in Europe, Africa, and Asia was studied by the diagnostic consensus carried out by "The Non-Hodgkin's Lymphoma Classification Project" In 1997, the concepts of the REAL classification were used to develop the classification of the World Health Organization (WHO), which is currently used. This distinguishes hematological neoplasms according to the cell line: lymphoid, myeloid, histiocytes or dendritic cells, and mast cells. Among lymphoid neoplasms, morphological and immunological characteristics distinguish Hodgkin's lymphomas from non-Hodgkin's lymphomas [22]. In the differentiation stage, the morphological, phenotypic, genotypic, and clinical characteristics distinguish the subtypes

of non-Hodgkin's lymphomas. The corresponding lymphomas and lymphoid leukemias are considered different phases (solid versus circulating) of the same disease [21].

2.4. Epidemiology

In the group of lymphomas originating from B lymphocytes, Follicular Lymphoma ranks second in frequency after Diffuse Large Cell Lymphoma B, representing 20% of all lymphomas worldwide [24]. The annual incidence of the disease has increased rapidly in recent decades and has increased by 2-3 / 100. 000 inhabitants in 1950 at 5/100. 000 [25]. The average age of presentation is in the sixth decade of life and presents a slight predilection for the female sex. In individuals under 18 years of age, it represents 1 to 2.5% of all lymphomas and 15 to 20% in patients between 18 and 40 years of age [26]. Other rare variants occur in the pediatric population and others are located in the gastrointestinal tract and skin [27]. Although numerous risk factors have been proposed, most have not been validated in independent studies, so no consensus has been reached on which risk factors affect the development of follicular lymphoma. Relatives of people with follicular lymphoma have a slightly higher chance of having the disease themselves, although this risk is still rather modest [28]. Age, stage, bone marrow involvement, B symptom presence, lactic dehydrogenase levels, and the existence of anaemia have all been linked to the clinical course, but more work is needed to identify risk factors and treatment approaches. The FLIPI categorises individuals into high, intermediate, and low risk groups for death due to follicular lymphoma [29]. Patients with scores between 0 and 1 are considered low risk, those with scores between 2 and 5 are considered intermediate risk, and those with scores between 3 and 5 are considered high risk. The risk factors for lactic dehydrogenase elevation include age greater than 60 years, involvement of more than 4 ganglion sites, haemoglobin less than 12 G / dL, advanced stage, and elevated levels. Multiple studies have shown the accuracy of this score in predicting both PFS and OS. Patients who need therapy have been advised to employ a FLIPI2 evaluation, which incorporates niveles microglobulin levels, biggest lymph node width, bone marrow involvement, and haemoglobin levels, since it may offer additional information on progression-free survival [30].

Table 2.1. Risk factors of the follicular lymphoma international prognostic index (FLIPI) [31-30].

Parameter	FLIPI 1	FLIPI 2
Ganglion sites	> 4 GL regions	Largest GL diameter> 6 cm
Age	> 60 years	> 60 years
Serum marker	Elevated LDH	Elevation of beta 2 microglobulin
Stadium	Advanced (III-IV according to Ann Arbor classification)	Bone marrow involvement
Hemoglobin	>12 g/dL	> 12 g / dL

0—1 risk factors: low risk, 2 risk factors: intermediate risk, 3-5 risk factors: high risk; NHL in Iraq. In Northern Iraq, referral institutions in the Northern Iraq region of two primary histology, defined 270 lymphoma patients by morphology and adequate immunohistochemistry [32]. Non-lymphoma Hodgkin's was 205 (76%) while Hodgkin's lymphoma was 65 (24%) [33]. NHL included 91% B cells and 9% T cells. Diffuse large B cell lymphoma (DLBCL) was the most frequent kind of NHL, making up 52.2% of all NHL cases. This was followed by Burkitt's lymphoma (BL), which made up 14.6% of all NHL cases [34]. These were largely main intestines. Follicular lymphomas (FL) were discovered to account for just 2.9 per cent of NHL. Extranodal was primaried are seen in 48.3% of NHL patients [35]. Hodgkin's lymphoma (HL) had 48 percent nodular (NS) and 37 percent mixed cellularity (MC) [36]. Every HL was the principal node. The high prevalence of DLBCL, extranodal primary, intestinal BL, and FL in North Iraq are comparable but considerably different, to research from neighboring countries in the West or the Far East, indicating a similar geographical influence on the patterns of non-lymphoma Hodgkin's in the NHL [35]. In contrast to previous Iraqi and regional HL studies, NS outperformed MC, the most common histological subtype in northern Iraq. This tendency most likely reflects the region's expanding urbanisation [35]. In the study of its expression pattern and correlation and comparisons between immunosethole detections of P53 and ki67 as well as different clinical and histological parameters of patients with NHL, Harith Sami Ali et al. [37]. Will determine the frequency and correlation of P53 & Ki67 protein in the case of immunohistochemistry in lymphatic biopsies of Iranian NHL patients [38]. A total of 85 Iraqi non-patient Hodgkin's lymph node biopsies were performed, with 54 men and 31 women participating [39]. The most common grade was middle-grade NHL (65%), while the lowest frequency of instances was low-grade NHL (65%), (15.3 percent) [40]. High-quality lymphoma was discovered in

(18.8 percent). The entire frequency of both P53 and Ci67 protein positivity was covered in NHL patients (63.5 percent) and (68.2 percent). Age, sex, biopsy, and sclerosis of a lymph node, which are present in specific morphological subtypes of NHL, were not substantially altered by the P53 and Ki67 findings [41]. However, statistically, significant differences appeared between P53 or Ki67 and certain NHL histological grades, with a significant increase in both P53 positive and Ki67 expression [42]. Since 2000, a customised LMB 96 protocol has been in place at Baghdad's Children's Welfare Teaching Hospital for the treatment of paediatric B-cell non-Hodgkin lymphoma [43]. First observed that this Protocol was successful and feasible in Iraq (2000–2005) [44]. Additional adjustments were introduced in 2006 to try to reduce the toxicity associated with therapy. The findings of the second cohort of 190 children (2006–2010) are presented, as well as a comparison to the previous study [45]. Of the 180 patients treated, 120 had a complete response; 51 died, and 9 were discharged [46]. Overall survival (OS) was 64.7 percent at 60 months and event-free survival (EFS) was 56.3 per cent [47]. There were no differences in the 24-month OS and EFS between the 2000–2005 and 2006–2010 cohorts (66.3 percent vs 65.1 per cent; p= 89 percent and 53.3 percent vs 57.3 percent; p= 28). The second cohort demonstrated a superior therapy group B, though not significantly more so than the first cohort (EFS 62,9% vs. 53,8% ; p= 0.088). The mortality rate linked with treatments was substantial [48]. Fifty-six FFPE tissues were taken from Baghdad Medical City and utilised as a source of diarrhoea and B-cell lymphoma cloning confirmed by polymerase chain reaction (PCR) and heteroduplex studies, according to the BIOMED 2 protocol, as a diagnostic tool for B. The gathered data included FFPE tissues from 37 NHL patients and 19 reagent lymphoid utilized as polyclonal controls [49]. Positive clonality was found to be monoclonal in 70% of the cases (26/37), and polyclonal in 29% of the cases (11/37). DLBCL clonality was observed as monoclonal in 66 percent (10/15) of patients, 75 percent in SLL (3/4), 66 percent in BL (2/3), and 50 percent in MALT (1/2), and 100 percent in FL and NMZL identified individuals [50]. DLBCL clonality has been found in a few cases. Clonalities in reactive lymphoid hyperplasia were 100% polyclonal (19/19). The whole IGH rearrangement provides a valuable diagnostic tool for B cell lymphomas by determining the clonality of B cells [51]. Conducted research for Hemopoietic and Lymphoreticuar Malignancies that mostly used leukemia, lymphoma, and multiple myeloma. In the 1990s, the yearly incidence of non-Hodgkin lymphomas climbed by 3-4 percent in various parts of the industrialized globe, although rates of Hodgkin's disease, myeloma, and leukemia remained unchanged [52]. The goal of

this survey is to examine the pattern of hemopoietic and lymphatic malignanc vered with bone marrow tests in the broadest spectrum of Iraqi patients with prevalent hemopoietic and lymphocytic disorders [53]. With the exception of three northern provinces (Sulaimanyia, Erbil, and Dohouk), the Iraqi Ministry of Public Health recorded 63923 patients with various types of newly diagnosed cancer in the largest series over a five-year period [2000-2004] [54]. 10330 common cases [leukemia, lymphoma, and multiple myeloma] of hemopoietic and lymphoreticuarium [leukemia] accounted for 16% of Iraqi cancer patients. Leukaemia, accounting for 7% of all malignancies, was Iraq's third most common cancer [55]. There have been 4476 reports of leukemia, with 2618 (58.5 percent) for males and 1858 [41.5 percent] for women. Hodgkin's disease was found in 1502 cancer cases. 925 (61.6 percent) of males and 577 (38.4 per cent) of women. NHL accounted for 6% of all cancers in 3883 patients [56]. 469 cases represent 0.73 per cent of all cancers involving multiple lymphomas. In Iraq, unlike in many Western countries, the prevalence of leukemia is increasing, whereas trends for other hematpolymphopoietic malignancies remain unexpectedly stable [57]. From 1 June 2003 to 30 November 2004, 80 newly diagnosed HL patients [32]. Females and 48 males) were compared to 50 control patients with Non-Hodgkin lymphoma (NHL) and 50 control healthy people [58]. The National Cancer Institute obtained and analyzed more HL histopathology samples. The unique questionnaire style was created in order for all patients to have customized interviews. In this study, HL patients varied in age from 10-73 years old, with a median age of (29.311.73) years. In HL patients, 48 (60%) were male, while 32 (40%) were female. The incidence of aging below (45 percent) of the age is 67 (84 percent) and the incidence below 45 percent is 13 (16 percent) [59]. Individuals with LH were more likely to have a bigger cervical lymph node (70%) and an A symptom (27%) [60]. Mixed cellularity (MC) (61%) and stage II had the most subtypes (38 percent). Cigarette smoking and the risk of heart disease ($p > 0.05$) were not significantly associated [61].

2.5. Epstein–Barr virus

2.5.1. Epstein–Barr Virus and NHL

Over 100 viral proteins are encoded by the linear DNA molecule of Epstein–Barr virus (EBV) [62]. Contact with saliva may transmit Epstein-Barr virus. During an acute infection, EBV is most often found in the stratified squamous epithelium lining the oropharynx [63]. Later, a dormant B lymphocyte infection sets in (although the sequence of epithelial versus

lymphoid infection is a matter of debate). It is thought that the oropharyngeal lymphoid organs are the primary sites of EBV infection of B lymphocytes, and that the virus persists in circulating memory B cells in healthy carriers [64, 65, 66]. The ability of the virus to immortalise normally quiescent B lymphocytes in vitro, therefore converting them into immortally dividing lymphoblastoid cell lines, reveals its B-lymphotropic nature [67]. Initial infection is associated with increased viral shedding into saliva, although the virus may continue to be shed into saliva from the oropharynx for years [68, 69]. has been linked to a wide range of lymphoid and epithelial malignancies [70], including Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), Hodgkin lymphoma (HL), primary effusion lymphoma (PEL), and other T/NK-cell lymphomas. To protect the virus' genetic material, the viral envelope encloses a nucleocapsid. Before entering the B cell, the viral receptor CD21 (the C3d complement receptor) interacts with the major envelope glycoprotein gp350 [71]. Viral DNA replication, virion structural component production, and host immune response regulation When EBV infects epithelial cells in culture, the result is rapid viral production and cell death [72]. Onto the outside of B cells Other factors than CD21 can contribute to infection. When it comes to B cell infection, the major histocompatibility complex (MHC) class II molecule plays an important supporting role [73].

2.5.2. Non-Hodgkin Lymphoma and Epstein-Barr Virus

In most individuals, Epstein-Barr virus (EBV) causes a chronic infection of B cells and may cause cancer. Several rare types of non-Hodgkin lymphoma (NHL) including NHL in immunocompromised people have been linked to EBV, a known carcinogen. It is not generally considered to be a health risk [74]. Burkitt's lymphoma (98% of cases) is one of many lymphoproliferative disorders associated with the Epstein-Barr virus [75]. Angioimmunoblastic T-cell lymphoma (84.6%), natural killer cell lymphomas (40%) and Hodgkin's lymphoma (40%) [76]. Although the precise function of EBV in the development of various lymphomas remains unknown, it is hypothesised that HIV-1-positive, immunocompromised individuals are more likely to develop NHL due to immunosuppression, which in turn leads to increased replication of EBV and other carcinogenic viruses [77]. When the immune system is compromised in even a little way, EBV may become active again and replicate. Immune function may be diminished by, among other things, psychological stress and old age [78]. Subclinical EBV reactivation has been linked to both [79]. Reactivation of Epstein-Barr virus (EBV) may increase the risk of

NHL, or both factors may be triggered by the same external event that causes immunological dysfunction. However, we anticipate that in the future, greater antibody levels associated with reactivation (rather than seropositivity) will be linked to an increased risk of NHL. Conflicting findings have come from serologic investigations examining the relationship between EBV antibodies and the likelihood of developing NHL [80-84]. Multiple subtypes of NHL have been related to Epstein Barr virus (EBV), a well-known transforming virus [85]. EBV is often acquired by young children in developing countries. The infectious mononucleosis clinical syndrome is associated with primary infection during adolescence in developed countries. When EBV infects a person, it hides in their memory B cells and stays there for the rest of their lives. EBV readily transforms B lymphocytes in culture. Latent membrane protein-1 is thought to be the most important EBV protein for transformation, while other EBV proteins may be expressed by infected cells. In healthy infected individuals, the presence of functional T lymphocyte-mediated immunity limits the proliferation of EBV-transformed B cells [76].

2.5.3. Mechanisms Involved in Infection of EBV Associated NHL

Direct transformation caused by a pathogen: some forms of NHL are associated with long-term infections. Human oncogenic viruses have been studied since the discovery of Epstein–Barr virus (EBV) in 1985 [86]. Viruses have been shown to alter host cell homeostasis by expressing their own miRNAs and interfering with host miRNA expression [88, 89]. EBV, like other *Herpes viridae* viruses, has a lytic and a latent infection phase. To achieve latency, EBV preferentially targets B-cells, where it may remain dormant permanently as an episome [90]. Unfortunately, the mechanisms through which Epstein-Barr virus (EBV) causes lymphoid cancers are still unclear. The relevance of the virus is further clouded by the fact that in only a fraction of cases (10%) within each EBV-associated malignancy is a viral infection of tumour cells verified using standard techniques [91, 93]. The activation and differentiation of B-cells infected with EBV is triggered by the carefully regulated production of latent viral genes that hijack essential biological processes [94]. B-cells that have been infected with EBV develop into memory B-cells, which are characterised by low levels of gene expression and latent EBV infection [90]. Rarely, the normal EBV cycle in B-cells may take an unexpected turn, allowing infected B-cells to proliferate under the influence of viral oncogenes, eventually giving rise to B-cell lymphomas [95]. Human T lymphotropic virus 1 was the first human oncogenic retrovirus discovered (HTLV-1). Adult

T-cell leukemia/lymphoma, a particularly lethal type of T-cell malignancy, has been linked to it [96]. Although the mechanisms by which HTLV-1 and EBV cause lymphomas to develop are quite different, they share the feature that transformed cells harbour the transforming pathogen and that oncogenesis is driven by viral components inside the altered cells. An enormous step forward in unravelling the lymphoma-forming cascade has been made via illuminating the aetiology of EBV-associated lymphomas. When neoplastic B-cells get infected by EBV, the virus's latent oncogenic products may trigger the development of lymphomas. Infectious mononucleosis (IM) is a febrile illness characterised by a hyper-expansion of both lytic and latent antigen-specific T cell responses following primary EBV infection. Subsequently, these responses persist as memory T cells at lower levels in the blood of all virus carriers and are enriched as tissue-resident populations in or pharyngeal lymphoid tissues, where EBV reactivations are thought to occur [97, 98, 99]. To identify EBV latency, one must look at the tightly regulated pattern of EBV latent gene expression. Latent membrane protein 1 (LMP1), encoded by EBV, works in conjunction with BAFF/BLyS and APRIL [100] to trigger T cell-independent Ig heavy chain class switching. LMP1 mimics the signals normally associated with the engagement of CD40 on B-cells by CD-40L generated by T-cells during the GC response. In addition to EBV nuclear antigen2, other viral proteins, such as EBNA 2, transactivate several viral and cellular genes to stimulate B-cell proliferation. As a potent cellular oncogene, (MYC) is induced by EBNA 2. EBV nuclear antigen 2 (EBNA 2) is only one of several viral proteins that transactivate many viral and cellular genes, hence driving B-cell proliferation [101]. Type III latency is followed by type II latency in which (LMP1) and latent membrane protein 2 are expressed but (EBNA 2) starts to downregulate, and finally type I latency in which only EBV nucleic acid antigen 1 "EBNA 1" is expressed in memory B-cells [103]. For the viral episode to continue during each cell division, EBNA 1 is required [104]. EBNA 1 is not oncogenic per se, but it does play a role in lymphoid trans- genesis since EBV lacking EBNA 1 is much less effective at transforming cells [105]. Post-transplant lymphoproliferative disorders linked with Epstein-Barr virus are mostly B-cell lymphomas that arise in immunocompromised hosts with deficient CD8+ T-cell activity [106].

2.6. Treatment

Histology and disease progression play defining roles in NHL therapy. The severity of the illness is classified according to the Ann Arbor staging method [107]. However, few

treatment procedures really adjust the original therapeutic suggestion to account for the IPI's classification into discrete prognostic categories [108]. Most treatments, including radiation therapy, combination or single-agent chemotherapy, immunotherapy, or radioimmunotherapy, are effective against NHLs [109]. Chemotherapy is often used in treatment, and most medical professionals agree on how best to approach individual cases. However, there is considerable leeway in terms of which agent (or agents) are used, how long they are used for, and how much they are given to patients with NHL. If the lymphoma is located in the gastrointestinal system, surgery may be the first line of defence if the illness is limited or if perforation is a concern [110]. When used to localised illness, irradiation has the potential to be curative; when applied to bulky diseases, it may help reduce the recurrence rate; and when applied to palliative symptoms, it can help alleviate suffering. Standard treatment for aggressive non-lymphomas Hodgkin's was established in the 1990s using anthracycline-based chemotherapy (CHOP- Cyclophosphamide, doxorubicin, vincristine, and prednisone) [111]. More recent research has shown further benefits when rituximab is added to this regimen. It is possible for people with indolent lymphoma to go years without experiencing any symptoms. Treatment options for follicular NHL patients are varied and contentious, ranging from observation and monoclonal antibody therapy to combination chemoradiotherapy. Radiotherapy is the standard treatment for patients with early-stage (Ann Arbor I or II) follicular NHL, whereas chemotherapy, immunotherapy, immunochemotherapy, or radioimmunotherapy are used for patients in later stages of the illness when there is a therapeutic justification. Therapeutic strategies comparable to those utilised for diffuse large cell NHL are used for grade 3 follicular NHL because to its more aggressive nature. MALT NHLs tend to be slow-moving and show primarily limited illness [113]. Radiotherapy is used to treat MALT lymphomas that have spread to other organs, such as the lung, thyroid, salivary gland, breast, or orbit [114]. If *Helicobacter pylori* is involved, antibiotic treatment of gastric MALT lymphoma may be necessary. However, radiation and/or chemotherapy should be administered if submucosal involvement is present or if t translocation (11; 18) is present [115]. Anthracycline-based chemotherapy followed by involved-field radiation was formerly the treatment of choice for patients with locally advanced diffuse large B-cell NHL [116]. Patients without a substantial tumour burden in stages I and II of the Southwest Oncology Group Study were examined and randomly assigned to receive either eight cycles of CHOP or three cycles of CHOP in combination with radiation treatment [117]. When comparing patients two years following treatment,

those who had chemoradiation fared better in terms of both disease-free and overall survival. In contrast, 7 and 9 years of follow-up failed to substantiate the advantage shown with the combination treatment approach. Results from previous research comparing chemotherapy to no treatment or radiation have been mixed. Rituximab was not used in any of these investigations [118]. The combination of rituximab and CHOP, with or without field irradiation involvement, is the recommended method of numerous facilities until more research are conducted to establish the optimum treatment plan for localised stage illness [119]. Research has not established that one treatment regimen is significantly better than another for treating diffuse big stage advanced B-cell lymphoma. Given its simplicity of implementation, CHOP has become the gold standard [120]. However, this treatment option is subpar. Patients over the age of 60 were analysed by the French research organisation Groupe d'Etude DES Lymphomes de l'Adult [121] to determine if eight cycles of CHOP or eight cycles of CHOP supplemented with rituximab (R-CHOP) were more effective. Evaluation at the median follow-up of two years showed that the R-CHOP group had improved event-free and overall survival. It has also been shown that R-CHOP is better for young patients. Although R-CHOP has been shown to be effective, treatment is only curative in 50% of patients, therefore novel methods are especially required for those with poor prognoses [122]. A full response may be less common in individuals with peripheral T-cell NHL treated with the same chemotherapy treatment as those with B-cell NHL. Chemotherapy regimens including anthracyclines, such as CHOP, are still used despite the lack of data comparing their efficacy in the treatment of peripheral T-cell NHLs. Chemotherapy combined with radiation is utilised to treat T / NK nasal NHL cells [123]. TACTP (Autologous Peripheral Stem Cell Transplantation) following high dose chemotherapy is the treatment of choice for aggressive NHLs in recurrence that remain chemosensitive. Patients with aggressive NHL who did not achieve full remission in the first line of therapy but who demonstrated chemosensitivity should also be evaluated for TACTP [124]. Patients with chemoresistance or those whose illness recurs after TACTP should be offered investigational therapies. It is possible to provide allogeneic transplantation to the patient if the illness recurs after TACTP [125]. Lymphomas, which make up around 5% of all head and neck cancers, are a diverse collection of tumours that are divided into Hodgkin's lymphomas (LH) and non-lymphomas Hodgkin's (NHL) based on the presence or lack of Reed-Sternberg cells. Infectious agents (such as Epstein-Barr virus, human immunodeficiency virus, T-cell leukaemia virus 1 [126]. infection by the *Helicobacter pylori*

and hepatitis C), dysregulation of the cell cycle, and susceptibility factors associated with the host have all been implicated in the development of NHLs, the second most common malignant neoplasm of the head and neck (congenital or acquired). NHL is the second most frequent tumour of the head and neck, however it accounts for just 3.5% of oral cavity malignancies [127]. Tumors or ulcerated lesions, most often on the gums, the palate, the salivary glands, and the tongue, are the most prevalent symptom. These symptoms may be preceded by prodromal ones such tooth mobility and/or nerve paralysis [128], both of which point to a more advanced stage of the illness. Oral lymphomas are more common in people infected with the Human Immunodeficiency Virus (HIV), and they manifest with similar symptoms to those of other cancers, including swelling, discomfort, and ulceration [129]. Diffuse large B-cell lymphoma is the most frequent kind of tumour and may rapidly spread to the jaw bones. Extensive swelling and tooth loss are the primary clinical symptoms in the mandible. Correct staging is crucial because it determines the therapy that will be administered; head and neck lymphomas are typically diagnosed at an early stage, and NHLs in stages I and II can be treated using only RT, but NHLs in intermediate and advanced stages must be treated using a combination of QT and RT [130]. About 86% of oral cavity-expressing lymphomas are thought to be NHL, and their prevalence is higher in people with autoimmune disorders including immune suppression due to HIV and Sjögren's syndrome [131]. The condition may afflict people of any age, however it is most common in men between 20-79. Lesions in the soft tissues are often ulcerated, accompanied by discomfort, swelling, movement of the dental parts, loss of alveolar bone, lip paresthesia, and pathological fractures [132].

2.7. Classification of NHL

Non-lymphoma Hodgkin's is classified as indolent (low grade or slow developing) or aggressive (high grade or rapid growing).

1- Type: Comprises more than 50 subtypes, depending on the appearance of the cells;

2- Stage: Indicates the lymphoma's location and extent. The stage (I, II, III, or IV) is determined by the afflicted site: lymph nodes or other organs and tissues.

3- Non-lymphoma Hodgkin's is classified into the following stages:

4- Stage I: lymphoma cells are detected only in a single group of lymph nodes; 4-Stage II: lymphoma cells are discovered in at least two groups of lymph nodes on the same side of

the diaphragm (below or above), or lymphoma cells are identified in an organ and its nearby lymph nodes on the same diaphragm.

1. Stage III: The lymph nodes above and below the diaphragm are common sites for lymphoma to spread. You could also find it in a different organ or tissue close to a cluster of lymph nodes.
2. Stage IV: Lymphoma has metastasized when it is found in more than just the lymph nodes, or when it is detected in the liver, blood, or bone marrow.

If a patient has a fever above 38 degrees Celsius, loses 10 percent of their body weight in six months for no apparent reason, and sweats excessively at night, their prognosis is dismal. In order to assess the severity of these conditions, it is sometimes essential to do further diagnostic procedures, such as imaging (X-ray, CT, PET-CT), invasive testing (biopsies), and blood work. Insights on the patient's classification, type, and stage help clinicians arrive at an accurate diagnosis and formulate an effective treatment strategy [133-134].

3. MATERIALS AND METHODS

3.1. Sample Selection

Clinical and laboratory information was gathered from the medical records of 50 patients who had been diagnosed with FL. There were 20 women and 30 males in the sample, and their average age was 56 years and 1 month. No new intervention was necessary for this study (new collections of liquids or tissues), and only the principal investigator had information on the identity of the patients to proceed with the adequate collection of data and tissues. The subjects of this research had the data of clinical and laboratory evolution analyzed, and the availability of tumor tissue filed in paraffin blocks and blood collated to evaluate the level of EBV-EBNA-1 IgM and IgG in case and controls group the samples were collated from January 2020 to March 2021. Pathology Department of Hawler Medical University, Erbil (Hawler) in Iraqi Kurdistan Region. Patients with aggressive lymphoma concomitant to the diagnosis, individuals with infection by the human immunodeficiency virus, and localized skin disease were excluded. Only patients who received treatment with rituximab and were infected by Epstein Barr virus were included. The necessary clinical and laboratory data were collected from the patients' medical records. No new intervention was necessary for this study (new collections of liquids or tissues), and only the principal investigator had information on the identity of the patients to proceed with the adequate collection of data and tissues. The subjects of this research had the data on clinical and laboratory evolution, and the availability of tumor tissue filed in paraffin blocks. Patients with aggressive lymphoma concomitant to the diagnosis, In our practical internship at the anatomy-pathology and hematology services of the Baghdad teaching hospital lasting one month, we could do a descriptive study in two parts, the first of which is an epidemiological study of non-Hodgkin lymphoma while the second is exploitation of non-Hodgkin's lymphoma using the technique of immunohistochemistry.

3.2. Evolution of Epstein-Barr Virus IgG and IgM Antibody

3.2.1. The Tests Principles

An enzyme-linked immunosorbent test IgM and IgG ELISA Kit measures levels of antibodies specific to ebv in a solid phase (ELISA). In order to reduce competitive inhibition from particular IgG and remove rheumatoid factors, patient samples are diluted with sample diluent and treated with IgG-RFSorbent, which contains hyper-immune anti-human IgG-class antibody. Pretreatment like this helps to prevent unintended positive or negative results. The recombinant EBV nuclear-1 antigen is coated as a solid phase on microtiter wells. These wells are used to hold diluted patient specimens, as well as controls that are ready to be used. Antibodies against EBNA-1 from both test and control samples bind to immobilised antigens during incubation. Anti-human IgM antibodies coupled with horseradish peroxidase are then dispensed into the wells, following a washing step to remove unbound sample and control material. During a second incubation, this anti-IgM or anti-IgG conjugate binds specifically to IgM or IgG antibodies, forming enzyme-linked immune complexes. After a second wash to eliminate free conjugate, positive immune complexes are detected by incubation with TMB substrate, which causes them to become blue. When the enzymatic indicator reaction is stopped using sulfuric acid, the blue hue is transformed into a yellow one. The intensity of this colour corresponds with the concentration of EBNA-1-specific IgM and IgG antibodies in the patient samples. Measurements of absorbance at 450 nm are made using an ELISA microtiter plate reader.

3.2.2. Reagent Preparation

Before usage, bring to room temperature all reagents and the required number of test strips.

3.2.3. Washing Solution

Wash Solution 1+19 (10 mL + 190 mL), for example, should be diluted with clean, sterile redistilled water. A pH 7.2 dilution of wash solution, used at 0.5 mL per unit of measure, is described as follows: The crystals may be dissolved by gently heating the solution in a water bath to 37 degrees Celsius. Make sure the crystals are completely dissolved before using them. For 4 weeks, the diluted Wash Solution is stable at temperatures ranging from 2 to 8 degrees Celsius.

3.2.3.1. Dilution of Specimens

The samples from each individual patient need to be diluted with Sample Diluent before analysis. To absorb rheumatoid factor, these diluted samples are treated with IgG-RF-Sorbent.

1. Each patient specimen should be diluted 1:50 with Sample Diluent, thus a 10 litre specimen would be mixed with 0.5 millilitres of Sample Diluent. Use a good mixing technique.
2. Before using the IgGRF-Sorbent, thoroughly mix it.
3. This concentrated sample should be diluted further with IgG-RF-Sorbent by a factor of 1+1. Use a good mixing technique.
4. Keep at room temperature for 15 minutes, up to 2 hours, before recombining.
5. For the ELISA, take 100 L of these pretreatment samples.

3.2.4. Assay Methodology

All specimens and controls must be distributed and identified according to the protocol supplied in the kit, which includes diluting the Wash Solution and preparing patient samples as described in section 5.3.

1. Microtiter strips or wells, as the case may be, should be inserted into the holder. The substrate blank (well A1), the negative control (well B1), the cut-off control (wells C1 and D1), and the positive control (well E1) should all be separated from one another.

Users are responsible for identifying replicate patient and control samples.

2. Pour 100 L of Neg. 100 L of Cut-off Control into well B1 Control 100 L of Pos into wells C1 and D1. 100 L of each pretreatment sample with fresh disposable tips into well E1 and 100 L of control into well E1. Leave well A1 blank for the substrate!
3. Use the foil that comes with the box to cover the wells. Allow to incubate at 37 degrees Celsius for an hour.
4. Put the wells' contents through a brisk shake. Five times using 300 litres of diluted Wash Solution, rinse the wells. If there are any lingering drops, you may get rid of them by using a sharp tool to hit the wells on absorbent paper.

***Important note:** The right execution of the washing procedure has a significant impact on the sensitivity and accuracy of this assay!

5. Fill each well with 100 L Enzyme Conjugate, except A1.
6. Incubate at ambient temperature for 30 minutes (20°C to 25°C).

Avoid direct sunlight exposure!

7. Give the wells a good shake to mix the contents. Put 300 litres of diluted wash solution through the wells five times. Striking the wells with a sharp tool on absorbent paper can remove any last traces of moisture.
8. Fill each well with 100 L of substrate solution.
9. It is recommended to incubate the mixture for 15 minutes at room temperature (between 20 and 25 degrees Celsius) and in the dark.
10. To halt the enzymatic process, add 100 L of stop solution to each well.

Any blue hue that develops throughout the incubation process turns yellow.

Please keep in mind that very positive patient samples might result in black chromogen precipitates!

11. Use a microtiter plate reader to measure the optical density at 450/620 nm within 30 minutes after applying the stop solution.

3.2.5. Measurement

The substrate blank in well A1 should be used to zero out the ELISA microplate or microstrip reader. Well A1's absorbance value should be subtracted from all other absorbance values observed if the ELISA reader cannot be set to zero using the substrate blank in well A1. The absorbance of each well should be measured at 450 nm, and the results should be recorded in the distribution and identification plan with the control and patient sample numbers. If you're going to do dual-wavelength measurements, you should choose 620 nm as your standard. If possible, calculate the average absorbance of each copy.

3.2.6. Results Validation of the Test Run

If the following requirements are satisfied, the test run may be declared valid: A1 blank substrate: Absorbance less than 0.100 Neg. B1 management: Cut-off for absorbance values less than 0.200 C1/D1 control: Absorbance value between 0.350 and 0.850 Pos. In E1, the absorbance value should be between 0.650 and 3.000.

3.2.6.1. Calculation

As an indicator of absorbance, the Cut-off Control (CO) value is used. Take the average of the two (2) values you found for the Cut-off Control (for instance, C1/D1). Examples: (0.54 + 0.56) 2 = 0.55 = CO.

3.2.6.2. Interpretation

Absorbance values in the positive range for patients (mean) are those that are at least 10% higher than CO (Mean OD patient > 1.1 x CO).
Patient (mean) absorbance values 10 percent above and 10 percent below the CO Experiment redone in two to four weeks using fresh patient samples (0.9 x CO Mean OD patient 1.1 × CO). Another round of ambiguous results from the second test negative. Ten percent or more below CO in patient (mean) absorbance (Mean OD patient 0.9 x CO).

3.2.7. Results in DEMEDITEC Units [U]

(Average) Absorbance Value of Patients * 10 = (Units = U) CO

Using the above example, 1.580 * 10 = 29 U0.55

Results Analysis

Minimum detectable value = 10 U

Zone d'ombre = 9-11 U

Asserts a negative: 9 U

Verdict: 11 U

3.3. Anatomopathological Study

Our retrospective and descriptive study lasting one month is based on 50 cases with non-Hodgkin lymphoma with lymph node and extranodal locations.

3.3.1. Type of Sample

In our study, biopsies represented 79% of samples. Indeed, the biopsy is the sample that best diagnoses solid tumors. The most removed organ is the lymph node because the lymphadenopathy is the seat of a malignant tumor and is easily accessible to the biopsy. Each sample reaches the laboratory in formalin, and it must be accompanied by an information sheet or it is to mention the type of sample and the document number.

Our collected parts:

• Lymph node biopsy

• Gastrointestinal biopsy

• Bone biopsy

These biopsies were processed using direct histology and immunohistochemistry techniques.

3.4. Histological Study

3.4.1 Microscopy

Measurement and description of the parts. The detailed microscopic examination is an essential part of the study of an operative specimen: the specimen is examined, measured, weighed, palpated, and then dissected. This examination gives indications for the prognosis of the disease (in particular the size and the localization) cancer and it allows you to select the areas to be sampled for the microscopic study: injured areas, areas of healthy macroscopic appearance, and limited resection. After choosing the samples for microscopic analysis, the remains of the operative part are kept for a few days or weeks so that additional samples can be taken if necessary. Using a scalpel and under the respiratory hood, the doctor makes 1.5-thick cuts which will then be placed in cassettes bearing the last part number, these will be immersed in vials containing formalin diluted to 1/3 in order to fix the antigens and to oppose the premature dehydration of the cells and especially the putrefaction of the tissues.

3.4.2. Dehydration

The samples having completed their fixation are placed in cassettes, then they are placed in a dehydration device called Technicom (Fig. 3.1) by passing through 12 bins including 7 tanks: containing ethanol (the first three tanks at 70% 80% 90% (10h) and the rest 100% pure ethanol, (1h) 3 tanks: containing xylene (1h * 3) 2bins: filled with liquid paraffin at 56 ° C (1h * 2).

Figure 3.1: Dehydration device (Technicom).

3.4.3. Heated Paraffin Inclusion

The inclusion is manual, the tissue fragment is properly reoriented in the direction of the section in a paraffin mold (Fig. 3.2 A), the purpose of which is to allow fine and regular sections to be made. The most widely used inclusion medium is paraffin, our sample is immersed in a mold containing liquid paraffin melted by heating, which then infiltrates the entire part. Once the sample is fully immersed it is allowed to cool in a freezer to obtain a block. Hard paraffin inside which the removed part is included (Fig. 3.2 B).

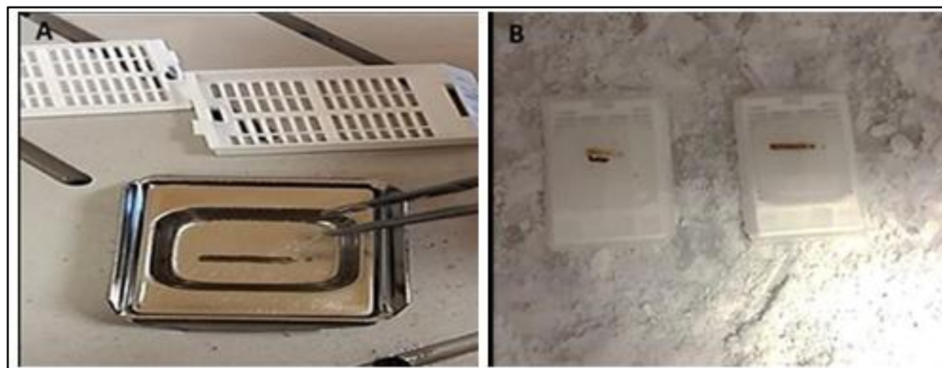


Figure 3.2: Inclusion in paraffin and cooling of the blocks.

3.4.4. Sections of the Paraffin Block

Using a microtome set at 35 µm which serves to remove excess paraffin until our fragment becomes visible then we set it to 2.5 µm in order to obtain section slices (sections) of 2 at 5 µm thick (Fig 3.3 A). Using a needle, the tape obtained is placed in a water bath to remove these folds so that they can then be spread and dried on a slide (Fig. 3.3 B). In order to ensure

good adhesion to the tissue slide before staining. Finally, we put our slides together in a rack (Fig. 3.3 C) in order to transfer them to the oven set at 65 ° for 2 hours with the aim of dewaxing and removing all traces of paraffin to facilitate staining (Fig. 3.3 D).

Figure 3.3: Preparation of sections for staining.

3.4.5. Coloring

Hematein Eosin (H.E.) combines hematein, which stains nuclei purple, with eosin, which stains cytoplasm pink, in routine stains.The stains used on slides amplify the contrasts, making it easier to identify the various aspects of the preparation. Because the dyes are in an aqueous solution, the sections must be rehydrated by running them through two alcohol baths (2min*2). Then we rinse with distilled water for 2 to 3 minutes.

* The hematein staining for one minute

* We rinse our slides with distilled water.

* Staining with eosin for one minute

* We dehydrate our slides to remove excess dyes in 2 ethanol baths after drying

* We immerse our slides in 3 xylene baths for 4 minutes (Fig. 3.4 A.B)

***** At the end, we obtain slides with pink purple coloring (Fig. 3.4 C)

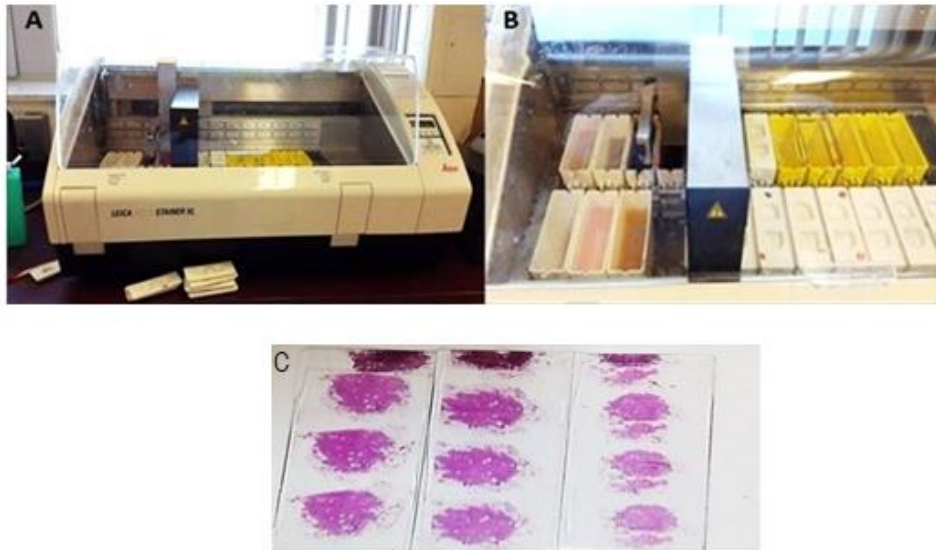


Figure 3.4: Staining with hematein eosin.

3.4.6. Assembly

After having undergone dehydration, the colored sections are mounted between slides and coverslips with a synthetic resin whose refractive index is close to that of glass (Fig. 3.5).

Figure 3.5: Assembly of the blades.

3.4.7. The Microscopic Study

The observation of the colored sections is carried out using an optical microscope (Leica DM500 and DM750). This device makes it possible to obtain an image magnified (20 to 1000 times) by an optical combination of the cup illuminated by a light passing through it.

3.5. The Immunohistochemical Study

• Principle: Immunohistochemistry is a method of localizing proteins in the cells of a section of tissue, by detecting antigens by means of antibodies or Ag-Ac binding is revealed by a tracer (fluorescent, enzyme) Immunohistochemistry exploits the fact that an antibody specifically binds to antigens in biological tissues. The antibodies can be of polyclonal or monoclonal origin.

• Deparaffinization: Using a microtome, sections of 2 to 3 are made, the latter is placed on silanized blades which, then, are transferred to:

1 xylene container for 15 min

1 container of pure ethanol for 15 min 1 container of distilled water for 10 min (Fig. 3.6).

Figure 3.6: The three dewaxing tanks.

Blocking of endogenous peroxidases: One proceeds by removing the excess washing buffer by tapping and carefully wiping the outline of the sample to remove all the remaining liquid and maintain the reagent inside the zone specified.

The sample is encircled with a DAKO Pen to prevent spillage (Fig. 3.7 A). Then 200 µl of the endogenous enzyme blocking solution is applied to cover the latter. Then our slides are allowed to incubate for 5 (± 1) minutes.

Finally, we soak our slides in a new wash buffer solution for 5 minutes after giving them a gentle washing with a wash buffer solution from a squeeze bottle (Fig. 3.7 B).

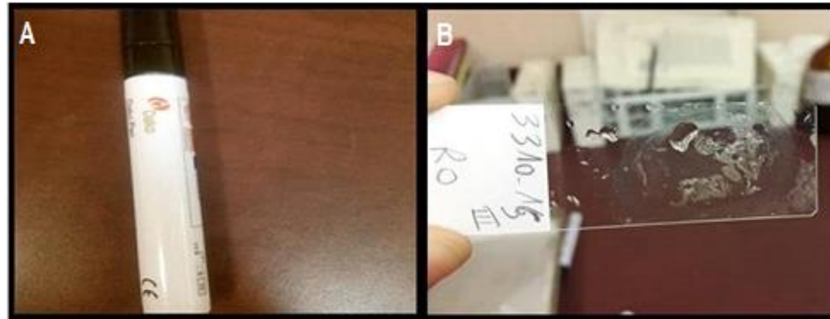


Figure 3.7: Strapping of samples with the DAKO PEN.

Endogenous peroxidase blocking is carried out in order to avoid background noise, due to the peroxidase staining of red blood cells.

• RQ: Solution for inhibiting endogenous enzymes, detergents, enzyme inhibitors, and a preservative, with a pH of 2.

PBS as a washing buffer

• Antigen unmasking: To unmask the antigen, submerge the slide in Tris-EDTA buffer, pH 9.0, 0.05 percent Tween-20 *, and incubate at 95 ° C in a bath for 45 minutes. 5 minutes in 0.05 M Tris-HCl buffer (pH 7.6) with 0.2 percent Tween-20, then allow the slide to cool at room temperature for 10 min. And finally, we rinse it with distilled water (Fig. 3.8) .

Figure 3.8: Unmasking solution.

• Application of the primary antibody: The excess wash buffer is removed by tapping the slides, then 200 µl of the rabbit or mouse primary antibody solution is applied to cover the sample. And incubate for 10 (± 1) minutes. Washing is carried out twice for 5 minutes in 0.05 M Tris-HCl buffer (pH 7.6) with 0.2% Tween-20 for 5 min.

• Application of the secondary antibody: Our slides are washed twice for 5 minutes in 0.05 M Tris-HCl buffer (pH 7.6) with 0.2% Tween-20 (Fig. 3.9 A.B).

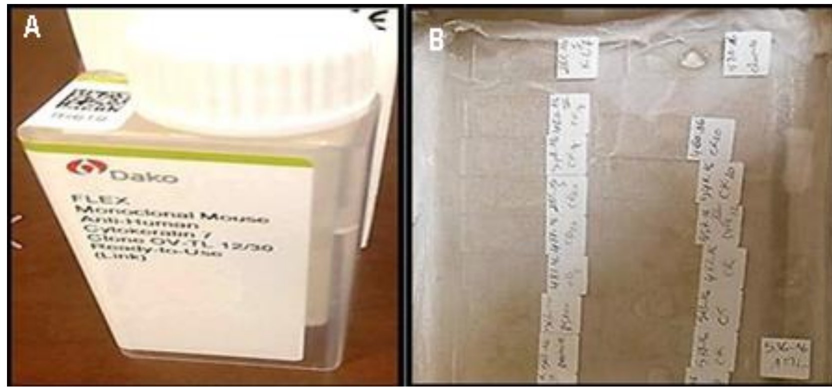
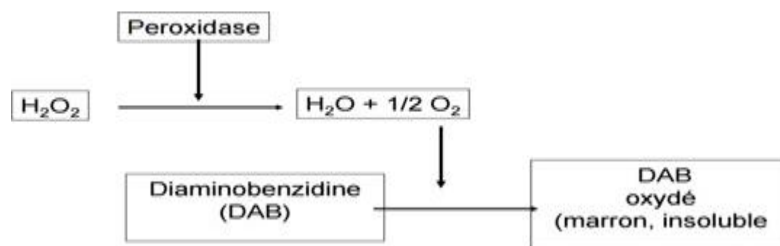


Figure 3.9: The application of ACs.

• Happened by diaminobenzidine (DAB): The secondary antibody is linked to a peroxidase, which converts DAB into a colourful product in the following way.

Immunohistochemistry method as nucleic acid staining material is given in the diagram below.



The excess wash buffer is removed by tapping and wiping the slides as before. Then apply 200 µl of the dosed DAB solution to cover the sample. And incubate for 5 to 15 minutes. And finally we rinse with distilled or deionized water from a squeeze bottle (Fig 3.10 A. B).

Figure 3.10: Application of diaminobenzidine.

• Counterstaining: We immerse our slides in a hematoxylin bath. The duration of incubation depends on the concentration of hematoxylin used and then they are rinsed thoroughly with

distilled water. We dehydrate our tissues in 2 baths of 96% benzyl alcohol for 5 min each, and finally, we wash them in 2 xylene baths for 2 minutes each.

• Mounting: Dako Glycergel TM Mounting Medium (C0563) or Paramount Aqueous Mounting Medium (S3025) were used for aqueous mounting.

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• Mounting: Dako Glycergel TM Mounting Medium (C0563) or Paramount Aqueous Mounting Medium (S3025) were used for aqueous mounting.

3.6. Histology

The tumor tissue samples included in paraffin and histological slides were recovered from the archives of the Institution's Department of Pathological Anatomy and re-evaluated histologically, colored with Eosin and Hematoxylin. The histological grade was evaluated, according to WHO 2008 criteria. Two areas of 1mm in diameter including tumor were selected to build a tissue microarray (TMA, from English, tissue microarray) and placed with the antibodies highlighted below for analysis. immunohistochemistry. In this way, the rest of the tissue was intact, without prejudice to future demands by the patient or guardian.

3.7. Immunohistochemistry

TMA sections were subjected to immunohistochemical study, with sample preparation, antigen recovery, dewaxing, and preparation with antibodies for cell proliferation markers, Ki-67 from manufacturer Dako, and Top-2 from manufacturer Leica. The slides were immersed in ethylenediaminetetraacetic acid (EDTA) / TRIS buffer, and taken in a water bath, at a temperature of 97.5ºC for 40 minutes. After cooling at room temperature for 20 minutes, the slides were washed in distilled water and placed in phosphate-buffered saline (PBS) with TWIN 20 (Sigma code P7949), with three baths of five minutes each. Then, the sections were incubated with the antibodies previously diluted in a solution of PBS-TWIN20 + 0.1% BSA. In the TMA slides, the markers were evaluated in a quantitative manner [number of positive cells per µm2], performed through images by microscopy, and captured

by a 20x camera of immunohistochemical preparations in the Aperio Scanscope® apparatus, based at the A.C.Camargo Cancer Center.

Each TMA slide was mapped and programmed for analysis by the “Positive Pixel Count (v.9)” algorithm, using the TMA Lab system. Areas of fibrosis or necrosis were eliminated using the "pen tool" system. The mentioned algorithm classifies the pixels, in each TMA spot, as “0” (negative), “1” (weakly positive), “2” (average positive), and “3” (strongly positive). These data were processed as follows:

Core markers of specific populations of the cellular microenvironment such as Top2a and ki67, a score for each case was performed and calculated by dividing the number of pixels of any positivity by the total number of pixels (fraction of positivity), according to the formula: = 3 (n. Strong Positive / n. Total Pixel) x 100 + 2 (n. Moderate Positive / n. Total Pixel) x100 + 1 (n. Weak Positive / n. Total Pixel) x100. The result will be between 1 and 300. The immunohistochemistry quantification of cell proliferation markers was connected to the histological grade traditionally assigned to overall survival, disease-free survival, and the likelihood of transition to aggressive lymphoma with FLIPI. This association was investigated bivariate and multivariate in order to determine if any of the proliferation markers, whether linked or not with other classics, had independent prognostic value.

3.8. Molecular Study

3.8.1. Quantitative Real-Time Reverse Transcription (RT-qPCR)

Gene expression was determined by using a quantitative real-time polymerase chain reaction (RT-qPCR) test to analyse cloned mRNA transcripts from blood samples; the expression of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as an internal standard. These are the procedures that were followed throughout this analysis.

3.8.2. Total RNA Extraction

For total RNA extraction, we followed the manufacturer's instructions for the Trizol kit produced by the Korean firm Pioneer:

When inactivating cells and solubilizing cell components, TRIZOL Reagent does so without disrupting RNA. After adding chloroform and centrifuging, transfer to Eppendorf tubes containing 0.5 mL of DEPC water.

Two minutes were spent centrifuging the tubes at 12,000 revolutions per minute.

After step 3, the tampon was removed, leaving just the tissue; then, 0.5 ml of Trizol was added, and the tissue was crushed with microplastics until mashed and homogenised thoroughly.

Four, we added one millilitre of Triazole and two hundred microliters of chloroform to the test tubes holding the tissue fragments.

After 5 minutes in the freezer, the tubes were shook using a vortex device.

Six, the tubes were centrifuged at 12,000 rpm for 15 minutes.

Add 500 l of isopropanol to the exudate after transferring it to a fresh Eppendorf in step 7.

In step eight, we used a Vortex shaker and chilled the tubes for 10 minutes.

As step 9, we centrifuged the tubes for 15 minutes at a speed of 12000 rpm.

The supernatant was discarded, and the precipitate was reduced to a pellet form by centrifugation at 12000 rpm for 5 minutes. Step 10 then saw the addition of 1 ml of 80 percent ethanol alcohol to the pellet, which was agitated continuously in a Vortex device. Supernatant was discarded and precipitate was collected.

Turn the tubes upside down on blotting paper and let the precipitate at room temperature for 10 minutes.

Twelve, add 50 l of DBC and let it sit in a water bath at 70 C for 10 min. After RNA has been isolated, it is kept at -20 degrees Celsius.

3.8.3. Measurement of RNA Yield and Its Quality

With the use of a specialised instrument called a Nanodrop spectrophotometer, we were able to determine the RNA concentration in ng/l and the RNA purity by monitoring the absorbance at a degree (260/280 nm) after the RNA was extracted.

1- After turning on the Nanodrop device, the RNA-type DNA measurement program was selected.

2- We filter the device by placing 2 microliters of Free nuclease water using a sterile micropipette on the surface of the scale substrate.

3- We press the OK button to start the process of measuring the concentration of RNA, using 1 microliter of each sample of the extracted RNA, and then we clean the substrate of the device scale again to measure the other sample.

4- The purity of the extracted RNA samples was determined by reading the absorbance in a Nanodrop Spectrophotometer at two wavelengths (260/280 nm), as the extracted RNA is considered pure when the absorbance ratio is (1.8 and 2).

3.9. Determination of DNase1 by DNase inactivation

DNase I was used to the mRNA extract to remove any lingering DNA from the purification procedure, as detailed by the enzyme kit in the table below (Table 3.1). The enzyme was deactivated by heating EDTA in a water bath to 65 degrees Celsius for 10 minutes.

Table 3.1: Several DNase I treatment.

Mix	Volume
Total RNA 100ng/ul	10 ul
DNase I enzyme	1ul
10X buffer	4 ul
DEPC water	5 ul
Total	20 ul

3.9. Complementary Synthesis cDNA

The Accupower Rockscript RT Premix kit from the Korean business Pion was used to create complementary DNA from the isolated RNA samples (Table 3.2).

Table 3.2: Components of RT master mix.

RT master mix	Volume
Total RNA 100ng/ul	10 ul
Random Hexamer 20pmol	1 ul
DEPC water	9 ul
Total	20 ul

Then the components of the RT master mix mentioned in the above table were added to the cDNA synthesis kit tubes containing the reverse transcription enzyme, and then all tubes were placed in a vortex centrifuge (Exispin) at 3000 rpm for three minutes. The tubes were transferred to the Thermocycler, and the thermal conditions for the cDNA manufacturing process were applied according to the way the kit works, as shown in the following Table 3.3:

Table 3.3: cDNA synthesis kit works.

Step	Temperature	Time
cDNA synthesis (RT step)	50 ° C	1 hour
Heat inactivation	95 ° C	5 minutes

Then the preservation samples were transferred at -20°C until they were used in the Real-time PCR assay.

3.10. Quantitative Real-Time PCR (qRT-PCR)

The cDNA qPCR test was carried out with the AccuPower 2x Green Star Real-Time PCR kit and the Pioneer ExicyclerTM 96 real-time Quantitative Thermal Block. This test is based on the interaction of the Syber Green dye with the amplified genes in the qRT-PCR machine, as shown in Tables 3.4, Table 3.5.

Table 3.4: Preparation of the reaction mix for qPCR for the L1PA2 target gene.

qPCR master mix		**Volume**
cDNA template		5 µL
Primers	Forward primer	1 µL
10 pmol	Reverse primer	1 µL
2x green star master mix		12.5
DEPC water		5.5 µL
Total		25 µL

Table 3.5: Preparation of the reaction mixture for qPCR for standard conservative GAPDH genes.

qPCR master mix		**Volume**
cDNA template		5 µL
Primers	Forward primer	1 µL
10 pmol	Reverse primer	1 µL
2x green star master mix		12.5
DEPC water		5.5 µL
Total		25 µL

Then these components mentioned in the above tables were added to the special qPCR tubes, and then all tubes were placed in a vortex centrifuge (Exispin) at rpm3000 for three minutes and then a plate was transferred to (the Miniopticon Real-Time PCR BioRad.USA). The qPCR Thermocycler conditions were applied for all genes according to the method of working the kit as in the following Table 3.6.

Table 3.6: qPCR thermocycler conditions for gene.

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	3 min	1
Denaturation	95 °C	20 sec	45
Annealing \Extension Detection (scan)	60°C	30 sec	
Melting	60-90 °C	0.5 sec	1

3.11. Real-Time PCR Data Analysis Method

The results from the polymerase chain reaction were quantitatively evaluated in real-time using the link technique described by [181]. which depends on extracting both the relative quantity and the absolute quantity through a correction process. Equation of target genes with control samples so that the results have a biological meaning.

The gene for conservatism In order to determine the relative gene expression or fold change in the target genes, we may employ GapdH as a corrective gene. The relative quantitative gene expression levels were then used to adjust the target gene's threshold cycle number (Ct) values using the Ct values of the reference gene (multiple changes). When using this approach, the relative expression levels are extracted using the CT method with the corrective gene, and one of the experimental samples serves as a calibrator (e.g., the control samples). In this particular case of Gene:

1. Correcting the Ct value of the conservative gene reference gen(ref) from both the target gene for the measurement sample and the target gene for the test sample as in the following two equations:

$$\Delta Ct\ (test) = Ct\ (target, test) – Ct\ (ref, test)$$

$$\Delta Ct\ (calibrator) = Ct\ (target, calibrator)– Ct\ (ref, test)$$

2. Correct the value of ΔCt for the target gene of the test sample from the value of ΔCt of the target gene for the measurement sample as in the following equation:

$$\Delta\Delta Ct= \Delta Ct\ (test) – \Delta Ct\ (calibrator)$$

3. The diploid change of relative gene expression is calculated according to the following equation:

$$\text{Fold change} = 2^{-\Delta\Delta Ct}$$

3.12. Primers

The primers in this study were designed using the NCBI GenBank Data website and using the Primer3 plus design program. These primers were prepared from Genaid DNA Blood Midi Kit (Korea) as in the Table 3.7 below :

Table 3.7: The gene primers that were used in this study.

Primer	Sequence		Amplicon
L1PA2	F	5-CGTGTGCATGTG TCTTTATAGC-3	86-bp
	R	5-GAAATACCATTTGACCCAGCC-3	
miR-532	F	5'-GCCCATGCCTTGAGTGTAG-3'	bp-78
	R	5'-GTGCGTGTCGTGGAGTCG-3'	

3.13. NCBI-Reference Sequence

Peripheral blood was collected from both healthy and ill people. A 4-milliliter (mL) aliquot of blood was drawn into a K3-EDTA-treated vial, and the tube was centrifuged within an hour. Centrifuged at 4 degrees Celsius and 1,000 g for 10 minutes, the blood was fresh. Following cautious transfer to a 0.5 mL Eppendorf tube, the plasma was centrifuged for 10 minutes to eliminate any remaining cell debris. Supernatants (plasma) were separated into aliquots and stored at -80° C.

3.14. The Diagnostic Value of Circulating L1PA2

We isolated DNA from 0.5 mL of plasma using the Genaid DNA Blood Midi Kit (Korea) and kept it frozen at -20º C. qPCR was used to determine the DNA concentration, utilising human multi-copy L1PA2 as the internal standard.

The primer sequences looked like this: According to [166], the forward and reverse sequences for an 86-bp fragment are 5-CGTGTGCATGTG TCTTTATAGC-3 and 5-GAAATACCATTTGACCC AGCC-3, respectively. Genomic DNA from healthy controls was diluted by a factor of 10 to generate a calibration curve for cfDNA concentration. Based on what was already published, we determined the concentration of genomic DNA by measuring its UV absorbance. The linear dynamic range of the standard curve was established at 0.1–100 ng of DNA. Utilizing a SuperScriptTM III One-Step RT-PCR System with PlatinumTM Taq DNA Polymerase, we amplified our samples by subjecting them to

an initial activation step at 95º C for 10 minutes, followed by 40 cycles of 15 s at 95º C and 1 min at 60º C. After each PCR cycle, the amplified product's specificity was evaluated using a melting curve analysis (Table 3.8).

Table 3.8: RT master mix components.

RT master mix	Volume
2X Reaction Mix	25X
Template RNA (.01 pg to 1 µg)	X ul
Sense primer (10 µM)	1 ul
Anti-sense primer (10 µM)	1 ul
SuperScript™ III RT/Platinum™ Taq Mix	2 ul
Autoclaved distilled water	Up to 10 ul

3.15. Qpcr Thermocycler Conditions

The qPCR plate was loaded in Real-time pcr machine and the following thermocycler protocol was done accoeding to qPCR kit protocol in the following Table 3.9:

Table 3.9: qPCR thermocycler conditions.

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	50 ºC	30 minutes	One cycle
	90ºC	2 minutes	
Denaturation	94ºC	15 seconds	40 cycles
	60ºC	30 seconds	
Annealing\Extension Detection (scan)	86ºC	60 seconds	
	68ºC	60 seconds	1 c
Melting	60-90 °C	0.5 sec 1	1

3.16. The Diagnostic Value of Circulating Mir-532-5p

TRIzol reagent was used to extract total serum RNA. Following the measurement of RNA purity and concentration, the obtained RNA was reverse transcribed into cDNA, and qPCR was carried out using a SuperScriptTM III One-Step RT-PCR System with PlatinumTM Taq DNA Polymerase) and SYBR green I Master Mix Kit (Table 3.10). The PCR has performed under the following conditions: initial denaturation at 95º C for 5 minutes; 30 cycles of 95º C for 30 seconds, 60º C for 30 seconds, and 72º C for 20 seconds; and a final extension at 72º C for 10 minutes. The 2–Ct technique was used to calculate the relative miRNA expression level. According to [172], the primers utilized were: miR-532-5p forward, 5'-GCCCATGCCTTGAGTGTAG-3', and reverse, 5'-GTGCGTGTCGTGGAGTCG-3'.

Table 3.10: RT master mix components.

RT master mix	Volume
2X Reaction Mix	25X
Template RNA (.01 pg to 1 µg)	X ul
Sense primer (10 µM)	1 ul
Anti-sense primer (10 µM)	1 ul
SuperScript™ III RT/Platinum™ Taq Mix	2 ul
Autoclaved distilled water	Up to 10 ul

3.17. Qpcr Thermocycler Conditions

The qPCR plate was loaded in Real-time pcr machine and the following thermocycler protocol was done accoeding to qPCR kit protocol in the following Table 3.11.

Table 3.11: qPCR thermocycler conditions.

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	50 ºC	30 minutes	One cycle
	90ºC	2 minutes	
Denaturation	94ºC	15 seconds	40 cycles
	63ºC	30 seconds	
	86ºC	60 seconds	
Annealing\Extention	68ºC	60 seconds	
Detection(scan)	Holding	-	

3.18. Statistical Analysis

The analyses were carried out using Stata Statistical Software® 12.0 (College Station, Texas, United States), with categorical variables distributed in percentages and continuous variables summarized by median and interquartile range (IQR) or means and intervals 95 percent confidence interval, based on the results of the Shapiro-Wilk test for normal distribution.

To assess the statistical difference between two groups, numerical variables were analyzed using the unpaired Student's t-test or its nonparametric analog Mann-Whitney test, and ANOVA to verify the statistical difference in variables with two or more groups, or their nonparametric analog Kruskal-Wallis test. Spearman's correlation was also used to calculate the correlation coefficient between these variables. The multiple analysis was conducted by Cox regression using the stepwise backward step-by-step method, based on an initial set of variables composed of those with $p < 0.20$ in the bivariate analysis or when they had plausibility biological.

The probability of survival was calculated using the time elapsed between the diagnosis of follicular lymphoma and the date of death, considering this outcome as a failure and its non-occurrence as censorship. Progression-free time was calculated using the time elapsed between the diagnosis of follicular lymphoma and the date of tumor progression, considering this outcome as a failure and its non-occurrence as censorship. The Kaplan-Meier curve was used to graphically represent the survival functions that were analyzed by the bivariate Log-rank test, considering the two-tailed $p \leq 0.05$ statistically significant.

4. RESULTS

4.1. Epidemiological Results

Our study concerns 50 cases grouped together in the haematology service of the Pathology Department of Hawler Medical University, Erbil (Hawler) in Iraqi Kurdistan Region from January 2020 until March 2021. 50 patients were evaluated, the average age of male patients was 56.1years (Min = 21; Max = 79) (95% CI = 52.5-58.7) and female patients equal to 56.4 years (Min = 19; Max = 91) (95% CI = 53.9-58.9), with no statistical difference. The level of The anti-EBNA-l antibody were measured in both cases and controls group. In our study we identified 4 age groups where the average age of our patients is 56.5 years. With extremes of 18 years up to 79 years. Almost half of our patients belong to the age group over 60 years old, or 42% of cases, on the other hand, the group of patients under the age of 20 only constitutes 8% of cases. In general, the average age of patients with NHL in developing countries is lower than that in Western countries because it is between 60 years according to Doyen et al. [80] in which was confirmed by our results.

4.2.Classification of NHL According To Sex:

The distribution of patients by sex showed a predominance of men, i.e. 60% and only 40% of women (Table 4.1).

Table 4.1: Distribution of patients by sex

Age in years (mean. 95% CI)	**56.4**	**(54.1 - 58.0)**
Sex		
Male	30	60
Female	20	40

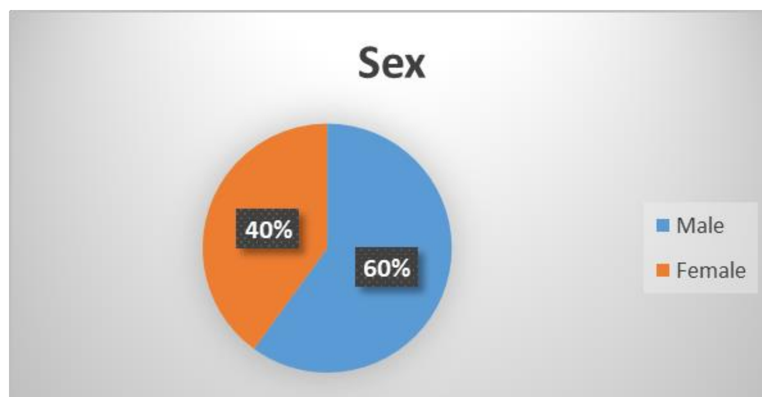

Figure 4.1: Distribution of patients by sex.

Our gender results showed 60% male dominance versus 330% female (Fig.4.1). This is consistent with the work published by: Eve Romon et al., Who reported that of the estimated 12.68 million new cancer cases worldwide during 2008, 6.64 million were males and 6.04 million were females by Boudjerra et al. [76] with a dominance of 80%.

4.3. Classification of NHL According to Geographical Origin

Our patients came from different regions of Iraq. This distribution noted the existence of a maximum of patients residing in the capital region (Baghdad) with a frequency of 30%, followed by the region of Erbil with a frequency of 20% and the rest distributed among the different regions of the Diyala:15%, Wasit: 15%, ThiQar: 10% and Al-Najaf: 10% (Fig 4.2).

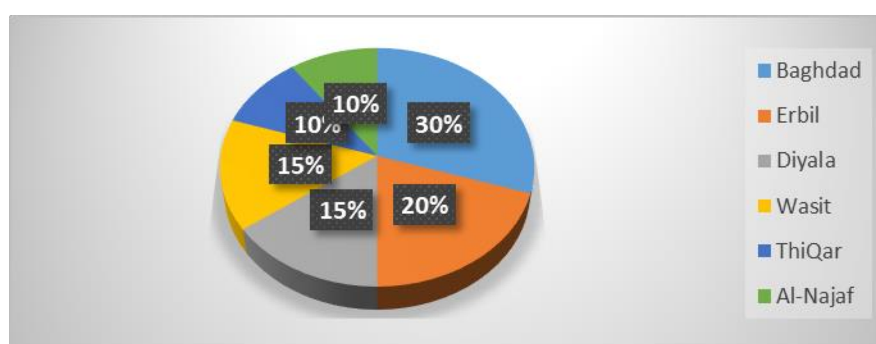

Figure 4.2: Distribution of patients by geographic origin.

It is suggested that this rise is due to the geography site of the Baghdad as much as it has the only haematological service in Iraq, as well as this service contains very sophisticated medical equipment and also well develop the competence of doctors. Classification of NHL according to characteristics clinical and pathological variables was shown in Table 4.2.

Table 4.2: Clinical and pathological characteristics of patients with follicular lymphoma.

Variables	N	%
Symptoms B		
Gift	17	34.0
Absent	32	64.0
Not available	1	2.0
Bulky		
Gift	15	30
Absent	35	69
Not available	10	20
Extranodal		
Yes	15	30
No	30	60
Not available	5	10
Staging		
1	5	10
2	5	10
3	15	30
4	25	50
Bone marrow involved in the diagnosis		
Yes	28	56
No	20	40
Not available	2	4
FLIPI		
Low risk	10	20
Medium risk	10	20
High risk	10	20
Not available	5	10
Ki-67 (median. IQR)	7.5	(15)
Top-2a (median. IQR)	7.5	(15)
Death		
Yes	6	12
No	44	88
Tumor progression		
Yes	7	14
No	43	86
Response to chemotherapy		
Complete	43	86
Partial	3	6
Progression	2	4
Refractory	1	2
Not available	1	2

Table 4.2 Continue: Clinical and pathological characteristics of patients with follicular lymphoma.

Subsequent maintenance with immunobiologicals		
Yes	28	56
No	22	44
Not available	0	0

95% CI: 95% confidence interval; IQR: interquartile range.

4.4. Frequency of Anti-EBNA-L Antibody In Cases And Controls Group

EBNA- l Abs were identified in all cases of NHL The degree of positivity ranged different in each age group.

The frequency of anti-EBNA-l antibody in case group was higher than controls group, howover the frequency of anti-EBNA- l IgG Ab in case group was higher than anti-EBNA- l IgM Ab may be due to from latent re-infection, so the highly frequency distribution of anti-EBNA- l IgG Ab were data 19 cases (47.5%) in age group (60-74)years, follow by 8cases (20.2%), in age group 45-59 and 5 cases (12.5%) recorded in age group >75 years and 30-44 years respectively while the lowest level 3(7.5%) show in age group 15-29 years (Table 4.3). The anti-EBNA- l IgM Ab in patient with NHL show highly frequency distribution 9 cases (42.8%) in age group 30-44 years follow by 4(19%) in age group 15-29 years and 60-74 years while the low level of anti-EBNA- l IgM Ab 2 cases (9.5%)showed in both age group 45-59 and in >75 years old, this different of level depended on the time of exposure to viral infection and the stage or re infection in latency phase so in our study noted the low level of IgM antibody in may be as result of complication of chemotherapy protocol to treatment NHL. Frequency distribution of viral infection was highly in males than females, where recorded 30 cases of NHL in males and 20 cases in females. The level of anti-EBNA- l IgG Ab showed 23 cases (57.5%) in males was higher than anti-EBNA- l IgM Ab 16 cases (76.1) while in females IgG was 17 cases (42.5%) and for IgM 5 cases (23.8).

Table 4.3: Frequency of anti-EBNA-l antibody in cases and controls group.

Age group		Case group		Control group	
		Frequency of anti-EBNA- l Ab		**Frequency of anti - EBNA- l Ab**	
		IgG	IgM	IgG	**IgM**
15-29		3(7.5%)	4(19%)	1(3.4%)	**4(26.6%)**
30-44		5(12.5%)	9(42.8%)	5(17.2%)	**7(46.6%)**
45-59		8(20.2%)	2(9.5%)	5(17.2%)	**2(13.3%)**
60-74		19 (47.5%)	4(19%)	8(27.5%)	**2(13.3%)**
>75		5 (12.5%)	2(9.5%)	10(34.4%)	**0(00%)**
Total		40(100%)	21(100%)	29 (100%)	**15(100%)**
Gender					
Males	30	23(57.5%)	16(76.1)	20(68.9)	**8(53.3)**
Females	20	17(42.5%)	5(23.8)	9(31.1)	**7(46.6)**
Total					

4.4.1. Evaluation According To The Antecedents

The majority of our patients (64%) present with ATCD or we notice that heart disease presents the large percentage either 30% followed by type 2 diabetes or 24% while hypertension and tuberculosis have only been presented. By 8% and 2% for each and only 36% of patients did not present with DCDA. The history review in our series found that heart disease is the most common in our findings compared to type 2 diabetes and other conditions. Indeed, these results agree with that of Boukhrissa (2009) [144]. These diseases characterize the elderly population and have no relation to cancer.

4.5. Assessment According To General Signs

The conditions of finding were various, with general signals dominating. Almost all of our patients (74%) displayed general symptoms of fever, nocturnal sweats, and weight loss. And only 26% of patients presented no symptoms. The presence of general signs or signs commonly called B symptoms such as fever, weight loss and sweating were present in the majority of our patients with 74%, which is consistent with the results of Idrissi et al. [136].

4.6. Evaluation According to The Clinical Stage

According to the Ann Arbor classification; We have observed that the large number of our patients have stage 4 NHL (i.e. 50%) followed by Fig. 4.3.

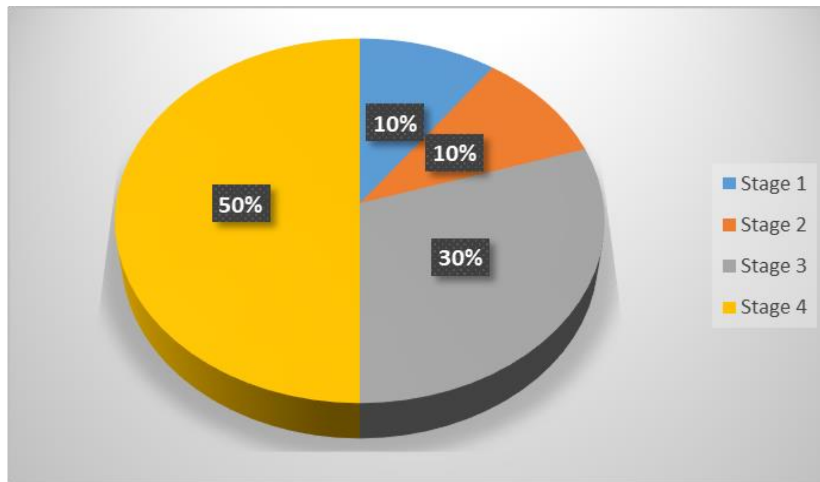

Figure 4.3: Distribution of patients by stage of Ann Arbor.

Likewise, the stage of disease extension has great prognostic value and remains one of the main parameters in the adaptation of treatment. According to the Ann Arbor classification and thanks to the radiological, scanno-graphic and endoscopic studies carried out; Our results agree with the literature, also with those of Boudjerra et al. [135].

4.7. Evaluation According To The Phenotype

In our series the majority of our patients have type B NHL with a percentage of 86% and only 14% phenotype T.

4.8. Evaluation According To The Secondary Location

Among the patients with extranodal localizations, the major localization is in the bone marrow (40%) followed by the liver (36%) while the spleen represents the least affected organ (6%). 6% of our patients do not present an extranodal extension. For lymph node localization, in our study it is followed by secondary localization in most of our patients. The bone marrow was the most affected organ in our population (40%) which explains the extent of the disease. This result is consistent with the study carried out by Badaoui et al. [137]. On a Moroccan population of 170 patients. Bone involvement is present in 38.82%.

4.9. Assessment According to the Sub Types Of NHL

In the distribution of our patients according to the NHL subtype we noticed that almost half of these patients present a large cell NHL (40%) followed by small B cell NHL (29%) while the other subtypes are present with small percentages (20.1%).

4.10. Assessment According to the Tumor Syndrome (ADNP)

Examination of the peripheral lymph node areas revealed the existence of ANDP in 82 % of cases. Cervical involvement is the most common, estimated at (50%), while axillary involvement is only present in 32% of cases, while only 18% of cases do not present with DNAP.

4.11. Results Relating to the Anatomopathological Study

The anatomopathological study was performed on 3 types of tissue :

- Lymph node tissue
- Gastric tissue
- Medullary tissue.

4.11.1. Investigation of Lymph Node Tissue

4.11.2. Histological Study

The photomicrograph below (Fig 4.4 A) illustrates the main histological features of the healthy lymph node, the presence of an external cortex (Cx) rich in strongly colored cells and an internal medulla (M), paler, in continuity with the hilum (H). The superficial part of the cortex contains numerous dense clusters of cells, the lymphoid follicles (F), most of which contain a clear germinal center. The deeper part of the cortex or para cortical zone (P). Extensions of the cortical cell mass enter the medulla in the form of medullary cords (CM). Several connective spans (T) enter from the capsule (C) into the body, the subcapsular sinus (S), located immediately below the capsule.

While the photomicrographs (Fig. 4.4 B, C) illustrates the histological appearance of the ganglion affected by diffuse large B-cell non-Hodgkin lymphoma, where in note that the

structure of the ganglion parenchyma is disorganized and erased by tumor cells (arrow) as well as lymphoma cells (CL) are large in size .

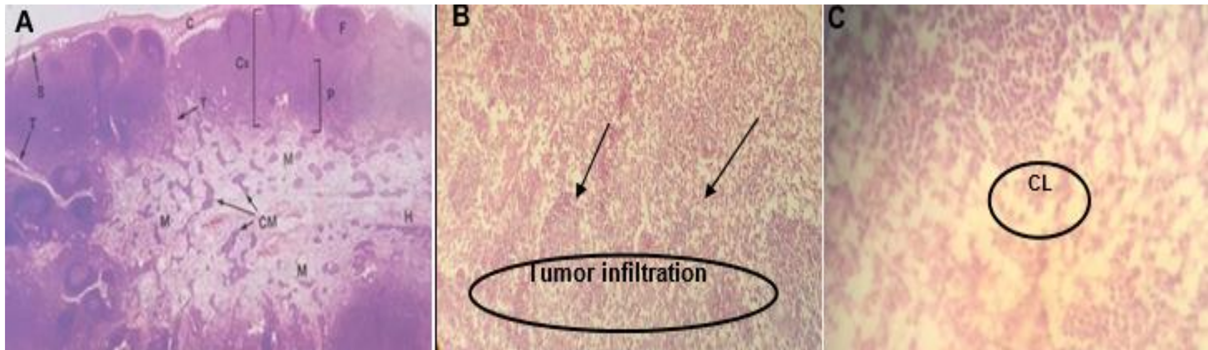

Figure 4.4: Histological sections of the lymph nodes. (A): Histological section of a healthy lymph node (Gx10). (B): Histological section of a lymph node affected by NHL (Gx10). (C): Histological section of a lymph node affected by NHL (Gx40).

4.11.3. Immunohistochemical Study

Confirmation of the diagnosis is carried out thanks to an immunohitohimical study which shows a positive labeling of the intense and diffuse anti CD20 Ab of all the tumor cells, the positive reaction is manifested by a color change from hematoxylin blue to brown color, which results in a lymph node localization of diffuse large B-cell lymphoma (Fig .4.5)

Figure 4.5: Immunohistochemical revelation of $CD20$ (A) $x4$, (B) $x10$, (C) $x40$.

4.11.3.1. Investigation of Gastric Tissue

4.11.3.2. Gastric Histology

The photomicrograph (Fig. 4.6) shows a healthy part of the gastrointestinal tract, which consists of a mucous membrane that includes 3 parts: an epithelium, a layer of supporting connective tissue or chorion (Ch), a thin layer of mucosal muscle smooth muscle (MM) that separates the chorion from the SM submucosa which contains very small parasympathetic nodes (GP) scattered within the submucosa. While the photomicrograph (Fig. 4.6) of a piece

of gastric mucosa shows that the chorion is largely ascended; at high magnification (fig 4.6) we note the presence of a polymorphic lymphoid population with a decrease in glandular mass and the presence of foci of cryptic abscess (arrows) as well as sectors of intestinal metaplasia sometimes in dysplasia.

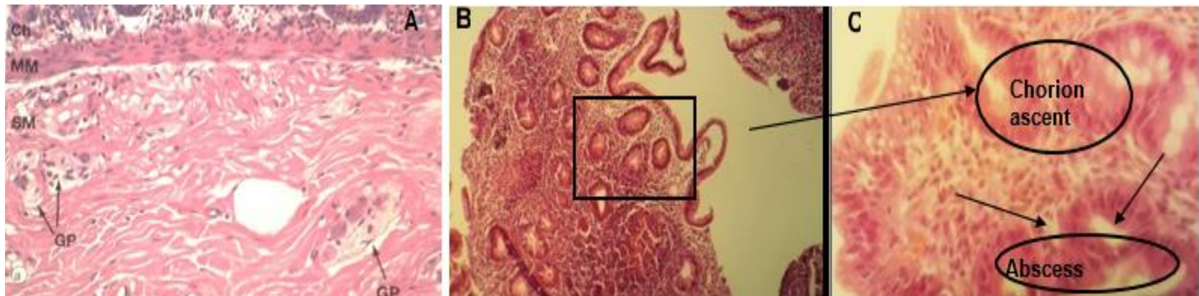

Figure 4.6: Gastric histological section. (A): Healthy gastro-intestinal histological section (Gx10). (B): Gastric histological section affected by an NHL (Gx10). (C): Gastric histological section affected by an NHL (Gx40).

4.11.4. Immunohistochemical Study

The immunohistochemical study shows that the intestinal mucosa whose surface epithelium is regular with some CD3 positive intraepithelial less than 20 lymphocytes per 100 entrocytes, the chorion is very inflammatory made up of regular polymorphic CD20 and CD3 lymphocytes and a chronic gastric with intestinal metaplasia. the presence of a CD20 positive lymphoepithelial lesion compatible with low-grade MALT-type non-Hodgkin lymphoma (Fig. 4.7A, B).

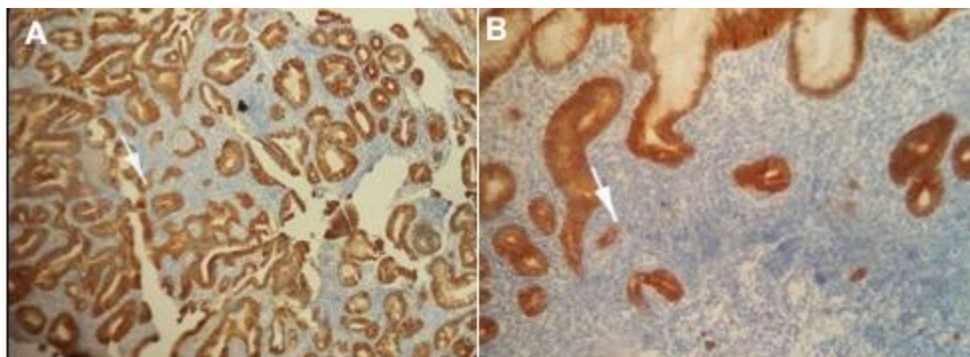

Figure 4.7: Immunohistochemical revelation of CD20. (A) x10, (B) x40.

4.12. Investigation of Medullary Tissue

4.12.1. Medullary Histology

The medullary photomicrograph (Fig 4.8 A) shows a fragment of trabecular bone (T) bone that contains a mixture of yellow MG marrow composed of adipose tissue and red hematopoietic marrow (HD) made up of the cellular precursors of blood cells in close contact with many thin-walled blood vessels (V) (sinusoids). While the photomicrograph (Fig4.8 B, C) shows a medullary space, the whole of which is infiltrated by a diffuse and dense lymphomatous proliferation made up of a rounded lymphomatous cell population of varying size .

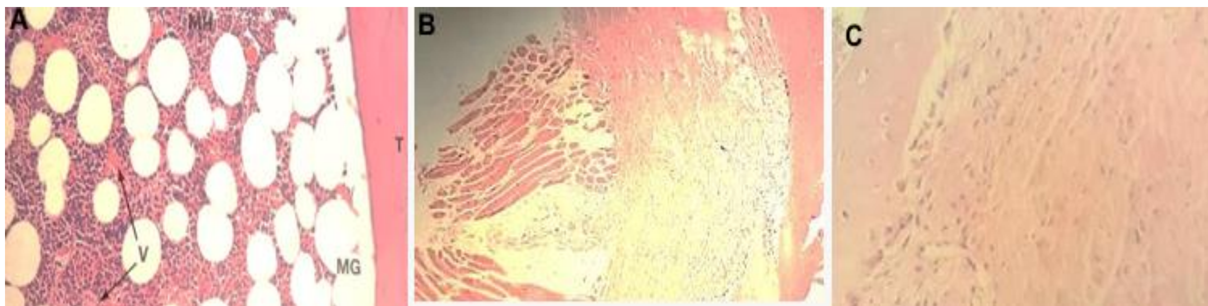

Figure 4.8: Histological section of the bone marrow. (A): Histological section of the healthy bone marrow (Gx10). (B): Histological section of the bone marrow affected by an NHL (Gx10). (C): histological section of the bone marrow affected by an NHL (Gx40).

4.12.2. Immunohistochemical Study

The immunohistochemical study shows a positive and diffuse staining of the whole malignant cell with CD20 which concluded in a medullary localization of a non-Hodgkin lymphoma of phenotype B expressing CD20 (Fig 4.9 A, B).

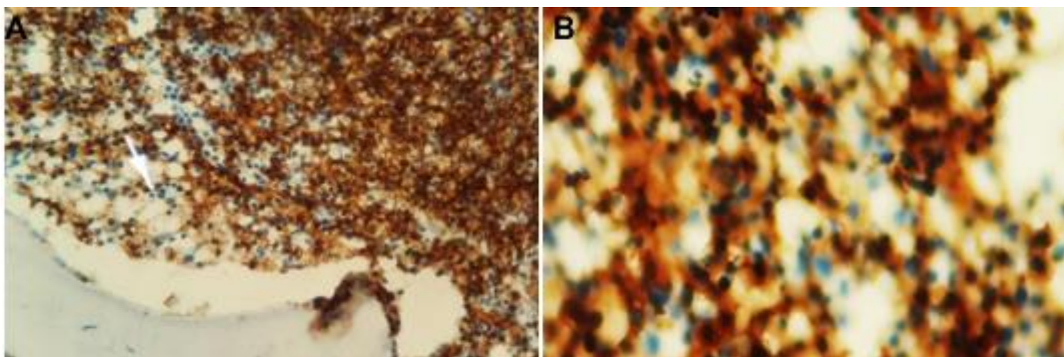

Figure 4.9: Immunhistochemical revelation of CD20. (A) x10, (B) x40.

In terms of diagnostic means, the morphological examination on the tumor biopsy as well as the technique of immunohistochemistry allowed us to confirm the diagnosis of 90% of cases. In our study, the labelling with the monoclonal antibody CD20 a revealed better results compared to that of CD3. This can be explained by the dominance of the B phenotype. Indeed, the histological and immunohistochemical study made it possible to draw up the anatomopathological profile of cases of NHL. In our study, type B NHLs were the most common with a percentage of 86% of cases while T lymphomas were found in 14%. This number is comparable to that of Barakat (2013) [138]. The usual distribution of these lymphomas according to the histopathological type and the Kiel classification shows in Europe a strong predominance of type B NHLMs (85%) confirmed by the studies of other studies [140]. In addition, the distribution of our cases, according to the histological grades of malignancy, shows a greater frequency of high histological grade lymphomas including diffuse large B-cell lymphomas (80%). Our results agree with those of Boudjerra et al. [140]. Patients whose response to chemotherapy was disease progression (8/170) had a median of the cell proliferation marker Ki-67 = 7.79 (IQR = 4.94 - 14.89) greater than patients who did not show progression of 2.39 (IQR = 0.91 - 5.71), with $p = 0.035$. The same behavior was also observed for the cell proliferation marker Top-2a, whose medians among patients with a progression of 194.37 (IQR = 156.59 - 227.59) in response to chemotherapy were statistically different ($p = 0.018$) and higher than among patients without progression in response to chemotherapy of 81.15 (IQR = 39.47 - 141.35), as can be seen in Table 4.4 below.

Table 4.4: Statistical comparison of medians and respective interquartile ranges of cell proliferation markers in patients with follicular lymphoma.

	Response to chemotherapy		
Cell proliferation markers	Progression (n = 8)	Others (n = 154)	p
Ki-67	7.79 (4.94-14.89)	2.39 (0.91-5.71)	0.035
Top-2[a]	194.37 (156.59-227.59)	81.15 (39.47-141.35)	0.018

The higher the values of the cell proliferation marker Ki-67, the shorter the tumor progression free time (TLP) was after the start of chemotherapy (rho = -0.28; $p < 0.01$). The correlations performed in this study can be seen in Table 4.5 below.

Table 4.5: Correlation matrix of the variables evaluated in patients with follicular lymphoma.

Variables	Ki-67	Top-2[a]	Stadium	Age	FLIPI	TLP
Ki-67	1					
Top-2nd	0.22*	1				
Stadium	0.06	0.11	1			
Age	0.23*	0.09	-0.11	1		
FLIPI	0.21	-0.02	0.40*	0.32*	1	
TLP	-0.28*	0.2	0.01	-0.33*	-0.21	1

TLP: progression-free time after starting chemotherapy (months); * p <0.05

Two years after diagnosis, the probability of survival among patients with follicular lymphoma was 86.8% (95% CI = 80.2 - 91.3). Elderly patients had an index of 74.3% (95% CI = 59.8 - 84.2). Thus, the elderly had a probability of survival 18.4% lower than non-elderly patients (92.7%; 95% CI = 85.2 - 96.5), after 2 years of diagnosis, with statistical difference (p = 0,0002).

The probability of survival among patients with the presence of symptoms B (67.1%; 95% CI = 52.2 - 78.3) was statistically lower (28.4%; p <0.01) than the probability of survival in patients without the presence of symptoms B (95.5%; 95% CI = 88.1- 98.3), after 2 years of diagnosis.

Patients who underwent maintenance with immunobiologicals (97.4%; 95% CI = 90.0 - 99.4) had a 22.2% greater probability of overall survival (p = 0.002) after 2 years of diagnosis than patients who did not (75.2%; 95% CI = 62.5 - 84.1). These data can be seen in Table 4.5 below and in the survival curve of Figure 4.10.

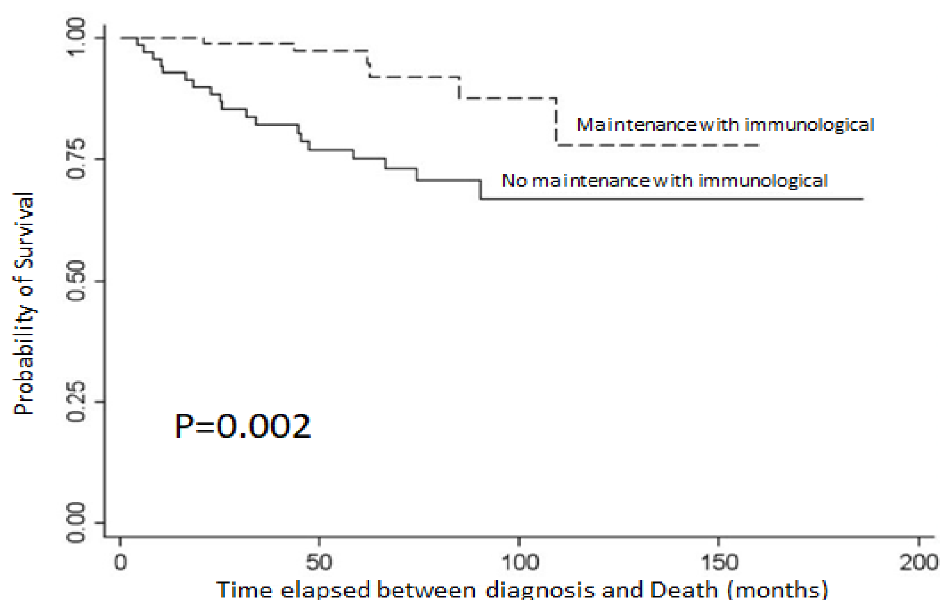

Figure 4.10: Kaplan-Meier curve with the probability of survival in patients with follicular lymphoma according to the use of maintenance with immunobiologicals.

The presence of symptoms B increased the risk of death 16 times in patients with follicular lymphoma, when compared to patients with this type of lymphoma, but without the presence of symptoms B. Patients who presented tumor progression in response to chemotherapy had significantly high risk of death.

After 2 years of diagnosis, the probability of no progression in patients with follicular lymphoma was 64.5% (95% CI = 56.0-71.8). Eight years after the diagnosis of follicular lymphoma was the time in which half of the patients (median probability) had tumor progression.

Two years after the diagnosis of follicular lymphoma, the probability of survival among patients with Ki-67≤ 7 was equal to 90.0% (95% CI = 81.0-95.0), while those with Ki-67 ≥ 7 the probability of survival was equal to 81.0% (95% CI = 68.9-88.7), as can be seen in Figure 4.11. Therefore, patients with follicular lymphoma who presented Ki-67 ≥ 7 had a 9% lower probability of survival than patients with the same pathological condition, but that the Ki-67 values were less than 7. This behavior was not observed for the Top-2a marker, finding no statistical difference between the cohort points as can be seen in Figure 4.12.

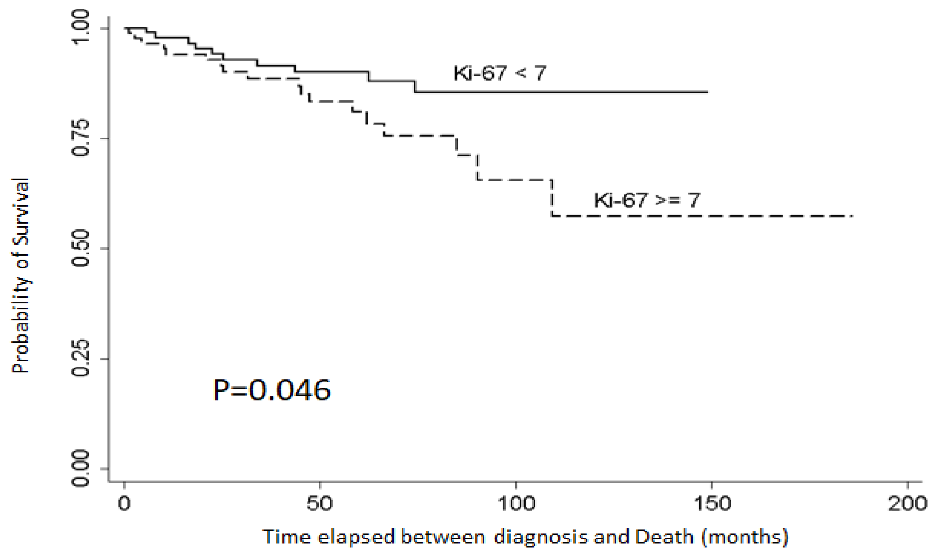

Figure 4.11: Kaplan-Meier curve with the probability of survival in patients with follicular lymphoma according to the presence of the cell proliferation marker Ki-67.

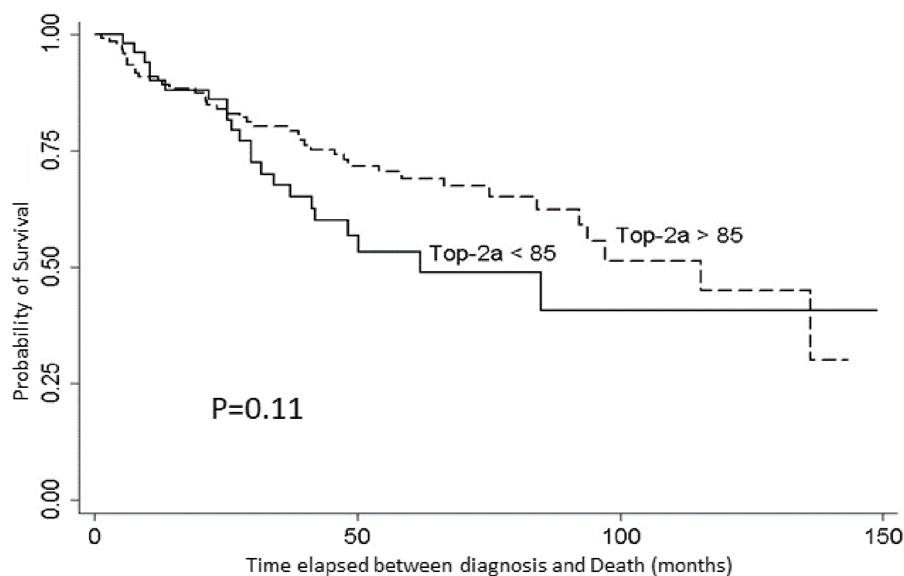

Figure 4.12: Kaplan-Meier curve with the probability of survival in patients with follicular lymphoma according to the presence of the cell proliferation marker Top-2a.

Patients with Ki67≥ 7 who were maintained with immunobiologicals also had a longer survival compared to those who did not ($p = 0.04$), as can be seen in Figure 4.13. The probability of survival after 2 years of diagnosis was equal to 69.8% (95% CI, 50.7-82.6) among patients with Ki67≥ 7 without maintenance and equal to 97.6% (95% CI, 84.1-99.6) among patients with Ki67 ≥ 7 with maintenance.

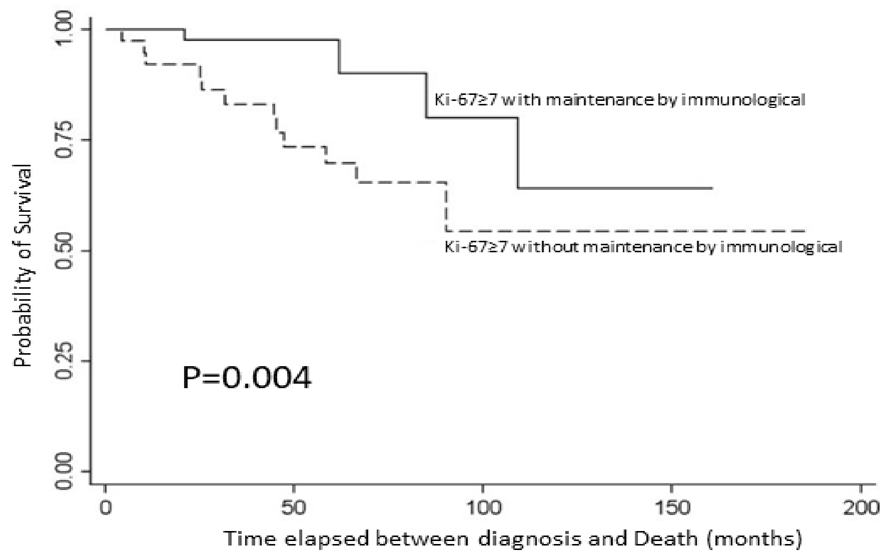

Figure 4.13: Kaplan-Meier curve with the probability of survival in patients with follicular lymphoma with Ki67≥ 7 according to the use of maintenance with immunobiologicals.

After 2 years of diagnosis, patients classified with high-risk FLIPI (50.5%; 95% CI = 34.7-64.3) were less than 20% less likely to remain without progression (p = 0.03) than those patients classified as medium risk FLIPI (70.0%; 95% CI = 56.9-79.8).

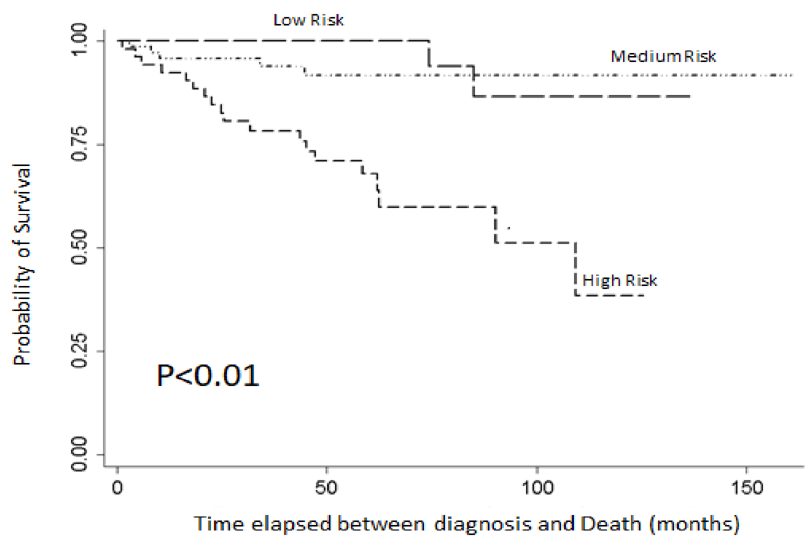

Figure 4.14: Kaplan-Meier curve with the probability of survival in patients with follicular lymphoma according to the FLIPI classification.

The median probability of survival (time in which half of the people followed died) in patients with follicular lymphoma classified as high-risk FLIPI occurred 8 years after diagnosis, as can be seen in Figure 4.15.

The time required for half of the patients followed to show tumor progression was almost 6 years shorter among those who had symptoms B (3.8 years) when compared with those who did not (9.6 years).

The presence of B symptoms in patients with follicular lymphoma tripled the risk of tumor progression when compared to patients without the presence of B symptoms (Figure 4.14). Regarding the response to chemotherapy, patients with follicular lymphoma who had the partial type had a 78% higher risk of progression than patients who had a complete response.

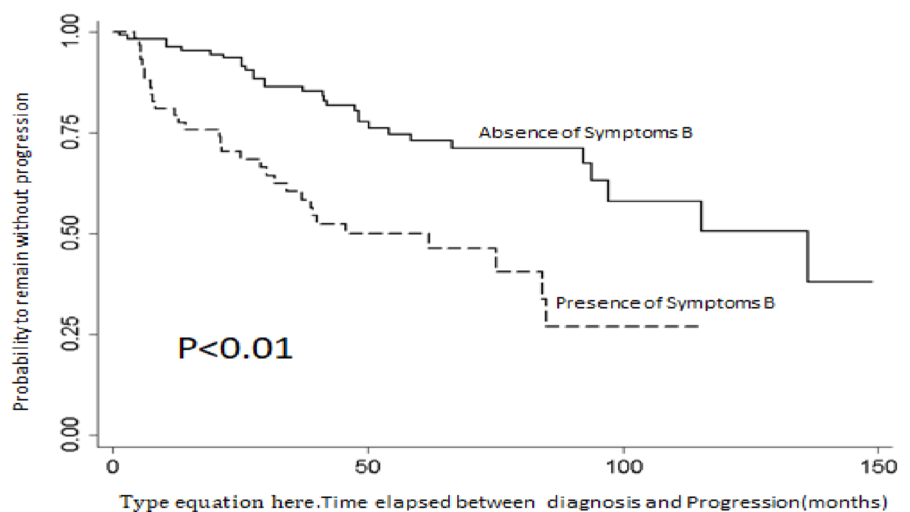

Figure 4.15: Kaplan-Meier curve with the probability of remaining without progression according to the presence of symptoms B.

4.13. Molecular Analysis

4.13.1. The Level of L1PA2 Gene Expression

The results of the current study of Fig. 4.16 confirmed that there was a significant increase in lymphoma patients at a significant ($P \leq 0.05$) level of L1PA2 gene expression in the blood tissues of patients with non-Hodgkin's lymphoma compared with the control group.

Non-lymphoma Hodgkin's patients and healthy controls were compared. Patients with lymphoma had elevated cfDNA levels. qPCR testing cfDNA levels in plasma of non-lymphoma Hodgkin's patients (N = 50) and controls (N = 20) (P 0.05).

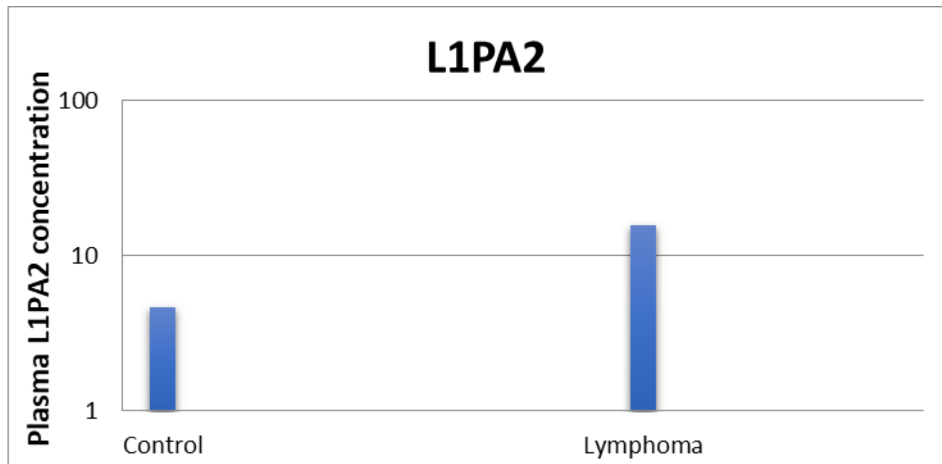

Figure 4.16: Shows the gene expression level of L1PA2.

4.13.2. The Level of Microrna-532-5p Gene Expression

The results of the current study in Fig. 4.17 showed a significant decrease in the level lymphoma patients of significance (P≤ 0.05) for the gene expression level of MicroRNA-532-5p in the blood tissue of patients with non-Hodgkin's lymphoma compared with the control group.

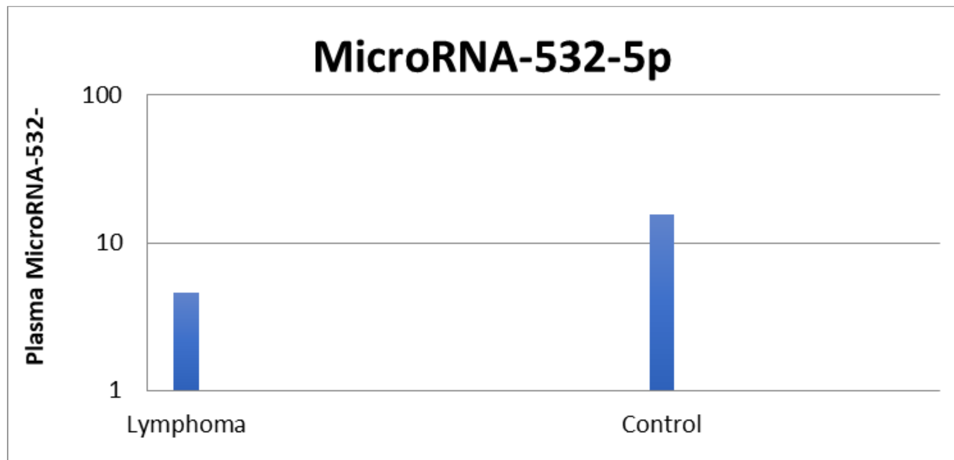

Figure 4.17: The Level of MicroRNA.532-5p Gene Expression

5. DISCUSSIONS

5.1. Epstein–Barr Virus Pathological

Epstein–Barr virus plays a pathogenetic role in several lymphoid and epithelial malignancies [139-140]. In our study the mean ± SD of case study was higher when compared with controls group, however the level of anti EPV antibody in patient with non-Hodgkin lymphoma observed positive of IgG and IgM, this finding was agreement with study that done by Kabyemera et al. [141] that show EBV-positive detection in Blood 21/35 (60%) in non-Hodgkin lymphoma patients , Despite the fact that anti-EBNA1 levels have been reported to be higher in chronic EBV reactivation and some forms of cancer [142], The higher risk of NHL linked with EBNA1 seronegative status may be due to a change in EBV activation. Alternatively, the existence of EBNA1 antibody may simply indicate a more stronger immune system, resulting in more efficient EBV infection control and greater ways of preventing lymphomagenesis.

An study of InterLymph consortium studies found that a history of infectious mononucleosis (induced by delayed initial EBV infection) was linked with an elevated risk of NHL, with the highest correlation reported for the CLL/SLL/PLL/MCL subgroup [143]. B Latently infected cells can change into continually growing blast cells, and then into lymphoma [144]. EBV LMP gene encodes an integral transmembrane protein. It is an oncogene that functions as a constitutively active CD 40 receptor, promoting B-cell proliferation. It encourages B-cell survival and multiplication, which happens on their own [145]. The EBV-induced proliferation and cellular transformation of B lymphocytes in vitro are mediated by the EBNA2 and LMP1 proteins [146]. Transactivation of additional cellular and viral genes requires EBNA2, which is necessary for transformation of primary B cells [147, 148]. There is an upregulation of CD21, CD23, LMP1, and LMP2 gene expression in B cells [147, 146]. Researchers compared the expression of three viral proteins, EBV RNA (EBER), EBV DNA, and their connections with CD21 and CD23 expression in lymphoid organs to better understand the role of Epstein-Barr virus (EBV) in the oncogenesis of B-cell non-lymphomas Hodgkin's in children.

As EBV may play a role in the emergence of B-cell NHLs, even in nonendemic regions, we sought to define the patterns of EBV latency in children in our country. Although NHL may affect anyone of any age, it most often strikes those in their 50s and 60s. Based on our data, the average age was 53.6313 years old (MeanSD). The results were consistent with those of

other researchers, including Mushtaq et al. [149], Jamal et al. [150], and Veelken et al. Average ages of [151] are 58, 55, 59, and 57 respectively (23-83 years). Studies by de Jong et al., Kai et al., and Lene et al. found that the average age was 68 (range: 22-93 years), 63 (14-90 years), and 65 (29-95 years), respectively. Patients in their sixties and seventies made up the largest age group in our study, at 26.8 percent. The results corroborated those of the vast majority of other studies, which found that the sixth and seventh decades were the most prevalent.

The results of our inquiry showed mean ages that were similar to those found in the Egyptian study by Karen et al. According to [152], over half of NHL patients are younger than 50. Patients above the age of 60 have a worse prognosis in terms of overall survival. Worldwide, males are more likely to be diagnosed with NHL than women. According to our findings, the gender breakdown of patients was also around 70.4% male to 29.6% female. These results were consistent with those found by Mushtaq et al. (2008). References: (p. 154) Jamal et al., Young et al., sixty-eight percent, seventy percent, sixty percent, fifty-nine percent, and sixty-five percent of the male cases were found in [153]. Two out of every eight cases tested positive for Epstein-Barr virus. Our study's elevated EBV expression in T-cell NHL is probably attributable to the small sample size. Our findings contrast with those of an Argentinean investigation that found EBV expression in NHL to be 25% on average. EBV was detected in 30.43 percent of B-cell NHL and 11.1% of T-cell NHL when NHL was subdivided by cell type [154-156]. In addition, Paola Chabay of Argentina found that 8% of T-cell NHL cases tested positive for EBV-LMP1 antibodies. Research was done by Paydas et al. One-fourth of all cases (11/177 NHL patients) had complete EBV-LMP1 antibody expression [161]. This result is consistent with what we found while doing our research. All of the positive individuals in the research, however, had B-cell NHL, which was an unexpected discovery. No cases of T-cell NHL were found to have elevated levels of EBV-LMP1 antibodies [161-155]. In Africa, 34.7% of patients with B-cell NHL had EBV-LMP1 antibody expression, according to a study that specifically looked at this subtype of the disease [157]. Because Burkitt lymphoma is characterised by a high level of EBV expression, it accounted for the vast majority of patients in our study. On the other hand, we identified just a single instance of Burkitt's lymphoma in all of our data. Compared to South East Asia, Africa has a higher incidence of Burkitt's lymphoma. Multiple further studies were undertaken by Oyama et al. This is according to Morales et al. [158]. Both [160] and Park et al. [159] are also relevant. Anti-EBV-LMP1 antibodies were detected in 9-15% of

patients with B-cell non-Hodgkin lymphoma. These proportions were consistent with our research. As the prevalence of EBV varies from region to region, it is possible that this accounts for the discrepancies in reported prevalence rates. Our findings, along with those of other researchers, imply that EBV may play a role in the pathogenesis of NHL due to the strong correlation between the two. For those who have FL Having a Ki-67 value of 7 was associated with a considerably better chance of surviving two years following diagnosis, in comparison to having a Ki-67 value of 7. For patients with Ki-67 levels below 7, this equates to a 9 percent decrease in expected survival time. A greater value of the cell proliferation marker Ki-67 was likewise related with shorter tumour progression-free time following the initiation of treatment. For the Top-2a marker, we could not identify any statistically significant differences across the different cohorts, thus we cannot draw any conclusions about its behaviour or its link to other markers. These findings on Top2a were consistent with those found in a breast cancer research. Another research found that the presence of B-cell symptoms and the location of involvement (in terms of extranodal origin) were significantly associated with IP Ki-67> 45 percent. Combined with other clinical indicators at diagnosis, these relationships highlight the importance of a high Ki-67 IP value in determining the proliferative activity of the tumour as a prognostic index [161]. Blood cultures from 11 of 15 patients with positive results showed the presence of a germ when C-reactive protein levels were greater than 10mg/L; similar results were evidenced by Avabratha et al., who evaluated episodes of febrile neutropenia in paediatric patients, showing that C-reactive protein is a reliable predictor marker of infection. At 49 percent specificity and 52 percent sensitivity, β2M was demonstrated to be ineffective. As a consequence, it is determined that levels of β2M in absolute terms are not appropriate for confirming or rejecting active NHL. There has been a lot of research done on the effectiveness of using prognostic variables like 2M. Based on our research, we conclude that 2M is not useful as a diagnostic marker for NHL.

In spite of its use as a prognostic marker in staging, we do not recommend determining it for re-staging or follow-up. Ferritinemia is a side effect of several types of cancer, including acute myeloblastic leukaemia, Hodgkin's lymphoma, and non-and Hodgkin's Hodgkin's lymphoma. Two distinct explanations were proposed for the abnormally high levels of ferritin in the tissues and serum of Hodgkin's patients. The function of ALC in cancer prognosis has previously been explored. Lymphocytopenia was proven to be an excellent predictor of clinical outcome in patients with metastatic breast cancer and advanced sarcoma

prior to the initiation of systemic treatment. In advanced Hodgkin's lymphoma, lymphocytopenia is generally recognised as a prognostic marker.
Proportions of Ki-67-positive cells have been shown to correlate strongly with the categorization of high and low aggressiveness in malignant NHL. In addition, the presence of a favourable link between prognosis and proliferation rates in chronic B and T cell lymphoproliferative diseases has also been found. Thus, the Ki-67 seems to offer early and reliable information on the predictive value in combination with clinical data. However, the data on the link between Ki-67 expression and several lymphoma subtypes are still conflicting and unclear. While some research has linked elevated Ki-67 expression to decreased survival, other studies have found no such link or have found the opposite. Our findings in the current research confirm the importance of this marker as a prognostic value, as it was seen that after two years of the diagnosis of follicular lymphoma, the chance of survival was larger among patients who had the lowest Ki-67 readings. In our research, the cell proliferation marker Ki-67 was considerably greater in individuals who had illness progression in response to treatment.
Top-2a, a marker of cell proliferation, had the same pattern of action (higher median values in patients who progressed in response to chemotherapy). This result has substantial implications since it enables selecting these indicators as prognostic variables and distinguishing a grouping with a bad prognosis. Overexpression of Top-2a and subsequent cell proliferation has been linked to p53 inactivation in neoplastic circumstances. However, the expression of p53 was not assessed in the current investigation. Both solid and haematological cancers are linked with a worse prognosis when the Ki-67 IP is high. Thus, Ki-67 has been deemed an effective marker to assess the growth percentage of a particular cell population. Samples from patients with FL exhibited a weak positive connection between Top-2a and Ki-67, with concordance only shown in those with disease progression in response to treatment. In spite of this, the literature still shows conflicting findings regarding the relationship between Top-2a and Ki-67 in various lymphomas. The two indicators have been linked in some research, but not much in the way of follicular lymphoma-specific investigations. Possible causes for these disagreements include variations in immunohistochemistry techniques, interpretation of results, and length of time paraffin samples were stored. Besides Ki-67, several proliferation markers were shown to be associated with Top-2a. Research by Brizova et al. [162] in mantle cell lymphoma established a linear correlation between TPX 2 and Top-2a. Similar patterns of enzyme

expression were seen throughout the many stages of the cell cycle in this investigation. Conversely, Ki-67 demonstrated that its existence is time-dependent inside the G1 phase of proliferation. Researchers determined that Top-2a and TPX 2 expression is a more accurate predictor of cyclic cells and proliferation for clinical prognosis of patients with mantle cell lymphoma than Ki-67 expression. Survival among patients with FL was equal to 86.8 percent; survival among elderly patients was 74.3 percent; the presence of symptoms B increased the risk of death by 16 times and tripled the risk of tumour progression, and these results are consistent with the literature, indicating that the sample was appropriate for analytic purposes. Patients who received immunobiological maintenance had a 22% better chance of survival than those who did not, however progression-free survival was not improved.

Progression-free survival was shown to improve with the administration of immunobiologicals, but not overall survival [163]. There may have been a disparity because of the small sample size (only 55% of patients were kept on immunobiologicals) and the need for extensive clinical follow-up to detect meaningful changes. Patients with high-risk FLIPI were shown to have a 20% lower chance of remaining progression-free compared to those with medium-risk FLIPI. Eight years after diagnosis, high-risk FLIPI patients had a 50% chance of surviving (the point at which the median survival rate dropped below zero). These results are consistent with what's been reported in the most recent medical journals [164]. Our research has limitations due to its retrospective nature and its inclusion of a population with varying treatment histories, in which only 55% of participants were maintained with immunobiologicals. It is still difficult in FL to keep up with the emergence of new markers.

Exams that can be readily taken again and again should be a major focus of future research. This provides an opportunity for further investigation of cell proliferation indicators such as Ki67 and Top2a, as well as other markers such as p53 and emerging technologies such as gene expression panels [170]. To ensure that these novel indicators can provide optimal therapy at the optimal time, prospective trials must be designed. This is in keeping with the trend toward personalised medicine in cancer. Our findings further support Ki-67 as an encouraging prognostic indicator, and we found a correlation between Ki-67 expression and overall survival. The results of this trial showed little promise for Top2 a.

5.2. Molecular Study (Gene Expression of L1PA2 Gene)

The absence of lymphoma Hodgkin's disease caused a dramatic increase in L1PA2 gene expression in the subset of individuals without cancer. Overall survival was shorter (median, 9 months; P = 0.022) for patients with diffuse large B-cell lymphoma (DLBCL) who had higher cfDNA concentrations than for those who had lower cfDNA levels, the study found [168]. Increased levels of circulating free DNA.

In [169], it is said that lymphoma patients often show increased levels of cell-free circulating DNA at the time of diagnosis, which may be accurately determined by real-time PCR and correlates with clinical features and prognosis. The amounts of DNA in the blood plasma were shown to be an excellent and separate prognostic indicator. The predictive impact of plasma DNA in lymphoma patients has not been studied before. Researchers have shown that elevated plasma DNA levels are associated with a lower risk of death from solid tumours like lung and colon cancer, as well as a higher risk of death from disease recurrence in prostate cancer. Real-time PCR measurement of circulating cell-free plasma DNA may become a valuable prognostic biomarker in DLBCL and HL, albeit our results need to be validated in larger and independent patient cohorts.

The research confirmed the claims made in [170]. which demonstrated abundant expression of the L1PA2 gene and suggested that most transposons have no discernible role in somatic tissues, About 45 percent of the human genome1 is made up of transposons, and they are important for regulating human gene expression and mediating epigenetic repression in epithelial cancers. When it comes to regulatory DNA, the L1PA2 transposon family is a major player. More than 27% of L1PA2 transposons had common localised binding sites, i.e. transcription factors that guide an enzyme (RNA Polymerase II) to the promoter (promoter) thereby separating the two DNA strands to facilitate cancer-related transcription in MCF7 cells, namely A cell line used for breast cancer modelling, and that L1PA2 transposons also contribute to transcription initiation sites of regulated transc. Altering Transcriptional Regulation in Breast Cancer, A deeper comprehension of the regulatory role of L1PA2 in breast cancer genomes will enhance our knowledge of cancer genome regulation and may inspire the discovery of new biomarkers for the diagnosis, prognosis, and treatment of this illness. Based on our findings, we can confidently say that L1PA2 transposons play a crucial role in breast cancer transcriptional control by providing binding sites for TFs that play a functional role in cancer-related transcriptional misregulation. As a result, they aided in the co-localization of different TFBS modules, which in turn led to the

combinatorial regulation of gene networks and the consequent rewiring of the cancer transcriptome. Functional similarity within the L1PA2 subfamily was revealed to be related to sequence similarity. The cancer-specific regulatory activities of L1PA2 transposons in breast cancer were revealed to be shared by its evolutionary relatives when we extended the TF binding profile to other primate-specific L1 subfamilies with varied degrees of sequence truncation and mutations. Our results demonstrate widespread exaptation of primate-specific L1 subfamilies for TF binding in the MCF7 breast cancer model.

5.3.Molecular Study (Gene Expression of Mir-532-P5 Gene)

Lymphoma decreased significantly in infected individuals in correlation with miR-532-5p gene expression. This finding is consistent with the data observed [171].

Recent study reveals that miR-532, which slows cancer development, may work to control various kinds of malignancies, including renal carcinoma, hepatocellular carcinoma, and colorectal carcinoma [172]. However, miR-532's specific functions in lymphoma are still unclear. Researchers have shown that miR-532 is overexpressed in lymphoma tissues and cell lines. Furthermore, miR-532 mimics inhibited tumour growth and development and triggered apoptosis, operating as a tumour suppressor in lymphoma cell lines and in vitro nude mouse model animals. Previous research has established miR-532 as a molecular marker for lymphoma identification, with findings showing that low levels of miR-532 in lymphoma patients block the development of cancer cells in lung and stomach cancer [173]. The findings are consistent with those of [175]. Very commonly expressed MiRNAs act as tumour suppressors by targeting mRNAs that encode oncoproteins and are involved in tumour molecular abnormalities [177]. Ovarian cancer patients with higher miR-532-5p expression seem to have better outcomes [178]. However, miR-532-5p acts as a tumour promoter to suppress RUNX3 expression in melanoma [176]. The role of miR-532-5p in promoting or inhibiting LNM in CC is yet unclear. First, we found and built mRNA for the CC signature using a predictive risk score technique. MiR-532-5p was shown to be downregulated in primary tumours with LNM and was proven to be considerably related with LNM. Similar to what was discovered in ovarian cancer, miR-532-5p was revealed to be an independent prognostic factor for CC, with a prediction accuracy of 82.7%. In addition, miR-532-5p slows the pace of LNM in vivo and suppresses lymphangiogenesis and metastasis in CC. As shown here, miR-532-5p has the potential to serve as a new predictive biomarker for CC and aid in clinical decision making about tumours. Preventing the

formation of LDs in cancer cells with PLIN2 siRNA reduces their potential for proliferation [180], whereas overexpression of miR-532-5p restricts LD accumulation in CC cells and alters lipid metabolism reprogramming. Both miR-532-5p and miR-532-3p are strongly associated with a poor prognosis in renal cell carcinoma, as shown in the research [179] for the pre-detection of RCC and the TCGA cancer genome atlas. The water channel protein aquaporin9 (AQP9) was found to be directly regulated by microRNAs miR-532-5p and miR-532-3p, and high levels of AQP9 expression were significantly associated with poor prognosis of RCC patients. Furthermore, multivariate analysis revealed that AQP9 expression is an independent prognostic factor for RCC patients.

6. CONCLUSIONS

Knowing the key epidemiological, clinical, and histological aspects of follicular lymphoma is crucial since it is the second most frequent kind of non-Hodgkin lymphoma. Follicular lymphoma is a disease with wide variability in its form of clinical presentation, so it is important to know them, in order to take the pertinent therapeutic measures according to the case. It is necessary to continue with the investigation of new therapeutic measures that consider the molecular bases of the disease, since follicular lymphoma continues to be incurable with currently available chemotherapy.

- Our study supports the value of ki-67 and Top2a expression in disease progression in patients with follicular lymphoma, since higher rates of these markers are significantly related to shorter disease progression time after chemotherapy.

- Only Ki-67 was significantly related to the overall survival in these patients, with those with higher rates having less favourable survival.

- In almost every country in the world, a worrying increase in the incidence rate of non-Hodgkin's lymphoma has been observed for several decades. At present, this increase remains unexplained because no etiological factor alone can explain it.

- These lymphomas represent 3 to 4% of all cancers diagnosed worldwide as well as they represent 5 to 10% of new cases of cancer, the fifth cancer, but also the fifth cause of death.

- Our study is carried out at the Hospital, where 50 cases of cancer were selected over a period of 2 years. FLs represented 4.4% of diagnosed cancers.

- Epidemiologically, the median age in our population is 51.5 years with a male predominance of 62%, the majority of whom reside in the Constantine region.

- The clinical symptomatology is increased by the poly lymphadenopathy and the attack of the general state by a slimming of more than 10% of the weight of the body in less than 6 months, a fever higher than 38° C for more than 15 days in the absence of documented infection as well as profuse night sweats.

- A diversity of locations of FL with an increase in lymph node locations followed by bone marrow.

- The extension assessment carried out on the basis of clinical and paraclinical examinations made it possible to classify patients according to the stage of their lymphoma, thus stage IV was highly representative, i.e., 48% of cases.

The techniques of histology and immunohistochemistry play a very important role in the diagnosis and the management of these tumours, which allowed us to classify our patients according to the histological type of NHL where it was concluded that the majority of cases have type B NHL with a percentage of 86%.

- It is essential to educate people about lymphoma through the media, and posters.
- It is necessary to create a file for each patient including regular and prolonged follow-up after treatment. It is based on medical consultations, blood tests and scans. The schedule and duration of this follow-up are adapted on a case-by-case basis by the specialist team who carried out the treatment, in conjunction with the attending physician.
- It is favorable for the histological use of other markers in order to facilitate and better specify the diagnosis.

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ATTACHMENTS

Annex 1: Ethics Committee Report

وزارة الصحة
دائرة صحة الديوانية
قسم التدريب والتنمية البشرية
شعبة ادارة المعرفة والبحوث
قرار لجنة البحوث

استمارة رقم ٠٢ / ٢٠٢١

رقم القرار: ٤١

تاريخ القرار : ٢٠٢١/٤/١٨

قرار لجنة البحوث

درست لجنة البحوث في دائرة صحة الديوانية مشروع البحث المقدم من قبل السيده الباحثة (وقار عدنان عزيز) احد طلبه الماجستير في جامعة اهي افيران كيرشهير وبرسالته الموسومة:-

Assessment of Epstein-Barr Virus molecular polymorphism and tumor biomarkers associated with non –Hodgkin in Iraqi patients

والمقدم من قبل الباحثة الى قسم التدريب والتنمية البشرية /شعبة ادارة المعرفة والبحوث / لجنة البحوث في دائرة صحة الديوانية بتأريخ ٢٠٢١/٤/١٨ قررت اللجنة :-

قبول مشروع البحث اعلاه كونه مستوفياً للمعايير المعتمدة في وزارة الصحة والخاصة بتنفيذ البحوث ولا مانع لدينا من تنفيذه .

المرفقات / تعديلات وملاحظات لجنة البحوث / لايوجد

البحث مستوفي الشروط العلمية ومطابق لأخلاقيات البحث العلمي

ولامانع لدينا من تسهيل واجراء البحث في (مستشفى الديوانية التعليمي)

جمهورية العراق
وزارة الصحة
دائرة صحة الديوانية
قسم تدريب والتنمية البشرية
العدد / ١٤٩
التاريخ / ١٨/٤/ ٢٠٢١

إلى / مستشفى الديوانية التعليمي

م / تسهيل مهمة بحثية

نهديكم أطيب تحياتنا....
استنادا الى اقرار البحث المقدم من قبل الطالبة الماجستير (وقار عدنان عزيز) في جامعة اهي افيران كيرشهير التركية بيحثها الموسوم :

Assessment of Epstein-Barr Virus molecular polymorphism and tumor biomarkers associated with non –Hodgkin in Iraqi patients

لامانع لدينا من تسهيل مهمة الطالبة على ان لا تتحمل مؤسساتنا اي تبعات مالية او قانونية

Annex 2. Article acceptance letter

JUNDISHAPUR JOURNAL OF MICROBIOLOGY
Ahvaz Jundishapur University of Medical Sciences

ACCEPTANCE LETTER

Paper ID: JJM-10208
DATE: 16-06-2022

Dear Authors,

Wiqar A. A. Abu-Omaima[1] *, Harun Çiftçi[2] and Mohammed A. AL-Askeri[3]

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With reference to your paper submitted **"The study of evaluating the Epstein-Barr virus molecular polymorphism and tumor biomarkers associated with non-Hodgkin's lymphoma in Iraqi patients. "** we are pleased to inform you that your article has been provisionally accepted for publication in **Jundishapur Journal Of Microbiology, Vol. 15 No. 1 (2022) .**

We thank you for considering our journal as a venue to publish your research work.

AUTOBIOGRAPHY

Personal Information	
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Date of birth	
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Graduation Year	

master	
University	Kirsehir Ahi Evran University
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Department	Molecular Medicine
Program	
Date of graduation	-

Articles and Papers
1. Adnan Aziz AbuOmaima, W., Ciftci, H., Abdulwahab Ati Al-Askeri M. “The Study of Evaluating the Epstein - Barr virus Molecular Polymorphism and Tumor Biomarkers Associated with Non-Hodgkin's Lymphoma in Iraqi Patients Jundishapur Journal of Microbiology Vol. 15 No. 1 (Accepted 2022).