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Association Between Human Herpes Virus -8 and IL-1R1 Gene Polymorphism Among Lymphoma Patients

A Thesis

Submitted to the College of Science/University of Babylon as Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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DEDICATION

To my dear father and mother …

I dedicated this study Report to my **Father** the first teacher I have ever had, to my **Mother** the holy human that who burn every day to light my life, to my family and any one teaches me even one letter.

Dear brothers and sisters…

You were and still my support, lean and fame in all stages of life.

Dear Friends…

My companion in my life.

To You…….

Ameer
2023

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Last but not least thanks to all those who helped me by every way, even if it is a good word.

Ameer

2023

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List of Abbreviations

Symbol	Description
AHC	Apparently healthy control
AIDS	Acquired Immunodeficiency Syndrome
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CHL	Classic Hodgkin's Lymphoma
CNS	Central Nervous System
DC-SING	Dendritic Cell- Specific intercellular adhesion molecule -3- Grabbing Non-integrin
DNA	Deoxy ribose nucleic acid
EBV	Epstein Barr Virus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
GI	Gastroenteritis
HHV-8	Human Herpes Virus 8
HIV	Human Immunodeficiency Virus
HL	Hodgkin's Lymphoma
HLA	Human leukocyte antigen
HRS	Hodgkin and Reed Sternberg
ICTV	International committee on taxonomy of viruses
IgG	Immunoglobulin G
IgM	Immunoglobulin M

IL-1R	Interleukin 1 receptor
IL1RN	interleukin -1 receptor antagonist
ILSG	International Lymphoma Studying Group
INFs	Interferons
LANA	Latent Nuclear Antigen
LY	Lymphoma
MHC	Major histocompatibility complex
mRNA	Messenger Ribose nucleic acid
NCBI	National Center for Biotechnology Information
NHL	Non-Hodgkin's Lymphoma
NK cell	Natural killer cell
NLPHL	Nodular Lymphocyte Predominant Hodgkin's Lymphoma
OBP	Origin binding protein
ORF	Open reading frame
PCR	Polymerase chain reaction
PEL	Primary Effusion Lymphoma
RNA	Ribose nucleic acid
Rta	Replication Transcription Active Protein
Th2	T-helper 2
TNF-α	Tumor necrosis factor alpha
VNTR	Variable Number of Tandem Repeats
VTM	Viral transport media
WHO	World Health Organization

SUMMARY

Summary

Lymphoma (LY) is a common hematopoietic cancer. Immunosuppression is one of the main risk factors for the development of lymphoma. Human herpesvirus 8 (HHV-8), stimulates lymphoproliferation by activating the signaling pathway of the interleukin-6 receptor, along with several other regulatory mechanisms. The interleukin (IL)-1 receptor antagonist IL1RN, which binds to the IL-1receptor, moderates a variety of immune responses related to IL-1. Polymorphisms in the gene cluster of Interleukin-1 (IL-1) have been identified as significant contributors to the regulation of inflammation.

This study was aimed to determine the percentage of Human Herpes Virus-8 (HHV-8) and whether polymorphisms at IL-1 receptor (IL-1ra) locus modulate the risk of developing malignant lymphoma of a group of Iraqi population sample.

A cross- sectional case control study including 200 blood specimens (25 cases Hodgkin and 75 cases Non-Hodgkin lymphoma and 100 apparently healthy control); (72 males and 128 females) who were recruited from different Teaching Hospitals in Mid-Euphrates and Baghdad Governorates of Iraq. Studied groups were had different ages that range from 15-80 years. The study was carried out at the department of Biology, College of Science, University of Babylon during the period between October 2022 and June 2023.

Five ml of blood specimens (2ml EDTA Blood and 3ml in gel tube) were collected and then viral genome and total DNA were obtained and stored at -20°C/-80°C till used.

The mean age of the patients with Non-Hodgkin lymphoma (**46.42**±**12.06** years) was higher than the mean age of the Hodgkin lymphoma (**45.12**± **14.49** years). While, the mean age of apparently healthy control (AHC) was (**44.9**± **12.51**) years.

Sixty-four percent (64%:16) of Hodgkin lymphoma were females, while the rest (36%: 9) were males. Regarding the patients whom suffering from Non-Hodgkin lymphoma, the percentage of females was also higher (64%:48) than the percentage of males (36%: 27), and the ratio of Female / Male was found (1.78:1).

In Hodgkin lymphoma group, the most affected age group were from 21- 40 years were constituting (60%:15) case. While, Non-Hodgkin lymphoma, the most commonly affected age group in 61- 80 years was constituted (40%:30) case.

Human Herpes Virus-8 were detected in HL and NHL patients by amplification of K1-HHV-8 gene and the results revealed that 46/75 (61.3%) and 16/25 (64%) of NHL and HL patients respectively.

According to PCR, only 43.7% (7 out of 16) of the HL specimens results are positive for K1-HHV-8 gene detection. While, in NHL 45.7% (21 out of 46) of the specimens have K1-HHV-8 gene**.**

The results of *IL-1R1rs1419620062* gene polymorphism showed that DNA polymorphism distribution were DNA polymorphism distributions according to A\A; A\C; and C\C were (15 Out of 25: 60%); (1 Out of 25: 4%) and (9 Out of 25: 36%), respectively in patients with LY and (8 Out of 15: 53.33%); (0 Out of 15 : 0.00%) and (7 Out of 15 : 46.67%); respectively in AHC group. The difference in frequency of genotype distribution of the polymorphism between patients and controls groups was statistically significant.

The mean of serum IL-1R concentration for AHC and patients with LY groups were 8.00 ±0.31pg./ml and 14.00 ±0.59 pg./ml, respectively, Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum IL-1R concentration among these study groups.

In view of the relatively the present results indicate the possibility that K1-HHV-8 and IL-1R1 polymorphism may play a role in the tumor biology of lymphocyte and may contributed to their development.

CHAPTER ONE

INTRODUCTION

1.1. Introduction

Cancer is one of the most common causes of death and health problems in the world. The numerous reasons, from environmental to genetic factors contribute in the pathogenesis of Lymphoma (Xia *et al*.,2019).

Lymphoma is a malignant neoplasm of lymphatic system. Evidences show cumulative factors are contributed in lymphoma pathogenesis including autoimmune diseases, HIV/AIDS, inherited immunodeficiency syndromes (e.g., ataxia–telangiectasia), Organ transplantation associated to the immune deficiency and infections (Shilkofski *et al*.,2020).

There are two types of lymphoma; Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL), the first type is recognized by the presence of special cells called the Reed-Sternberg cells where it can constitute only 12.5% of all lymphomas. The other type (NHL) is the most common type of lymphoma. The typing of non-Hodgkin's lymphoma depends on the type of cell which affected: either B-cell or T-cell. The B-cell lymphoma is more common than T-cell lymphoma. Non-Hodgkin's lymphoma is also classified as either slow growing (known as low grade or indolent lymphoma) or fast growing (known as high grade or aggressive lymphoma) (Parente *et al*.,2020).

The viral contribution to oncogenesis has to be addressed by determining how the viral life cycle and the host response, including their alterations, can be integrated within the multistep process of tumor development. Viruses that are well adapted to humans can participate in the angiogenesis process because they have not undergone a negative evolutionary pressure, since cancer develops at an age well above the median age of human till the twentieth century, However, the benefits of interferingor preventing infectious agents implicated in cancer are very large as demonstrated in hepatic, gastric and cervical carcinomas (Krump and You,2018).

However, researches have focused on some factors that may contribute to the development of lymphoma, including genetic factors, impaired immune system, viruses such as HIV, EBV, HCV or HHV-8, bacterial causes (e.g. *Helicobacter pylori*), exposure to chemicals and heavy smoking. Lymphoma represents a spectrum of lymphoid neoplasm with varying prognosis (Calabrò *et al*.,2018; Sanchez *et al*.,2020).

Human herpesvirus type 8 (HHV8) is a gamma herpesvirus known for its role in the development of varied lymphoid neoplasms. Studies have shown that when B lymphocytes are infected by HHV8 in vitro, B cell transformation does not occur. Cell lines have been derived from PEL specimens infected with HHV8 and they are being used for studying the molecular effects of HHV8 gene expression on B cells. Genomic studies have shown that multiple copies (50-150 copies/cell) of episomal HHV8 genomes occur in PEL cells and when tested for presence of HHV8, all PEL cells were infected with HHV8 hence suggesting a role of HHV8 in PEL (Keyvani *et al*.,2017; Sanchez *et al*.,2020).

Some studies indicated immune-regulatory cytokines play an important role in hematologic malignancies. Cytokines are soluble proteins which contribute to the inflammatory cell's recruitment and immune responses (Al-Khatib *et al*.,2020).

IL1RN is a member of the IL-1 superfamily that functions as a competitive antagonist of the cell surface IL-1receptor, thereby moderating a variety of IL-1related immune and inflammatory responses .IL1RN is produced by several cell types such as immune cells, epithelial cells, and adipocytes (Wang *et al*.,2019).

The IL1RN gene has an 86 bp variable number of tandem repeats (VNTR) polymorphism in the second intron. Associations between this polymorphism and some cancers such as lymphoma have been reported in previous studies, but with controversial results (Wu *et al*.,2015; Huang *et al*.,2016).

1.2 Aim of Study

This study was aimed to determine the percentage of Human Herpes Virus -8 (HHV-8) and *IL-1R1 rs1419620062* gene polymorphism in lymphoma patients., through achieving the following objectives:

1. Determine the percentage of HHV-8 in blood specimens of apparently healthy control and Patients suffering from NHL and HL by PCR technique.
2. Estimation of the genetic polymorphism of *IL-1R1 rs1419620062* gene in patients with NHL and HL as well as apparently healthy control by sequencing.
3. Studying of IL-1R level concentration in study groups by ELISA.
4. Find the association between these *IL-1R1rs1419620062* gene polymorphism and IL-1R level concentration as well as HHV-8 infection in patients with NHL and HL.

CHAPTER TWO

LITERATURE REVIEW

2. Literature Review

2.1 Lymphoma

2.1.1. Introduction

Lymphoma is a heterogenous group of lymphoid neoplasms with marked differences in clinical course and response to treatment. Lymphomas are grouped by their postulated normal cell type of origin (B-cell vs. T-cell), and morphology (Hodgkin vs. non-Hodgkin) as well as the degree of cellular differentiation. With advancements in molecular medicine, more than 100 discrete lymphomas have been identified and grouped by genetic and morphologic characteristics. Under the most recent classification system, there is increasing emphasis on genetic and pathologic markers in characterizing distinct lymphomas and on using these data to drive clinical management. While lymphoma will ultimately be managed by a hematologist/ oncologist in the United States, the initial lymphoma diagnosis and early clinical management is typically performed in a primary care or general hospital setting (Paquin *et al*.,2023).

Lymphoma can also have a variety of atypical presentations, for example, extra-nodal lymphoma could present with GI symptoms, CNS symptoms, cutaneous symptoms, or other generalized or organ specific symptoms.6–8 Despite this, lymphoma is a rare diagnosis with an incidence of 2.6 and 19.6 cases per 100 000 people per year for Hodgkin and non-Hodgkin lymphoma (NHL), respectively (Thandra *et al*.,2021).

2.1.2. Hodgkin's lymphoma (HL)

Hodgkin lymphoma (HL), initially called “Hodgkin’s disease”, was first reported in 1832. HLs primarily affect lymph nodes, and are characterized by a mixture of large dysplastic tumor cells and small non-neoplastic inflammatory cells.

The biological nature of HLs has long been a mystery, as the neoplastic cells lack both B-cell markers and T-cell markers in most cases. Based on their constellations of morphologic and biologic properties, nodular lymphocyte-predominant HL (NLPHL) and classic HL (CHL) are currently recognized as distinct disease entities, although they share a paucity of neoplastic cells and a rich inflammatory background of non-neoplastic cells, mainly T cells. NLPHL expresses B-cell markers and retains a B-cell phenotype, which led to its recognition as a B-cell neoplasm overlapping with T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) (Takahara *et al*.,2022).

In the International Consensus Classification of Mature Lymphoid Neoplasms (ICC classification), NLPHL was renamed as nodular lymphocyte-predominant B-cell lymphoma. CHLs exhibit reduced expression of B-cell markers, although they are reportedly derived from crippled germinal center (GC) B cells (Campo *et al*.,2022).

2.1.2.1. Cellular Origin of Hodgkin Lymphoma

For many years, the cellular origin of CHL has been controversial because the HRS cells of CHL have a morphology and immunophenotype that does not match any type of immune cells. Genetic analysis has revealed that in almost all CHL cases, the HRS cells have a clonally rearranged immunoglobulin (IG)gene. Furthermore, a significant proportion of HRS cells have somatic hypermutation at the IG gene locus. In around 30% of cases, IG gene rearrangements render the product nonfunctional through the introduction of stop codons, deletions generated within the GC (Asano *et al*.,2011). In the other cases, HRS cells frequently lack Ig gene transcription ability due to functional defects in the Ig gene regulatory elements. These genetic analysis findings indicate that HRS cells originate from GC cells, which normally cannot survive without B-cell receptor (BCR) signaling.

Notably, a minority of CHL cases have clonal TCR gene rearrangement and/or express T-cell markers (Takahara *et al*.,2022).

2.1.2.2. Genetic Alterations

The most frequent genetic alteration found in CHL is a copy number gain of 9p24.1 at the locus including *PD-L1/L2* and *JAK2*, which is found in up to 97% of CHL cases. A copy number gain of *PD-L1/L2* increases its transcripts in HRS cells. Additionally, a copy number gain of *JAK2* leads to constitutive activation of JAK/STAT signaling, and thereby also induces PD-L1 expression on HRS cells. A minority of CHL cases (4/200, 5%) exhibit unbalanced translocations involving 9p24.1, which might upregulate PD-L1 expression by stabilizing *PD-L1* mRNA. An inactivating mutation of the Beta 2 microglobulin gene (*B2M*) is another prevalent gene mutation in CHL (up to 40%), which also contributes to escape from immune surveillance by $CD8^+$ T cells, by limiting the cell surface expression of major histocompatibility complex class I (MHC class I). These immune-evasion-associated genetic alterations are more frequently observed in EBV^+ CHL than in EBV^-CHL, and presumably inhibit the T-cell response to the virus-derived antigen of EBV^+CHL tumor cells. In addition to *JAK2* copy number gain, the JAK-STAT pathway is also activated by inactivating mutations of *SOCS1* and *PTPN1*, which are both negative regulators of JAK/STAT signaling. Activating mutation of *STAT6* is observed in~30% of CHL cases. In total, almost all CHL cases harbor genetic alterations that affect the JAK/STAT pathway (Takahara *et al*.,2022).

CHL also frequently exhibits constitutive NF-κB signaling due to genetic alterations. A copy number gain of *REL,* a component of the NF-κB signaling pathway, is observed in over 50% of CHL cases, making this one of the most frequent copy number alterations in CHL. Additionally, over 50% of CHL cases exhibit genetic deletion and/or inactivating mutations involving *TNFAIP3,* a

negative regulator of the NF-κB pathway. Truncations of other NF-κB regulator genes, including *NFKBIA* and *NFKBIE,* are less frequently reported (Tiacci *et al*.,2018).

Disruptive mutations of *GNA13* (encoding G protein subunit alpha-13) and ITPKB (encoding inositol-trisphosphate 3-kinase) are observed in around 30% of CHL, and reportedly induce Akt activation. Since Akt activation plays a central role in tonic BCR signaling, and rescues BCR knock-out in B-cell lymphoma cell lines, these genetic alterations may replace the function of BCR signaling among neoplastic cells in CHLs lacking BCR expression (Wienand *et al*.,2019).

2.1.3. Non-Hodgkin's lymphoma (NHL)

Non-Hodgkin Lymphomas are a heterogenous group of lymphoproliferative malignancies that are much less predictable than Hodgkin's lymphomas and have a far greater predilection to disseminate to extra nodal locations. Nearly 25% of NHL cases arise in extra nodal locations and most of them are seen involving both nodal and extra nodal sites. The most common NHL subtypes by far in developed countries are diffuse large B-cell lymphoma (about 30%) and follicular Lymphoma (about 20%). All other NHL subtypes have a frequency of less than 10%.NHL is the sixth most common cause of cancer-related death in the USA after prostate, breast, lung, colorectal, and bladder cancer. Oropharyngeal Lymphomas are the second most common malignant disease in the oral region after squamous cell carcinoma (Al-Naeeb *et al.*,2018).

On the basis of morphologic and laboratory data available, the International Lymphoma Study Group (ILSG) codified and published a "revised European-American lymphoid neoplasms (REAL)" classification. This classification proposed 34 biologically well-defined lymphoma entities and placed the emphasis on the underlying biologic aberrations of the specific lymphoma

subtypes. Recently, this REAL Classification has been modestly revised to take an even more global stature as the World Health Organization (WHO) Classification (Singh *et al.*,2020).

2.1.3.1. Non-Hodgkin's lymphomas on the basis of location(Singh *et al.*,2020).

1. Oral cavity, Waldeyer's ring, and Pharynx

• Small B-cell lymphoma

- Marginal zone B-cell lymphoma

- Mantle cell lymphoma

- Follicular lymphoma - Extra nodal plasmacytoma.

• Diffuse large B-cell lymphoma

• extra nodal NK/T-cell lymphoma, Nasal type (Secondary extension).

2. Nasal cavity and par nasal sinuses

• Small B-cell lymphoma

- Lymphocytic lymphoma

- Follicular lymphoma

- Mantle cell lymphoma

- Marginal zone B-cell lymphoma

- Burkitt's lymphoma

- NK/T cell lymphoma, Nasal type.

3. Larynx and trachea

• Small B-cell lymphoma

- Marginal zone B-cell lymphoma

- Extramedullary plasmacytoma.

• Diffuse large B-cell lymphoma

2.1.3.2. Etiology of non-Hodgkin's lymphoma

I. Pathogenesis

Central pathogenesis mechanisms include immunosuppression, especially in relation to T-cell function and loss of control of latent EBV infection, and chronic antigen stimulation. B and T lymphocytes are important members of the immune system that above all serve to protect against infectious agents. In general, B-cells produce antibodies with antigen-binding capacity, whereas T-cells recognize antigen presented by other cells. Immunosuppression in a variety of medical conditions increases the risk of NHL. The most well-established risk factors for malignant lymphomas are characterized by dysregulation or suppression of T-cell function (HIV/AIDS, organ transplantation) that allows for Epstein-Barr virus (EBV) driven B-cell proliferation and transformation. Chronic antigenic stimulation increases B-cell proliferation, which in turn increases the probability of a random genetic mistake, particularly related to immunoglobulin gene rearrangements (Meena *et al.*,2019).

Factors that induce proliferation thereby potentially lead to more errors. In cases in which a virus acts as the foreign stimulant, the virus itself may infect a normal cell and integrate viral DNA into the host genome, thereby transforming the cell into a malignant cell capable of self-replication (Singh *et al.*,2019).

In either case, antigenic stimulation can also lead to a compensatory downregulation of the T-cell response, that is, an immunosuppressive state.

Lymphoma tumor cells are a malignant form of these precursor lymphocytes arrested at a specific stage of differentiation. Chromosomal translocations, usually balanced reciprocal recombination's, are the genetic hallmark of lymphoid malignancies; their presence has been confirmed in up to 90% of NHL cases. At a molecular level, these translocations with or without additional chromosomal deletions and mutations may precipitate oncogene activation or tumor suppressor gene inactivation (Singh *et al.*,2020).

II. Risk factors for NHL (Singh *et al.*,2020).

1. Immunosuppression

2. Ultraviolet radiations

3. Viruses and other pathogens (EBV, HTLV, HHV8, Hepatitis C, SV40, and Helicobacter pylori).

4. Autoimmune and chronic inflammatory disorders (Rheumatoid arthritis, Sjogren syndrome, and SLE).

5. Occupational exposure (pesticides like phenoxy acids, organophosphates, and organochlorines).

2.2. Human Herpes Virus-8 (HHV-8)

2.2.1. Historical Preview

Human Herpesvirus-8 (HHV-8), also known as KSHV, was discovered in early as 1984, scientists reported seeing herpesvirus-like structures in KS tumors examined under electron_microscopy. Scientists had been searching for the agent causing KS, and over 20 agents were proposed as the possible cause, including cytomegalovirus and HIV itself. The pathogen was ultimately identified in 1994 by Yuan Chang and Patrick S. Moore, a wife and husband team at Columbia

University, through the isolation of DNA fragments from a herpesvirus found in a KS tumor in an AIDS patient. Chang and Moore used representational difference analysis, or RDA, to find KSHV by comparing KS tumor tissue from an AIDS patient to his own unaffected tissue. The idea behind this experiment was that if a virus causes KS, the genomic DNA in the two samples should be precisely identical except for DNA belonging to the virus. In their initial RDA experiment, they isolated two small DNA fragments that represented less than 1% of the actual viral genome. These fragments were similar (but still distinct from) the known herpes virus sequences, indicating the presence of a new virus. Starting from these fragments, this research team was then able to sequence the entire genome of the virus less than two years later (Chang et al.,1994; *Moore and Chang*,1995; Ferla et al.,2023).

The discovery of this herpesvirus sparked considerable controversy and scientific in-fighting until sufficient data had been collected to show that indeed KSHV was the causative agent of Kaposi's sarcoma. The virus is now known to be a widespread infection of people living in sub-Saharan Africa; intermediate levels of infection occur in Mediterranean populations (including Lebanon, Saudi Arabia, Italy, and Greece) and low levels of infection occur in most Northern European and North American populations. Gay and bisexual men are more susceptible to infection (through still unknown routes of sexual transmission) whereas the virus is transmitted through non-sexual routes in developing countries (Ennaji,2022).

2.2.2. Taxonomy and Classification

More than 100 herpesviruses have been discovered, of which all are double-stranded DNA viruses that can establish latent infections in their respective vertebrate hosts; however, only eight regularly infect humans. The Herpesvirinea family is subdivided into three subfamilies: the Alpha-, Beta-, and Gammaherpesvirinea. This classification was created by the Herpesvirus Study Group of the International Committee on Taxonomy of Viruses using biological

properties and it does not rely upon DNA sequence homology. HHV-8 belongs to the γ Herpesvirinae subfamily (Etta *et al*.,2018).

Unranked: *virus*

Relam: *Duplodnaviria*

Kingdom: *Heunggongvirae*

Phylum: *Peploviricota*

Class: *Herviviricetes*

Order: *Herpesvirales*

Family: *Herpesviridae*

Subfamily: *Gammaherpesvirinae*

2.2.3. HHV-8 particles

HHV-8 virions have morphological features typical of other herpesviruses, with a size of approximately 100–150 nm. HHV-8 capsids possess a typical herpesvirus icosahedral capsid shell composed of four structural proteins. Nucleocapsids with an electron-dense core are found in the nucleus of the induced cells and enveloped virions are found in the cytoplasm (Juillard *et al*., 2016).

A proteinaceous layer of tegument surrounds the capsid and contains several organized capsid-associated proteins, several loosely-associated proteins, and viral RNAs. A host-derived lipid bilayer termed the viral envelope is the last layer that surrounds the whole particle. Viral envelope glycoproteins transverse the viral envelope and are responsible for the initial virus–host interactions. Viral envelope glycoproteins K8.1A, glycoprotein-B (gB), and the heterodimer of glycoprotein- H and glycoprotein-L (gHgL) are widely regarded as the most important for virus entry and are the best understood of the KSHV glycoproteins figure (2-1) (Dollery ,2019).

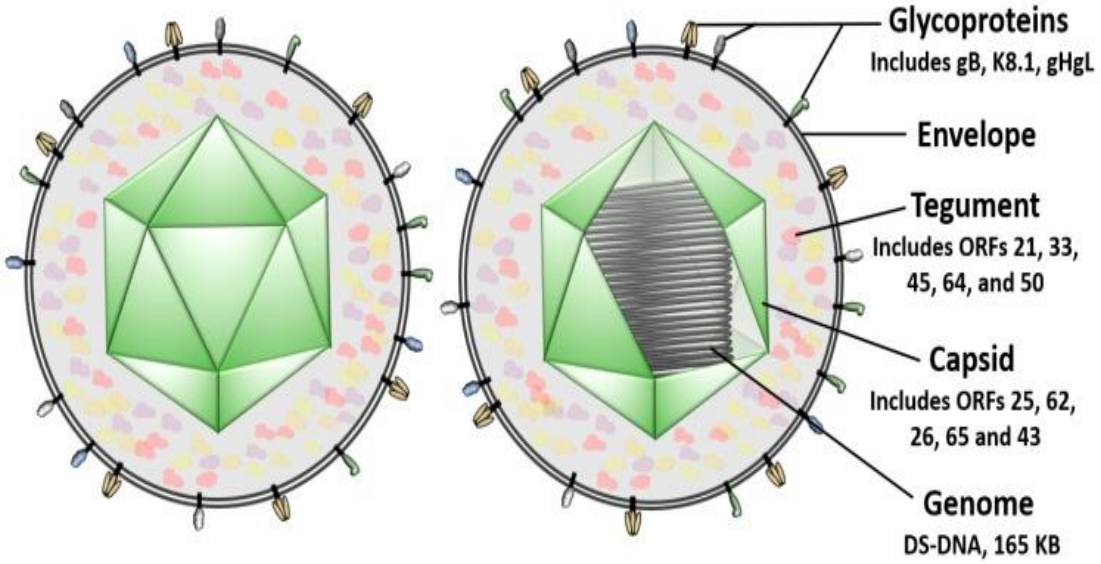

Figure 2.1. A diagrammatic representation of a Kaposi's sarcoma-associated herpesvirus (KSHV)virion (Dollery,2019).

2.2.4. Genome Organization of HHV-8

The viral genome consists of a 160 kbase long unique region, encoding all of expressed viral genes, which is flanked by 20-30 kbases of terminal repeat sequences. The coding region is flanked by variable numbers of direct terminal repeats. HHV-8 possesses approximately 26 core genes, which are highly conserved across the alpha-, beta-, and gammaherpesviruses. These genes are responsible for gene regulation, nucleotide metabolism, DNA replication, and virion maturation and structure figure (2-2) (Finkel *et al*.,2020).

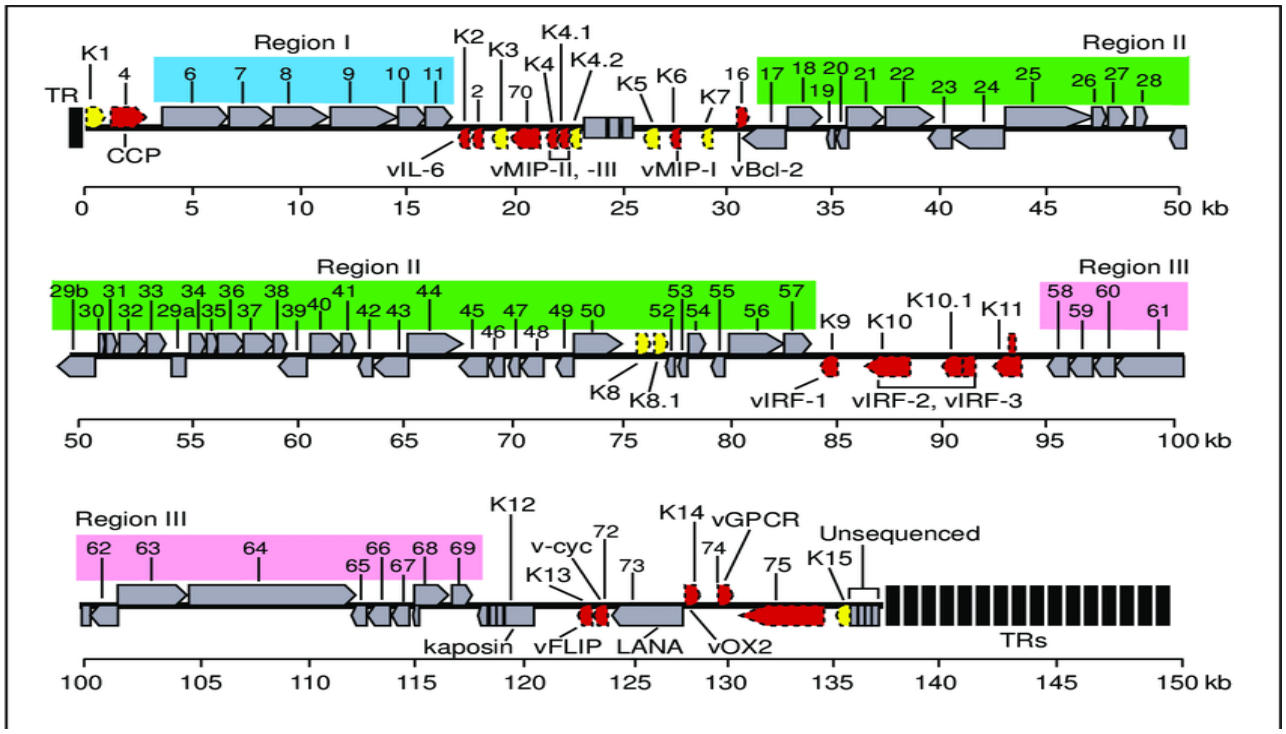

Figure 2.2. Human Herpes Virus-8 Genome (Finkel *et al*.,2020).

HHV-8 also has at least 12 human host gene homologs, not shared by other human herpesviruses. Some of these genes retain host function, or have been modified and implicated in oncogenesis. These include genes encoding viral Bcl-2, cyclin D, interleukin-6, G-protein-coupled receptor, and ribonucleotide reductase. In addition, HHV-8 has a number of genes and regulatory proteins that interact with the host immune response, functioning in evasion of host cellular defense mechanisms (Heming *et al*.,2017; Finkel *et al*.,2020).

2.2.5. Replication Cycle of HHV-8

2.2.5.1. HHV-8 Attachment

HHV-8 to spread to diverse tissues lies its wide tropism demonstrated in vivo for epithelial and endothelial cells, fibroblasts, B and T lymphocytes, monocytes, macrophages, and dendritic cells (Light *et al*.,2021).

2.2.5.2. Entry and Un-coating

After HHV-8 attaches to cells via its glycoproteins that protrude from the virus surface and engage in numerous low-affinity interactions with ubiquitous cellular factors such as heparan sulfate proteoglycans. The virus is trafficked toward the endosomal compartment, where the capsids are released into cytosol upon merger of the viral and endosomal membranes (Dollery,2019).

The membrane fusion process is mediated by the envelope glycoprotein B (gB) and the noncovalent heterodimer made of glycoproteins H and L (gH/gL), which constitute the conserved core fusion machinery of all herpesviruses. The gB is the fusogen protein, while gH/gL plays a role in the regulation of gB activity. Engage diverse cellular receptors (gB binds to integrins and DC-SIGN, gH/gL to EphA receptors), increasing the HHV-8 target repertoire and providing the virus with a set of tools for well-orchestrated entry (Connolly *et al*.,2021).

2.2.5.3. Latent Phase of Infection

During latency, the virus genome depends on the host replication machinery and replicates as closed circular episome ("plasmid") using sequences within the terminal repeats as a replication origin. Viral replication is suppressed, resulting in the formation of a quiescent state of dormancy, with which minimal gene expression occurs. HHV-8 is able to establish a predominantly latent, life-long infection in host monocytes, dendritic cells, B lymphocytes, and endothelial cells (Yan *et al*., 2019).

As with EBV, infection of B cells by HHV-8 triggers the expression of several latency-specific genes. These genes encode proteins that function primarily in the maintenance of episomal viral genome in latently infected cells to transform the cells to ensure the long-term survival in a short-lived cell. Latently expressed genes include ORF71, which encodes a homologue of the antiapoptotic factor vFLIP, and

ORF73, the latent nuclear antigen (LANA) which functions to maintain genome integrity and episomal persistence (Liu *et al.*,2018).

2.2.5.4. Lytic Phase of Infection

For viral propagation to occur, the virus should undergo reactivation from the latency into the lytic phase, when active viral replication occurs and newly synthesized virions are released into the extracellular space. The herpesvirus lytic replicative phase can be divided into four phases, immediate early (IE), early (E), partial late, and late, depending on the transcription pattern prior or after lytic life cycle induction (Edelman,2005). Immediate-early (IE) or? genes require no prior viral protein synthesis. These genes are involved in transactivating transcription from other viral genes. The most notable HHV-8 IE gene, ORF50 encodes R transcriptional activator (Rta), which functions in the earliest phases of reactivation from latency into the lytic cycle. Early or? genes, are expressed independently of viral DNA synthesis. Encoded by K2, vIL-6 is a homolog to the cellular IL-6, and stimulates multiple cellular pathways to induce cell proliferation, immunomodulation, and anti-apoptotic activity (Liu *et al.*,2018).

Polyadenylated nuclear (PAN) RNA stabilizes unspliced transcripts, increasing the abundance of RNA in the nucleus and blocking the assembly of mRNA-protein complexes. Partial late or ?1 genes are expressed in concert with the beginning of viral DNA synthesis. This is a subset of late genes, and their transcription rate is enhanced during DNA synthesis. ORF18, which encodes a transfactor that is essential for late gene transcription of gammaherpesviruses, is considered to be a ?1 gene. Late or ?2 gene expression is totally dependent upon synthesis of viral DNA. Virion structural genes encoding for capsid proteins and envelope glycoproteins areconsidered as late genes. K8.1 is a late gene that encodes a viral envelope glycoprotein at a late phase of infection (Yan *et al.*, 2019).

2.2.5.5. HHV-8 Releasing

The capsids exit the nucleus, acquiring an intermediate envelope by budding through the inner part of the nuclear membrane, are de enveloped by fusion with the external part of this membrane, and appear as tegumentary forms in the cytoplasm. The acquisition of the final envelope carrying viral glycoproteins occurs in the trans-Golgi network, and mature virions are released by exocytosis. The occurrence of a complete replication cycle, which lasts about 3 days, has a major impact on host cell functions and morphology. Infected cells engaged in this virus-producing process ultimately die by apoptosis and/or necrosis (Henri *et al*.,2015; Nishimura *et al*.,2020).

2.2.6. Pathophysiology of HHV-8 infection

Circulating blood mononuclear and endothelial "Progenitor cells" when infected with HHV-8 are reprogrammed to resemble lymphatic endothelium, which upregulates several lymphatic associated genes such as lymphatic vessel endothelial receptor 1 (LYVE1) podoplanin, and vascular endothelial growth factor receptor 3 (VEGFR3). Viral genes go through three stages to be transcribed and expressed: immediate early genes, early genes, and late genes. The nucleocapsid is ultimately formed and binds to the proteins of the tegument in the nucleus cell before acquiring the envelope in cytoplasmic vacuoles derived from the Golgi apparatus. These vacuoles then release virions into the cellular membrane by exocytosis. This replication cycle corresponds to the lytic (or productive) cycle that leads to the death of host cells and to virus production that will disseminate through the organism at the time of primary infection or during reactivations. Following primary infection, HHV-8 latently persist in various sites and cells of the organism, especially in monocytes-macrophages (Agut *et al*.,2016).

HHV-8 encodes a variety of gene products, some involved in cell survival processes such as transformation, proliferation, cell signaling, antiapoptosis and angiogenesis, and involvement in immune modulation, including cytokine production, and immune evasion. All these mechanisms may be involved in the promotion of oncogenesis and viral persistence. Various hypotheses have been put forward to explain this latency mechanism; all of these mechanisms might be present among various cell: presence of the sole viral genome in its episomal form with a potentially limited expression of some genes, replication cycle blocked at the intermediate stage or replication cycle completed but controlled and 5 compatibles with the prolonged survival of the host cell. From this latent state, a productive cycle may be reactivated and may lead to a new production of infectious viruses in the blood and in other body compartments such as saliva. However, the selective expression of some viral genes may be enough to induce modifications of cell functions, even in the absence of a complete replication cycle as demonstrated in an experimental study. Serum antibodies react to various viral proteins, and some of these proteins seem to be dominant antigens in serological tests. Cellular immune response may be detected with the proliferation of CD4+ and CD8+ T cells following exposure to viral antigens (Agut *et al*.,2015).

2.2.7. Transmission of HHV8

Transmission of HHV-8 in adults has been suggested to occur via blood, bodily fluids, and sexual contact. In industrialized countries, sexual transmission is highest in the population of men who have sex with men (MSM). A study conducted on a San Francisco cohort of MSM, HHV-8 found significant rates of sexual transmission. Disparity among several studies suggests that transmission of HHV-8 via transfusion of blood or blood products is possible, but unlikely (Vamvakas,2010; Gobbini, 2012). The reports of transmission via blood transfusion products vary greatly with geographical area. Incidence rates are low in North America, and higher

in areas of endemic HHV-8. A Ugandan study demonstrated that 41 of 991 blood transfusion recipients seroconverted to be HHV-8 positive post transfusion, with an increase in risk noted in those receiving HHV-8 seropositive blood (Lee *et al*.,2020).

2.2.8. Laboratory diagnostic of HHV-8

Immunologic assays are the most widely used in HHV-8 diagnostics. Currently four antibody detection assays are common of viral DNA detection in population screening. Immunologic assays are the most widely used in HHV-8 diagnostics. Currently four antibody detection assays are commonly used in HHV-8 serodiagnostics: enzyme-linked immunosorbent assay (ELISA), immunofluorescent assay (IFA), Western blot, and immunohistochemistry (IHC). Antibodies recognizing lytic proteins will result in a whole cell fluorescent staining, whereas recognitions of latent proteins result in a more restricted, punctate nuclear appearance, demonstrating recognition of the latently expressed protein, LANA. Also, commonly used in IFAs are recombinant proteins expressed in insect cells (Ana-Lia *et al*., 2020).

Polymerase chain reaction (PCR)-based methodology of HHV-8 DNA detection gives the greatest specificity for diagnosis, compared to all other tests currently used for measuring HHV-8 exposure or infection. Viral genomic copy number varies according to cell type and disease status (*McCall et al*., 2020).

In addition, Chromogenic In Situ Hybridization (CISH) was done for detecting the T1-1 probe for the viral mRNA of HHV-8 (Todorović., 2021).

2.2.9. Human Herpes Virus-8 and Lymphoma

Rare large B-cell lymphomas that develop as serous effusions in the body cavity without detectable tumor masses are usually positive for human herpesvirus 8 (HHV8), and primary HHV8-positive effusion-based lymphoma (EBL) is defined

as primary effusion lymphoma (PEL) according to the current World Health Organization classification (Kaji *et al*.,2020).

However, there have been several case reports and literature reviews on primary HHV8-negative EBL, and terms such as PEL-like lymphoma, HHV8-unrelated PEL, and primary HHV8-negative EBL have been coined to describe those cases (Chen *et al*.,2017; Kim *et al*.,2017; Kojima *et al*.,2017).

In addition to these well-characterized HHV8-related lymphoproliferative disorders, occasional cases with atypical and overlapping features among entities have been recognized such as lesions intermediate between multicentric Castleman disease and germinotropic lymphoproliferative disorder in HIV-positive patients or cases of germinotropic lymphoproliferative disorder that progress to high-grade EBV and HHV8-positive lymphoma (Gonzalez-Farre *et al*.,2017).

2.3. Interleukin-1Receptor (IL-1R)

2.3.1. Definition

IL-1R is a cytokine receptor which binds interleukin 1. IL1R1 is a glycoprotein that is expressed on various cells such as endothelial cells, lymphocytes and dendritic cells. IL1R1 encodes cytokine receptor for IL1, through combining with IL-1 on the cell surface affects NF-κB signaling and upregulates inflammation. Two forms of the receptor exist. The type I receptor is primarily responsible for transmitting the inflammatory effects of interleukin-1 (IL-1) while type II receptors may act as a suppressor of IL-1 activity by competing for IL-1 binding (Tettamanti *et al*.,2018).

2.3.2. Location of Interleukin-1Receptor (IL-1R)

IL-1Ra are closely associated in the region of 2q12-q21 of human chromosome 2 (Cauci, *et al*.,2019).

2.3.3 Structure of IL-1R

IL-1Ra is a 17 kDa polypeptide composed of 12 β-strands and two very short 3–10 helices, similar in architecture to IL-1α and IL-1β (Fields *et al*.,2019).

2.3.4. Function of IL-1R

IL-1R may be responsible for the formation and development of cancers. IL-1R1 initiates inflammatory responses when binding to the ligands IL-1α and IL-1β and has been reported to be expressed by T- lymphocytes, fibroblasts, epithelial cells, and endothelial cells (Zhu *et al*.,2019).

IL-1 binds to IL1R1 and initiates signal transduction by the cytoplasmic domains of IL1R1 and IL1R2 receptors (Vasilyev *et al*.,2015).

2.3.5. Diagnostic of IL-1R polymorphism

2.3.5.1. Single Strand Conformation Polymorphism (SSCP)

SSCP analysis is a simple and sensitive technique for mutation detection and genotyping. The principle of SSCP analysis is based on the fact that single-stranded DNA has a defined conformation. Altered conformation due to a single base change in the sequence can cause single-stranded DNA to migrate differently under no denaturing electrophoresis conditions (Satyanarayan *et al*.,2019).

2.3.5.2 Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to

distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence. The term may refer to a polymorphism itself, as detected through the differing locations of restriction enzyme sites, or to a related laboratory technique by which such differences can be illustrated. In RFLP analysis, a DNA sample is digested into fragments by one or more restriction enzymes, and the resulting restriction fragments are then separated by gel electrophoresis according to their size (Chaudhary and Kumar,2019).

2.3.5.3. Amplification Refractory Mutation System (ARMS)

In ARMS technique, one PCR comprises one allele-specific oligonucleotide primer at 5′-end and a common primer at 3′-end. If the presence of an amplified mutant is detected by agarose gel electrophoresis, it suggests that the target sequence contains the mutant allele. Similarly, if the result displays an absence of the amplified mutant, it indicates the presence of the normal DNA sequence on that specific point. In the same way, a normal primer at 5′-end together with a common primer at 3′-end was used in another PCR. If normal amplified product is present, it reveals the existence of a natural DNA sequence, whereas if normal amplified product is absent, then it reveals the presence of a mutant allele (Yang *et al*.,2017).

2.3.5.4. Sequencing of PCR products

The term DNA sequencing refers to methods for determining the order of the nucleotides bases adenine, guanine, cytosine and thymine in a molecule of DNA. The first DNA sequence was obtained by academic researchers, using laboratories methods based on 2- dimensional chromatography in the early 1970s. By the development of dye based sequencing method with automated analysis, DNA sequencing has become easier and faster. The knowledge of DNA sequences of genes and other parts of the genome of organisms has become indispensable for

basic research studying biological processes, as well as in applied fields such as diagnostic or forensic research (Hao and Stephen ,2018).

2.3.6. Association between IL-1R polymorphisms and lymphoma

Some studies indicated immune-regulatory cytokines play an important role in hematologic malignancies. Cytokines are soluble proteins which contribute to the inflammatory cell's recruitment and immune responses (Al-Khatib *et al*.,2020).

Studies regarding the role of interleukin gene polymorphisms in Hodgkin's Lymphoma have revealed anti-inflammatory functions of IL-1R (Kornman ,2006; Schoof *et al*.,2013). (Sarani *et al*., 2021) was found association between the IL-1A, IL-1B and IL-1R polymorphisms and lymphoma.

CHAPTER THREE

MATERIALS & METHODS

3. Materials and Methods

3.1. Subjects

3.1.1. Patients

This case control study was done for a two hundred (200) specimen's collected from patients subjected to Lymphoma (LY) included HL(25) ; NHL (75) specimen's and apparently healthy persons (AHC) as a control group (normal persons) from general hospitals as well as many private clinical in Middle Euphrates – Iraq. The age range of the study population was 15 years to 80 years. The specimens were collected during period from October 2022 to June 2023.

Blood from each study group of patients suffering from Lymphoma should be enrolled, that classified into: -

1. One hundred blood specimens from persons suffering from Lymphoma included HL(25) ; NHL (75) specimen's.
2. One hundred blood specimens of apparently healthy persons as a control group.

All these specimens were submitted for genetic part for screening human Herpes virus-8 (HHV-8) in patients and apparently healthy person control groups by polymerase chain reaction (PCR). However, the second part for detection SNPs of *IL-1R1rs1419620062* gene polymorphism by sequencing.

3.1.2 Specimens collection

Blood specimens were collected from patients and healthy persons.

5ml venous blood were collected aseptically from all patients by using gel tubes and EDTA tubes for gating blood serum and total DNA extracction, respectively; then stored at (-20°C).

3.2 Study Design

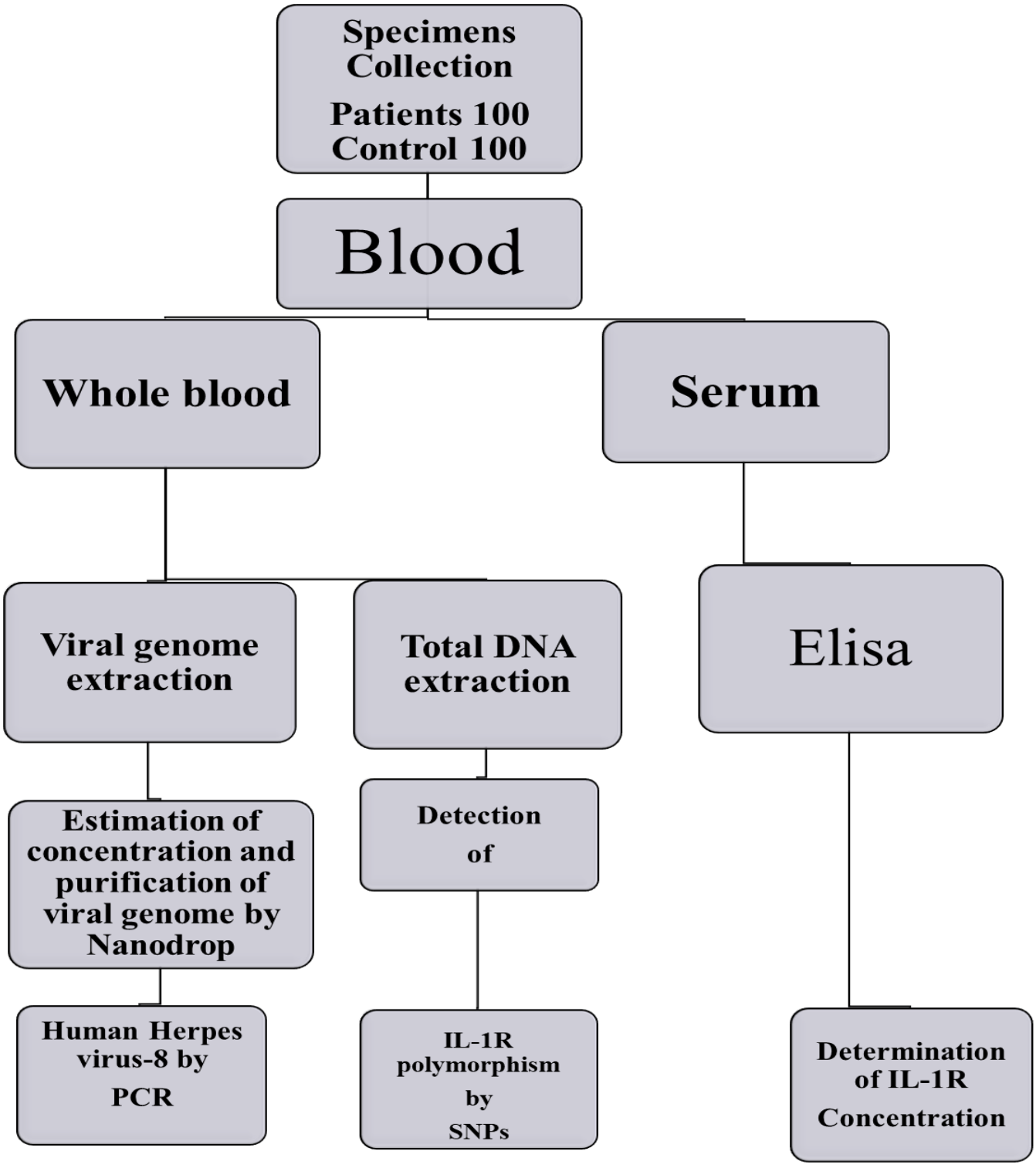

Figure 3.1 Study Chart Flow

3.3 Materials

3.3.1 Instruments and Equipment

Instruments and equipment used in this study are listed in table (3-1).

Table 3.1: Instruments and Equipment used in this study with their Manufacturer Company and origin

Instruments and Equipment	Manufactured Company (Origin)
Centrifuge	MSE / England
Centrifuge Eppendorf; Vortex	Eppendorf / Germany
Micro centrifuge	Hettich centrifuge, Sigma / Germany
Deep freeze (-70 C); Electric microwave	Sanio electric / Japan
Electrophoresis system	Fisher Scientific / USA
ELISA reader	Bio Tech / USA
Hood	Bio Lab / Korea
Electrical Oven; Incubator	Memmert / Germany
Nanodrop	Optizen / Korea
PCR device	Biometra / Germany
Refrigerator	Arcelik / Turkey
Sensitive balance	Sartoris / Germany
Timer with alarm	Junghans / Germany
Gel documentation system	Cleaver Scientific / UK
Eppendorf tubes with different size (2ml, 1.5ml); Disposable tips; PCR tubes	Extra gene / Taiwan
Baker; Inserted cylinder; Disposable (Gloves, Syringes, Gel tubes, EDTA tubes)	China
Micropipettes various sizes (0.5-10, 20-200, 100-1000) µl	Extra gene/Taiwan
Ice-box	Germany

3.3.2 Reagents and Buffers

Reagents and buffers used in this study are listed in table (3-2)

Table 3.2: Reagents and buffers used in this study with their Manufacturer Company and origin.

Reagents	Manufactured Company (Origin)
10X TBE DNA sequencing Grade	Intron /S. Korea
Absolute Ethanol alcohol	Merck – Germany
Agarose E	Conda / Spain
DNA Ladder (1500-100bp)	Intron /S. Korea
Proteinase K	Intron /S. Korea
RNase A	Intron /S. Korea
De-ionized sterile distilled water	BioNeer / Korea
Master mix	Promega / USA
Nuclease free water	Promega / USA
DNA loading dye	Intron / Korea
Safe stain (Red Safe)	Intron / Korea

3.3.3 Kits and Markers

Kits and marker used in this study with their Manufacturer Company and origin are listed in table (3-3).

Table 3.3: Kits and markers used in this study with their manufacturer company and origin

Kits	Manufacturer Company/ Origin	CAT.NO=
G-Spin Total DNA Extraction kit	Intron / Korea	14001
Human IL-1R1 ELISA kit	Elabscience Biotechnology Inc.\USA	E-EL-H2402
Viral Nucleic Acid Extraction kit	Intron / Korea	17151

3.3.4 Primer pairs of Human Herpes Virus-8 (K1-HHV-8) gene and *IL-1R1 rs1419620062 gene*, SNP.

Primers sets used in this study to detect the HHV-8, SNP of *IL-1R1* polymorphism with their product size and source are listed in table (3-4).

Table 3.4: Primers sets that used for detection of Human Herpes Virus-8 (*HHV-8*), *IL-1R1 rs1419620062* gene polymorphism.

Gene	Sequence (5′-3′)	Product size (bp)	Source/origin	Ref.
K1-HHV-8 (IF)	CAGTCTGGCGGTTTGCTTTC	**592 bp**	**IDT / USA**	**designed**
K1-HHV-8 (IR)	GTAGGTGCGGTTGCAAATGT			
***IL-1R1rs1419620062* (IF)**	TGTTCCTGCTAAGGTGGAGG	**480 bp**	**IDT / USA**	**designed**
***IL-1R1rs1419620062*(IR)**	AGGTTCCCTATCAAGTTTCACCA			

3.4 Methods

3.4.1 Detection of HHV-8 by Polymerase Chain Reaction (PCR)

3.4.1.1 Principles of Assay

Polymerase Chain Reaction (PCR) is based on two major processes: **Firstly, isolation** of viral genome (DNA\ RNA) from specimens, and **Secondly**, PCR amplification for each sample. In PCR, the accumulating amplified product can be detected at each cycle with fluorescent dyes. This increasing signal allows to achieve sensitive detection and quantification of pathogens.

3.4.1.2 Extraction of Viral Nucleic Acid from Clinical Specimens

By using specific viral DNA/RNA extraction kit (Intron/Korea); the viral genome was extracted, purified and migrated using agarose gel from the blood specimens as a first step to amplify the target Human Herpes virus-8 DNA.

3.4.1.2.1 Assay Procedure

The procedure was carried out in accordance with the manufacturer's instruction with some modification as following:

1. Three hundred (300) microliters from blood plasma was transferred into 1.5ml micro centrifuge tube.
2. Six hundred (600) microliters Lysis buffer was added, then the lysate mixed by vortex for (25sec). Mixture was incubated at room temperature for (15 min).
3. Six hundred (600) microliters from binding buffer was added, and completely mix well by gently vortex. This step is conducive to efficient passage of cell lysates through a column and to increase binding onto column resins and it is important for effective deproteinization.
4. The lysates of cell were placed in a spin column that provided (2ml) collection tube.
5. Loaded lysates on the column and centrifuged for 2 min at 13,000 rpm.
6. Discarded solution in collection tube and place the spin column back in the same (2ml) collection tube.
7. Five hundred (500) microliters of washing buffer A was added to spin column and centrifuged for 2 min at 13,000 rpm.
8. The solution was discarded in collection tube and places the spin column back in the same (2ml) collection tube and centrifuged for 2 min at 13,000 g and then discarded solution.
9. Five hundred (500) microliters of washing buffer B was added to the spin column and centrifuged for 1min at 13,000 rpm.
10. The solution was discarded in the collection tube and places the spin column back in the same (2ml) collection tube. Centrifuged for 1min at 13,000 rpm. It is

important to dry the membrane since residual ethanol may interfere with downstream reactions.

11. Placing spin column in an RNase-free (1.5ml) micro- centrifuge tube; then 50 µl of Elution buffer was added directly onto the membrane and was incubated at RT for 2min, then centrifuged for 2min at 13,000 rpm.
12. At this stage the supernatant was containing viral genome (DNA), then stored at -20°C.

3.4.1.3 Estimation of The Extracted DNA Concentration and Purity

After extraction of viral DNA from specimens; the concentration of DNA and purity were measured by using Nano drop (Korea), by applying 5 microliters of the extracted DNA in the instrument curette. Extracted with purity in between (1.8-2) at absorption wave length (260/280) was included in this study, otherwise; DNA extraction of the sample was repeated.

3.4.2 Detection of *IL-1R1rs1419620062* gene polymorphism by sequencing.

Total DNA for SNPs of *IL-1R1 rs1419620062* gene polymorphism were extracted from peripheral blood from patients and then using sequencing technique.

3.4.2.1 Extraction of Total DNA from Clinical Specimens

The G-spin™ Total DNA extraction kit is suitable for use fresh or frozen whole blood which has been treated with citrate, heparin, or EDTA. Pre-separation of leukocytes is not necessary. Purification does not require phenol/chloroform extraction or EtOH precipitation, and provides the simplest protocols. DNA is eluted in Buffer CE, TE (10:1), 10mM Tris (pH 7.5 ~ 8) or water, is prepared for direct addition to PCR or other enzymatic reactions. Alternatively, it can be safely stored at (–20°C) for later use. The purified DNA is protein-free, nucleases-free and does not include other contaminants or inhibitors. G-spin™ Total DNA extraction kit is

optimized for extraction of (20-30kb) DNA fragments and able to extract up to 50 kb fragments.

All reagents required for the total DNA extraction were provided with DNA extraction kit (G-Spin total DNA extraction kit, Cat.No. 14001 Intron / Korea).

3.4.2.2 Kit Contents

The contents of G-Spin total DNA extraction kit are listed in table (3-5).

Table 3.5: List of reagents and buffers of G-Spin Total DNA extraction kit used in this study.

Label	Contents 50 Columns	Contents 200 Columns
Buffer CL	25 ml	90 ml
Buffer BL	25 ml	90 ml
Buffer WA	40 ml	160 ml
Buffer WB	14 ml	56 ml
Buffer CE	20 ml	40 ml
Spin Column / Collection Tube	50	200
RNase A (Lyophilized powder)	3 mg x 1 vial	3 mg x 4 vials
Proteinase K (Lyophilized powder)	22 mg x 1 vial	22 mg x 4 vials

3.4.2.3 Assay Procedure

The procedure was carried out in accordance with the manufacturer's instruction as following:

3.4.2.4.1 Extraction of Total DNA from Blood Specimens

1. Two hundred (200) microliters of whole blood was pipetted into a (1.5 ml) micro- centrifuge tube.
2. Adding 40 µl proteinase K Solution and 5 µl of RNase A solution into specimen tube and gently mixed.

3. Two hundred (200) microliters of Buffer BL was added into upper specimen tube and mixed thoroughly. This step is important in order to ensure effective decomposition, and it is important that the BL sample and buffer blood are thoroughly mixed to give a dissolution solution.
4. Placing the mixture at room temperature for (2minutes).
5. The lysate was incubated at 56°C for 10 min. For complete lysis, mix 3 or4 times during incubation by inverting tube. If it breaks down perfectly, the red color of lysate became dark green.
6. The 1.5 ml tube was centrifuged briefly to remove drops from inside the lid.
7. Adding (200 µl) of absolute ethanol into the lysate, and then mixed well by pulse vortex. After mixing, briefly centrifuged the (1.5 ml) tube to remove drops from inside of the lid. This step is an equilibration step for binding genomic DNA to column membrane. Also, this step conduces to pass efficiently cell lysate through a column.
8. The mixture from step 7 was applied carefully to the spin column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuged at 13,000 rpm for 1 min. Discarded the filtrate and placed the spin column in a new (2 ml) collection tube (additionally supplied).
9. Seven hundred (700) microliters of Buffer WA was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. Discarded the flow-through and reused the collection tube.
10. Seven hundred (700) microliters of Buffer WB was added to the spin column without wetting the rim, and centrifuged for 1 min at 13,000 rpm. Discarded the flow-through and placed the column into a new (2ml) collection tube (additionally supplied), then it was centrifuged again for additional 1 min to dry the membrane and discarded the flow-through and collection tube altogether.

11. Placing the spin column into a new (1.5 ml) tube (not supplied), and adding (50 µl) of Buffer CE directly onto the membrane, then incubated for (1 min) at room temperature and then centrifuged for 1 min at 13,000 rpm to elute, then stored at -20°C.

3.5 Detection of HHV-8 and *IL-1R1rs1419620062* gene polymorphism by polymerase chain reaction (PCR)

3.5.1 Primers pairs dilution

The primers' source was from IDT / USA. Primers are commonly transported in a lyophilized state. The units of a lyophilized primer are given as a mass, in Pico moles. To create a stock of primers, one would reconstitute the primer in sterile, nuclease-free H_2O. The company supplies the amount of sterile, nuclease-free H_2O to be added to each primer to obtain master stock (100 mol/µl) that will be used again to obtain working solution. As following: The tube was spin down before opening the cap, then the desired amount of water was added according to the oligoes manufacturer to obtain a (100 pmol/µl master stock). Vortex properly for re-suspend the primers evenly. Then 10µl of the master stock was transferred to a (2ml Eppendorf tube) that contains 90µl of sterile, nuclease-free H_2O (Working Solution 10 pmol/µl). The master stock is stored at -20 C^o and the working solution is stored at -20 C^o. Finally, the working solution was thawed on ice and vortex before using in PCR and then stored at -20 C^o.

3.5.2 PCR Experiments

PCR amplification was done using conventional thermal cycler (Biometra - Germany) as follows: Sample DNA (about 4 µl) was added into PCR master mix tubes. Forward and reverse primers were added (2 µl) into PCR master mix tubes

(for each one). Distilled nuclease free water was added (5 µl) into PCR master mix tubes to a total volume of (25 µl) as the table (3-6).

Table 3.6: Recommended volumes and concentration for applying PCR into AccuPower® PCR tubes.

No.	Content of PCR Reaction Mixture	Volume/ µl
1	Master mix	12 µl
2	Forward primer	2 µl
3	Reverse primer	2 µl
4	Sample DNA	4 µl
5	Nuclease free water	5 µl
Total		**25 µl**

3.5.3 Thermal cycles conditions

Reactions were placed in a thermal cycler (Biometra-Germany) that had been preheated to 94°C and beforehand set up to the desired cyclic conditions. The target regions of HHV-8 and *IL-1R rs12234650* polymorphism were amplified using specific primers according to the mentioned conditions in table (3-7).

Table 3.7: Amplification conditions of HHV-8 and *(IL-1R1 rs1419620062*) gene in patients with Lymphoma.

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
K1-HHV-8	95C^0 /5 min	95C^0 /1 min	53 C^0 /45 sec	72 C^0 /1 min	72 C^0 /5 min	40
IL-1R1 rs1419620062	95C^0 /5 min	95C^0 /1 min	52 C^0 /45 sec	72 C^0 /1 min	72 C^0 /5 min	40

PCR products of target regions HHV-8; *IL-1R rs1419620062* polymorphism were electrophoresed on 1.5 % agarose at 85 V for 1h and visualized by safe stain. Photos were taken using gel documentation system.

3.6 Agarose Gel Electrophoresis Technique

The agarose gel electrophoresis was performed according to the method of Robinson and Lafleche (2000). This technique was used to detect viral genome; total DNA extracts, and PCR products (Robinson and Lafleche 2000).

3. 6.1 Preparation of Solutions and Buffers

3.6.1.1 Loading Buffer

3.6.1.2 TBE buffer (1X)

To prepare 500 ml of 1X TBE buffer, 50 ml of TBE (10X) stock solution was mixed with 450 ml of dH2O.

3.6.2 Gel Electrophoresis Protocol

1. Device setup: The casting gates were sited on the ends of the gel tray and locked in place firmly against casting tray. This was done by engaging the "claws" of the gate in the recess of the side wall of the tray. The comb was sited into the slots of the gel tray, (1.0 mm above the base of gel casting tray) so that the sample wells are near the cathode.
2. Gel dissolving: 1g of agarose was dissolved in 100ml of (1X) TBE solution by melting to 100°C to prepare 1% agarose gel for migrated genomic DNA extracts, also 1% or/and 2% agarose gel was prepared in 1X TBE buffer for migrated PCR products or digested DNA by restriction enzymes respectively.
3. Gel casting: After agarose gel dissolving completely, it is let to cooling to approximately 60°C and (5µl) of the red safe stain stock solution was added, then slowly pour the agarose into the gel- casting tray, and any air bubbles were removed. The comb was positioned at approximately (1.5cm) from one edge of the gel. The agarose was allowed to solidify at room temperature at least 30 min. After that, the claws were disengaged from the gel tray and the

comb was separated gently. Then the gel was placed in the gel tank in such a way that the wells should be on end with the cathode. (1X) TBE buffer (depending the purpose) was added to the buffer tank until it was about (5 mm) above the top of the gel.

4. Loading the samples: Each (5µl) of the genomic DNA sample was mixed with (3µl) loading dye briefly and loaded into the wells. Whereas, the PCR products were loaded without loading dye because of the PCR master mix contained loading dye.
5. Gel electrophoresis conditions: After sample loading the electric field was turned on at 5 V/cm (85V) for (60) min until red safe dye reached at the end edge of the gel.
6. The gel was photographed using gel documentation system (Cleaver Scientific - UK).

3.7 Sequencing

3.7.1 Nucleic Acids Sequencing of PCR Amplicons

The resolved PCR amplicons were commercially sequenced from both directions, forward and reverse directions, following the instruction manual of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (Applied Bio system) sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed nucleic acid sequences of local samples with the retrieved nucleic acid sequences, the virtual positions, and other details of the retrieved PCR fragments were identified.

3.7.2 Interpretation of Sequencing Data

The sequencing results of the PCR products of the targeted samples were edited, aligned, and analyzed as long as with the respective sequences in the reference

database using Bio Edit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The observed variations in each sequenced sample were numbered in PCR amplicons as well as in their corresponding position within the referring genome. The observed nucleic acids were numbered in PCR amplicons as well as in their corresponding positions within the referring genome. Each detected variant within the genome sequences was annotated by Snap Gene Viewer ver. 4.0.4 (https://www.snapgene.com).

3.8 Evaluation of *IL-1R1* Concentration in Blood Serum of Patients and AHC

The concentration of *IL-1R1* in the serum of female patients with Lymphoma was evaluated by enzyme linked immunosorbent assay (ELISA).

3.8.1 Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human *IL-1R1* antibody. *IL-1R1* present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human *IL-1R1* Antibody is added and binds to *IL-1R1* in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated *IL-1R1* antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human *IL-1R1*.The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450 nm.

3.8.2 Reagent Provided

Reagent provided of ELISA kits to evaluate IL-1R1 are listed in table (3-8).

Table 3.8: Reagent provided of ELISA kits to evaluate IL-1R.

Components	Quantity 96T
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Solution (1600pg/ml)	0.5ml x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human IL-1R1	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

3.8.3 Assay Procedure

1. Preparing all reagents, standard solutions and samples as instructed and Bringing all reagents to room temperature before use. The assay is performed at room temperature.
2. Determining the number of strips required for the assay and Inserting the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Adding 50µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Adding 40µl sample to sample wells and then add 10µl anti-IL-1R1 antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Removing the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for (30 seconds to 1 minute) for each wash. For

automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blotting the plate onto paper towels or other absorbent material.

6. Adding 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Adding 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determining the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

3.8.4 Calculation of Results

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

3.9. Statistical Analysis

Statistical analyses were performed using Statistical Package of the Social Sciences (SPSS) version 23.0. Two-way ANOVA, One-way ANOVA, a Chi-Square test (χ^2); Binomial test (Z- test); Kolmogorov Smirnov (Z) test and Spearman's rho and were done to establish relationships of expression immunological variables levels according to the ELISA test results between patients with Lymphoma and apparently healthy control .

The correlation matrix between the selected variables and HHV-8 infection in current study was estimated by using Spearman’s correlation coefficient analysis. Correlation coefficients were considered significant at P-values less than 0.05 by

using SPSS version 23.0. Asterisk (*) indicates that the differences were statistically significant when compared with control group with patient groups. Chi square test (X2) was used to compare the selected groups ** $p<0.01$; *** $p<0.001$. Hierarchical cluster analysis based on similarity coefficient was used in this study to identify the relatively homogenous results using expression immunological variables levels according to the ELISA test results between patients with Lymphoma and with apparently healthy control using PRIMER-E7 software package (http://www.primer-e-.com/)(Clarke and Gorley, 2014).

3.10 Ethical Approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 7/17/1336 (including the number M220904 and the date in 28/9/2022) to get this approval.

CHAPTER FOUR

RESULTS

4. Results

4.1. Clinicopathological Findings

4.1.1. Distribution of patients with Hodgkin and Non-Hodgkin lymphoma, according to their age

The blood specimens collected in this study were related to Non-Hodgkin lymphoma and Hodgkin lymphoma patients whom ages were ranged from fifteen years to eighty years. The mean age of the patients with Non-Hodgkin lymphoma (**46.42±12.06** years) was higher than the mean age of the Hodgkin lymphoma (**45.12± 14.49** years). While, the mean age of apparently healthy control (AHC) was (**44.9± 12.51**) years. However, there was no significant difference between HL and AHC and NHL and AHC in age distribution Table (4.1).

Table 4.1: Distribution of Study Groups According to the Mean and Range of their Age (years).

Studied groups	No	Mean Age / Year	Std. Deviation	Std. Error	Range	
					Min.	Max.
A.H. Control	100	44.9	12.51	2.56	20	68
HL	25	45.12	14.49	3.24	23	56
NHL	75	46.42	12.06	2.48	15	80
Total	200					

Note: A.H. Control = Apparently healthy control, HL = Hodgkin's Lymphoma, NHL = Non- Hodgkin's Lymphoma

*** Mini: Minimum, Maxi: Maximum**

4.1.2. Distribution of patients with Hodgkin and Non-Hodgkin lymphoma, according to their age groups

In Hodgkin lymphoma group, the most affected age group were from 21 – 40 years were constituting (60%:15), followed by the age group of 41 – 60 years (40%:10) and the lowest or not affected group of Hodgkin lymphoma was the age group of ≤ 20 and of 61 - 80 years where constituted (0.0%: 0).

While, in Non-Hodgkin lymphoma, the most commonly affected age group in both 41 – 60 and 61 – 80 years were constituted (37.34%:28) and (40%:30) respectively, followed by the age group of 21 - 40years (17.33% :13). Lastly the lowest affected group of Non-Hodgkin lymphoma was those in the age stratum ≤ 20 years (5.33% :4) as shown in the Table (4.2).

Table 4.2: Distribution of Age groups According to the Histopathological Diagnosis of Studied Groups.

Age groups /Year		Studied groups			Pearson Chi-Square (P-value)
		A.H. Control	HL	NHL	
≤ 20	N	2	0	4	P=0.01 Sign. (P>0.05)
	%	2.0%	0.0%	5.33%	
21 – 40	N	90	15	13	
	%	90.0%	60.0%	17.33%	
41 – 60	N	6	10	28	
	%	6.0%	40.0%	37.34%	
61 – 80	N	2	0	30	
	%	2.0%	0.0%	40%	
Total	N	100	25	75	
	%	100.0%	100.0%	100.0%	

***** Significant differences for all age groups using Pearson Chi- square test at **P**>0.05 level.

4.1.3. Distribution of The Patients with Hodgkin and Non-Hodgkin Lymphoma According to Their Sex

In this study, it was found that (64%:16) of Hodgkin lymphoma were females, while the rest 9 cases (36%) were males. Regarding the patients whom suffering from Non-Hodgkin lymphoma, the percentage of females was also higher (64%:48) than the percentage of males (36%: 27). The female/male ratios of the patients with Hodgkin lymphoma and Non- Hodgkin lymphoma were, 1.78:1.

The statistical analysis showed a highly significant difference (P>0.05) between lymphoma patients and control groups according to sex (Table 4.3).

Table 4.3: Distribution of Study Groups According to Their Sex.

Sex		Studied groups			Pearson Chi-Square (P-value)
		A.H. Control	HL	NHL	
Male	No.	36	9	27	P=0.01 High. sign. (P>0.05)
	%	36.0%	36.0%	36.0%	
Female	No.	64	16	48	
	%	64.0%	64.0%	64.0%	
Total	No.	100	25	75	
	%	100.0%	100.0%	100.0%	

*** Highly-Significant differences using Pearson Chi- square test at P>0.05 level.**

4.2. Detection of Human Herpes Virus 8 (HHV-8) by Conventional Polymerase Chain Reaction Technique (PCR)

4.2.1 Extraction of Nucleic Acid by Specific Viral DNA/RNA extraction kit

Forty- six NHL (46) specimens with a viral genome were detected in this study, accounting for 61.3% of the total number of NHL patients. While, sixteen (16) HL specimens with a viral genome were detected in this study, accounting for 64% of the total number of HL patients as shown in Figure (4-1). In the control group, 5 of the 100 blood specimens has viral nucleic acid.

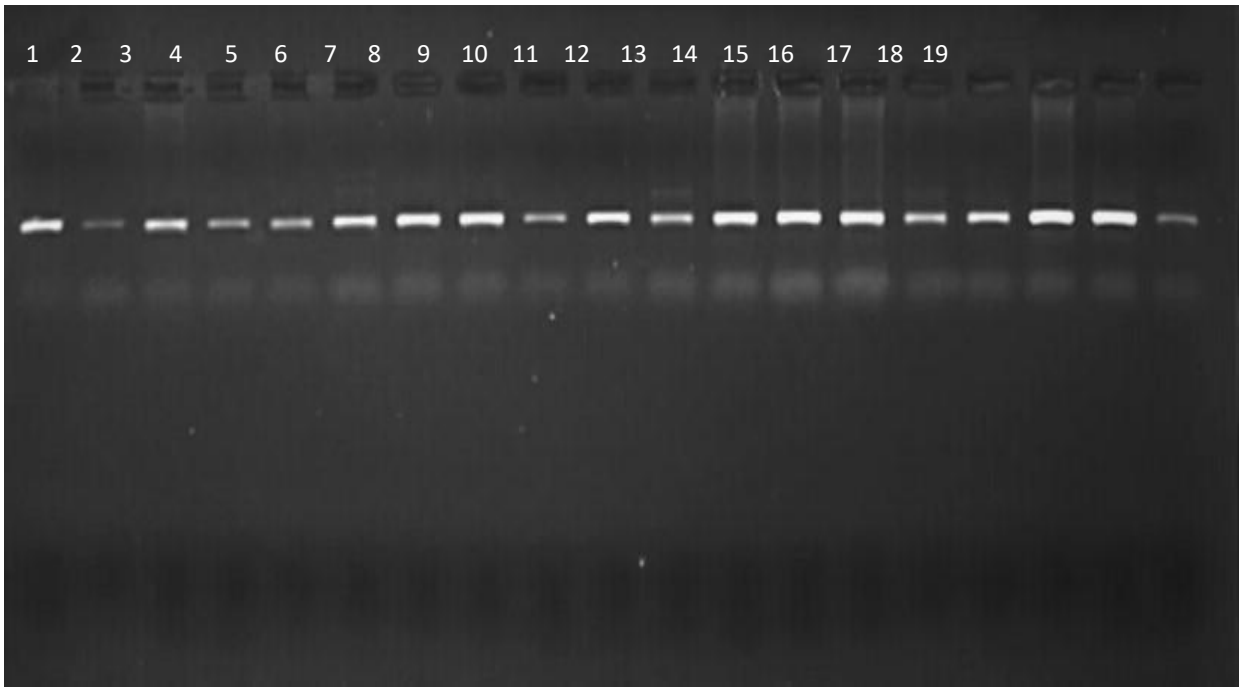

Figure 4.1: Percentage Extraction of viral genome from Lymphoma patients, 1.5% agarose gel electrophoresis, TBE 1X , at voltage 85 volt for 1h, lanes (1-19) were positive.

4.2.2. HHV-8 Associated with apparently healthy control

In this study, all cases were negative; therefore, they are excluded from the statistical analysis.

4.2.3. HHV-8 Genome Detection in Hodgkin lymphoma patients By Using PCR

According to PCR, only 43.7% (7 out of 16) of the HL specimens results are positive for HHV-8 genome detection, less than in patients with NHL was 45.7%

(21 out of 46) of the specimens have HHV-8 genome as indicated in Tables (4-4) and Figures (4-2). The results of these groups of patients showed statistically non-significant differences.

Table 4.4. Statistical analysis for HHV-8 Results in blood specimens from Patients with Hodgkin and Non-Hodgkin lymphoma

HHV-8		Studied groups		Pearson Chi-Square (P-value)
		HL N=25	NHL N=75	
Positive	No.	7	21	P=0.06 Non-Sign. (P<0.05)
	%	28.0%	28.0%	
Negative	No.	18	54	
	%	72.0%	72.0%	
Total	No.	25	75	
	%	100.0%	100.0%	

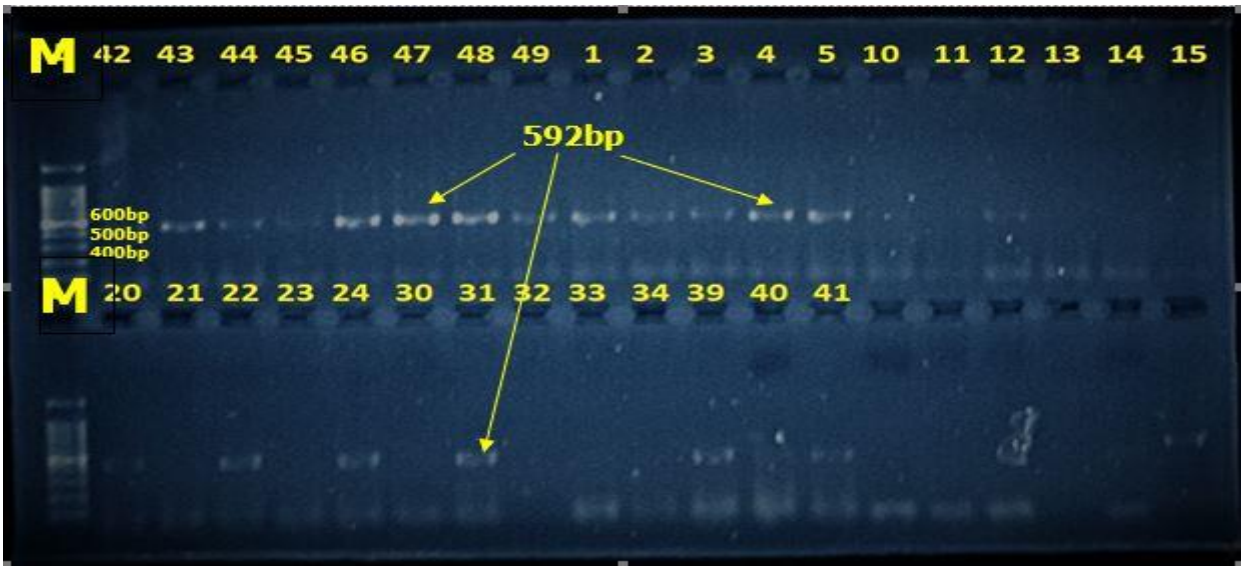

Figure 4.2: The electrophoresis pattern of HHV-8-K1 amplicon DNA (592bp) detection in blood sample patients with HL and NHL . Lanes (47,4,31 and others) refers to HHV-8 DNA specimens; Electrophoresis conditions, 1.5% agarose,85 V, for 1h.

4.3.4. The Results of HHV-8 in the Study Group According to the Age of Patients

Table (4-5) illustrated the positive results of HHV-8-DNA-PCR detection ,where 28% (21 of total 75) from NHL group found that positive signals included 6.7 % (2 out of 75 cases) in age group (≤ 20years), 9.3 % (7 out of 75cases) in age stratum (21 - 40 years); 8% (6 out of 75 cases) in age stratum (41 -60 years and4% (3 out of 75 cases) in age stratum (61 -80) years. The HL group revealed 28% positive signals (which represented 7 out of 25 cases) in this group included 16 % (4 out of 25 cases) in age stratum (7- 30 years), 7.5% (3 out of 40 cases) in age stratum (31-40 years), 16% (4 out of 25 cases) in age stratum (21-40 years), and 12% (3 out of 25) in age stratum (41-60 years) . The statistical analysis found that significant difference among age stratum and positive result of HHV-8-DNA-PCR (p-value > 0.05).

Table 4.5: HHV-8-DNA according to the age of patients with NHL and HL study groups.

Groups / Age		Groups				Total	P value
		NHL		HL			
		POSITIVE	NEGATIVE	POSITIVE	NEGATIVE		
Age group	≤ 20	1	3	0	0	4	0.05
		1.33%	4 %	0.00 %	0.00 %	4 %	
	21 – 40	5	8	5	10	28	
		6.67 %	10.6%	20%	40 %	28 %	
	41 – 60	7	21	2	8	38	
		9.3%	28 %	8 %	32%	38 %	
	61 – 80	8	22	0	0	30	
		10.67 %	29.3 %	0.00 %	0.00 %	30 %	
Total		21	54	7	18	100	
		28%	72%	28 %	72 %	100.0%	

4.4. The Results of *IL-1R1rs1419620062* SNPs.

4.4.1. Extraction Total Genome DNA From Blood Specimens

By using specific Total genome DNA extraction kit (G-Spin total DNA Extraction kit, Intron / Korea) the genomic DNA Figure 4-3 was extracted, purifying and migrated using agarose gel from the blood specimens of patients with (LY) as well as apparently healthy control (AHC) groups.

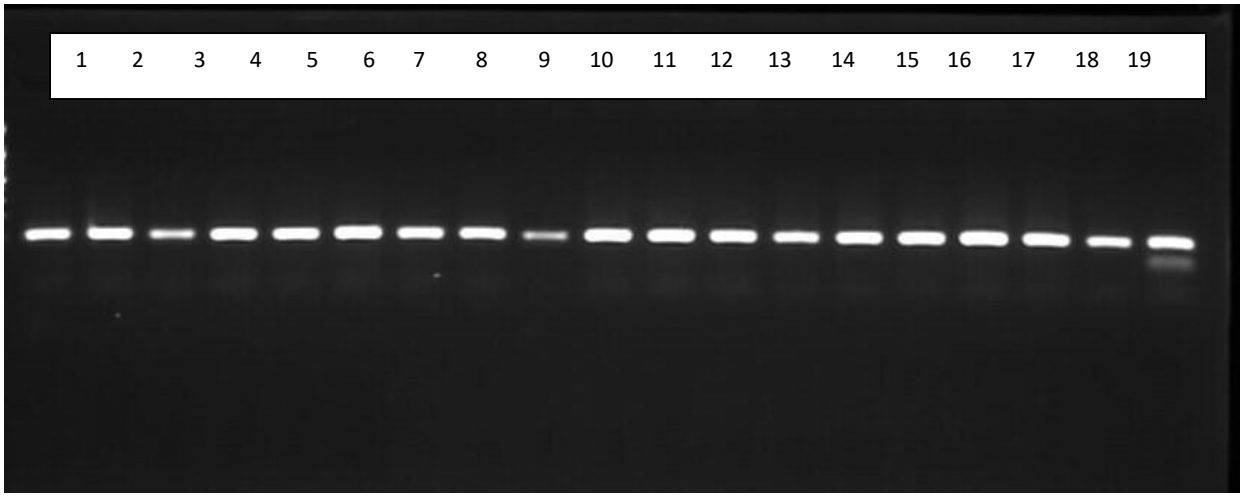

Figure 4.3: The electrophoresis pattern of gnomic DNA extracted from blood samples of lymphoma patients and healthy control groups. Lane 1 lane 19 refers to genomic DNA samples (1-10 patients with lymphoma & 11-19 control) ; Electrophoresis conditions, 1.5% agarose, 85 V, 20 mA for 1h (5 µl in each well), stained with red safe solution.

4.4.2 Genotyping of *IL-1R1419620062* Gene in LY and AHC

For *IL-1R1rs1419620062* genotyping, the genomic DNA was amplified using specific primers the results revealed that the presence a single band (480 bp) of the target sequence of IL-1R1rs1419620062 gene in agarose gel Figure (4-4).

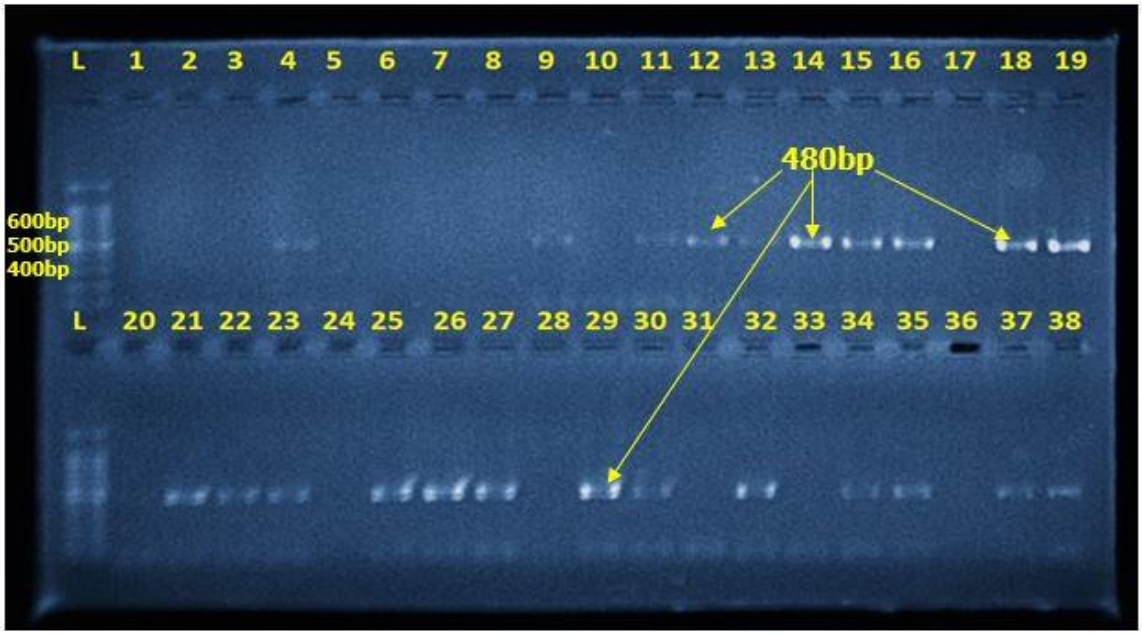

Figure 4.4: Agarose gel electrophoresis of an amplified product patterns of *IL-1R1rs1419620062 located on Chromosome 2 in* exon4 region. Electrophoresis conditions, 1.5% agarose, 85 V, 20 mA for 1h (5 µl in each well), stained with red safe solution.

4.4.2.1. Genotyping of *IL-1R1rs1419620062* Among Study Groups

To summarize the results obtained from the sequenced 480 bp fragments, the detailed positions of the observed variations are described in the NCBI reference sequences as shown in Table (4-6).

The results of *IL-1R1rs1419620062* gene polymorphism showed that DNA polymorphism distribution were DNA polymorphism distributions according to A\A; A\C; and C\C were (15 out of 25); (1 out of 25) and (9 out of 25), respectively in patients with LY and (8 out of 15); (0 out of 15) and (7 out of 15); respectively in AHC group. The difference in frequency of genotype distribution of the polymorphism between patients and controls groups was statistically significant Table (4-6).

Table 4.6: Comparison between patient with and without LY based on percentages of *IL-1R1rs1419620062* expressed gene polymorphism.

IL1 Rs1419620062	Patients	A.H.Control	Chi-square	p- value	Odd ratio (CI95)
AA	**15**	**8**	**Reference**		
AC	**1**	**0**	**0.52**	**0.47**	**0.65(0.48-0.87)**
CC	**9**	**7**	**0.32**	**0.57**	**1.45(0.39-5.39)**
Allele					
A	**31**	**16**			
C	**19**	**14**	**0.58**	**0.44**	**1.42(0.57-3.53)**

4.4.2.2. Sequences alignment fragment results of *IL-1R1rs1419620062* gene, exon-4 located on chromosome 2q12 region by bio edit program version 7.2.5

The sequencing results observed that many SNPs between the one resolved haplotypes and between the *IL-1R1rs1419620062*, exon4 for Primer3 plus reference sequence. The results appeared in the presence of fourteen SNPs Figure (4-5). Which revealed that which located at position 59 a substitution mutation (A→G) , position 99 substitution (T→ A) , position 336 substitution (T→ A) , position 387 substitution (A→ G) , position 421 substitution (A→ T) , position 423 substitution (G→A) , position 436 substitution (T→ G), and last one at position 456 substitution (A→ G) Figure (4-5) according to the reference sequence alignment of the human *IL-1R1rs1419620062* geneID:NG-047209.1(https://www.ncbi.nlm.nih.gov/Genbank /update .html).

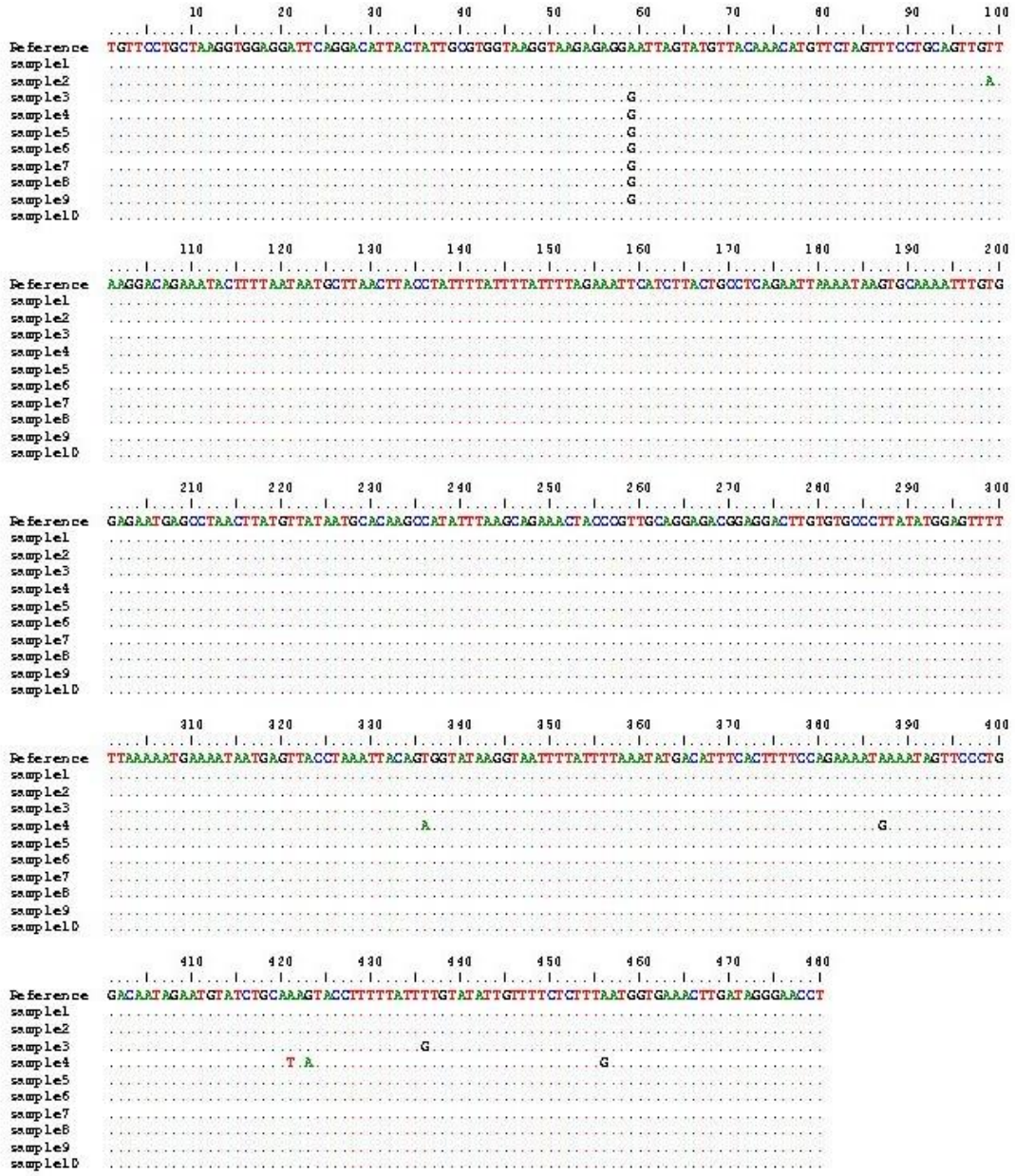

Figure 4.5: Sequences alignment fragment results of *IL-1R1rs1419620062* gene, are closely associated in the region of 2q12-q21 of human chromosome 2 by bio edit program version 7.2.5.

We have recording two new recording in gene bank NCBI & American bank Under ACCESSION NUMBERS: LC770967; LC770968

4.5. Evaluation of Serum IL-1R concentration By ELISA Among Study Population

Table (4-7) shows the mean of serum IL-1R concentration for AHC and patients with LY groups were 8.00 ±0.31pg./ml and 14.00 ±0.59 pg./ml, respectively.

Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum IL-1R concentration among these study groups.

Table 4.7: Results of serum IL-1R concentration by ELISA for AHC and patients with LY

IL-1R	AHC (pg/ml)	Patients with LY (pg/ml)
Mean± SE	**8.00 ±0.31**	**14.00 ±0.59**
LSD	**5.65**	
P value	***P*<0.05 (0.001) ***	

4.6. Typical data

This standard curve is only for demonstration purposes Figure (4- 6).

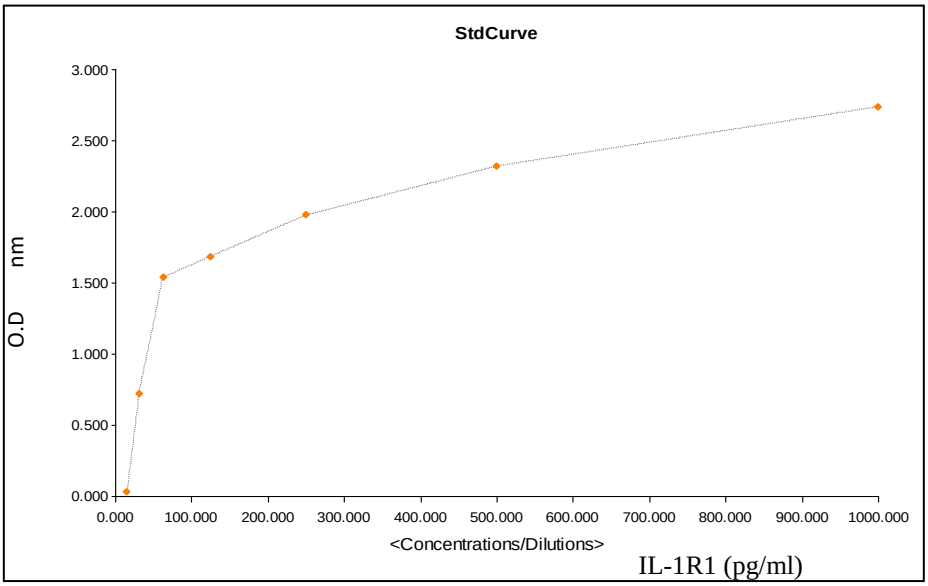

Figure 4.6: A standard curve for IL-1R1 assay.

CHAPTER FIVE

DISCUSSION

5. Discussion

5.1 Risk factors of Lymphoma

Lymphoma is a type of cancer that affects the lymphatic system, which is a part of the body's immune system. It occurs when abnormal lymphocytes, a type of white blood cell, grow out of control. Understanding the risk factors associated with lymphoma is crucial for early detection, prevention, and effective management of the disease. These possible causes and associations with at least some forms of NHL and HL include (Chiu *and* Hou,2015; Sud *et al*.,2020)

5.2. Evaluation of Some Clinico-Pathologic Criteria of Patients with Hodgkin and Non-Hodgkin lymphoma

The current study updates the recent insight of lymphoma with the advanced available techniques. The lymphoma is the second most aggressive form of tumors, and is characterized by lymphadenopathy with atypical lymphocytes involving the superficial and deep lymph node chains.

In the first part of the study, some clinical and pathological information of Hodgkin and Non-Hodgkin lymphoma were investigated to explore this challenging group of malignancies in relation to their existing information.

5.2.1. Distribution of patients with Hodgkin and Non-Hodgkin lymphoma to their age

On reviewing the 100 blood specimens which were included in this study, the age of the patients with Hodgkin and Non-Hodgkin lymphoma were ranging between 15-80 years (Table 4.1). In Hodgkin lymphoma, the mean age of patients was (**45.12**± **14.49** years) and the most affected age stratum is 21 - 40 years followed by the age stratum of 41 – 60 years (Table 4.2). From the present results, it was noticed that malignant tumors have also increased with the proceeding of age of patients and

our results are broadly agreed with the results obtained by SEER, (2016), they revealed that the most common age of diagnosis in HL is between 20 and 40 years old.

The present study agreed with study conducted by Adedayo *et al*., 2003, who found that the age range was 9–89 years. The present results are consistent with those reported world-wide where Hodgkin lymphoma was usually affecting the adults in the third decade and a second peak after the age of 50 (Hialgrim and Jarrett., 2020).

However, in Non-Hodgkin lymphoma, the mean age of patients was (46.42±12.06 years) and the most commonly affected age stratum in 61 – 80 years were constituted (40%:30 cases) for each group, followed by the age stratum of 41 - 60years (37.34%:28 cases) (Table 4.1 and 4.2).

Moreover, this study agreed with Jordanian study done by Al-masri *et al.,* (2004) who found that the median age of their NHL patients was 43.5 years.

This study also is consistent with Al-Alwany *et al*.,(2018) and AL-Lebawy et al., (2017) who found the median age of their NHL patients was (48.0±13.3) years and (42.5) years, respectively. These differences in age groups could be contributed to the differences in environmental and geographical risk factors affecting each study groups making HL and NHL affecting relatively middle age group in Iraq and neighboring countries. The reasons for these age-specific patterns are not entirely understood, but they may be related to variations in the immune system's function at different life stages or to other factors like exposure to certain infections during early childhood and the reasons for these age correlations are multifaceted and may involve both biological and environmental factors. Additionally, exposure to certain infections, environmental toxins, and genetic predispositions might play a role.

5.2.2. Distribution of the patients with Hodgkin and Non-Hodgkin lymphoma according to their sex

In this study, it was found that (64%:16) of Hodgkin lymphoma were females, while the rest 9 cases (36%) were males. Regarding the patients whom suffering from Non-Hodgkin lymphoma, the percentage of females was also higher (64%:48) than the percentage of males (36%: 27). The female/male ratios of the patients with Hodgkin lymphoma and Non- Hodgkin lymphoma were, 1.78:1 Table (4.3).

The results of the current study are in concurrence with the results of most other studies. Sup *et al*., (2005) and Jakovic *et al*., (2012) revealed that the number of male HL patients slightly more common than female HL patients. The percentage of male and female HL patients was 50.9% and 54%, respectively. Whereas the percentage of female HL patients was 49.1% and 46%, respectively.

In addition, Hussein *et al.*, (2005) was found that HL is more common in male (70%) than female (30%). Horesh and Horowitz, (2014) and Smith *et al*., (2015) who found in HL and NHL that the affected men were more than their counter part women. Also, Boffetta, (2011), was found that the NHL is the 8th most commonly diagnosed cancer in men and the 11th in women.

The present result are incompatible with these results reported by Hashemi and Parwaresh (2001) in Iran (M:F ratio was 1.5:1).Also, the present M:F predominance result is consistent with two other studies done in Pakistan (Lal *et al.*,2008 and Naz *et al.*,2011) who found M:F ratio of 2:1 and 2.6:1, respectively and a study done in India by Arora *et al.*,(2011) who found M:F ratio of 3.9:1 .

Furthermore, this study is similar to that study done by Groves *et al.* (2000) in USA who found the ratio of males to females in their studied cancer surveillance series of NHL in the period from 1978 through 1995 was 1.5:1 for total NHL group where 1.4:1 was reported in their white population Versus 1.7:1 for black citizens.

Moreover, our results agreed with the results of several studies. Møller *et al*., (2000); Mounier *et al*., (2003); Küçükzeybek *et al*., (2013) and Albejow *et al*., (2015) who found that the percentage of male slightly higher than female. The male rates were 50%, 60%, 53%, 68%, respectively. Whereas the female rates were 50%, 40%, 47% and 32%, respectively.

On the other hand, the majority of male sex as documented in this study and many other series of studies is contrasted by the majority of females in relation to males in NHL was reported by another researchers in Turkey, (Ali et al., 1999) and Germany (Pulte et al., 2008). Dorak and Karpuzoglu in (2012) found in Hodgkin lymphoma, the sex ratio reverses toward adolescence.

The pattern that autoimmune disorders are more common in females, whereas the little differences among these studies with current study regarding the ratio between two sexes may be related to the sample size of patients enrolled in these studies. Lymphoma and infections in males higher than females suggest that the well-known differences in immunity may be responsible for this dichotomy. Besides immune surveillance, genome surveillance mechanisms also differ in efficiency between males and females. Other obvious differences include hormonal ones such as estrogen **because** estrogen receptors are presented on lymphocyte which are the cell involved in lymphoma development and the number of X chromosomes.

Also, the difference might be due to the peculiar characteristics of the referral centers, smaller case numbers, or geographic distribution.

However, still there is need to generate more data regarding variation in sex predominance in our population for better studies.

5.3. Human Herpes Virus -8(HHV-8)-Infected Lymphoma

Primary effusion lymphoma (PEL) is a rare and distinct type of non-Hodgkin's lymphoma typically characterized by serous effusions without any detectable tumor

masses. It accounts for ~4% of all human immunodeficiency virus (HIV)-related non-Hodgkin lymphoma (NHL) cases and <1% of all non-HIV-related NHL cases in the United States (between 2001 and 2012) (El-Fattah,2017). According to the 2017 World Health Organization (WHO) Classification of Tumors of Hematopoietic and Lymphoid Tissues (Swerdlow *et al*.,2017), human herpesvirus 8 (HHV8) infection is commonly detected in neoplastic lymphoid cells.

However, up to our knowledge this study of HHV-8 in lymphoma is the first research work in Mid-Euphrates Governorates of Iraq that was designed to analyze the association of HHV-8 with HL and NHL by using the PCR technique.

5.3.1. Results of HHV-8 in patients with Hodgkin lymphoma

HHV8 is associated with three main categories of disease: Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease and the plasmablastic proliferations that arise from it. The presence of fibrotic bands and Hodgkin and Reed–Sternberg-like cells in a reactive background initially raised the differential diagnosis of classical Hodgkin's lymphoma, but the negativity for B-cell markers, CD30, and CD15 ruled out this possibility. Hodgkin's lymphoma is also consistently negative for HHV8 (Ferry *et al*.,2009).

In the current study, only 43.7% (7 out of 16) of the HL specimens results are positive for HHV-8 genome detection, while 9 out of 16 (55.3%) are negative for HHV-8 genome detection, as indicated in Tables (4-4)

The presence of lymphadenopathy in an apparently immunocompetent patient with EBV and HHV8-positive B-cells would suggest GLPD as first differential diagnosis. These cases usually present with a localized nodal enlargement and EBV and HHV8-positive plasmablasts involve preferentially the germinal centers, lack B-cell specific markers and generally have a good prognosis (Said *et al*.,2017). In contrast, our case presented with multiple lymphadenopathies and no

germinotropism could be demonstrated. Moreover, IGH molecular analysis in GLPD usually renders a polyclonal or oligoclonal pattern (Gonzalez-Farre *et al*.,2017; Said *et al*.,2017), whereas in the current case an IGH clonal peak was detected and the patient presented a fatal clinical course.

Sanchez *et al*., (2020) who explained the HHV8-positive, EBV-positive Hodgkin lymphoma-like large B-cell lymphoma may represent an unrecognized pattern of HHV8 and EBV-associated LPDs. Alternatively, both cases may represent an early lymph nodal involvement by an extracavitary variant of PEL, even though the clinicopathological context does not support this scenario. The presence of recurrent pulmonary infections with a fatal outcome in our case, might suggest the possibility of an under diagnosed immune deficiency, favoring the development of HHV8 and EBV LPDs.

HHV-8 infection can lead to the transformation of B lymphocytes, contributing to the development of lymphoproliferative disorders. The exact mechanisms by which HHV-8 causes these lymphomas are not fully understood, but it is believed that viral proteins and interactions with host immune responses play a role in the oncogenic process.

Kankaya *et al*., (2009) who found KS is an indolent endothelial neoplasm for immunocompromised patients developed by the oncogenic effect of HHV-8 which is not restricted to endothelial cells. It also infects B lymphocytes and is associated with two B-cell lymphoproliferative diseases, primary effusion lymphoma and multicentric Castleman’s disease.Individuals with immunodeficiency are not at increased risk for intravascular large B-cell lymphoma, and HHV8-positive intravascular large B-cell lymphoma.

5.3.2. Results of HHV-8 in Patients with Non- Hodgkin lymphoma

Typical PEL occurs in immune-compromised patients, particularly in those with HIV infection. HHV-8 universally infects malignant lymphoid cells and encodes proteins that are essential for the proliferation and survival of tumor cells. Although EBV co-infection is found in most cases of HHV8 infection, it is not considered to play an important role in the pathogenesis. The etiology of HHV8-negative effusion-based lymphoma is still unclear (Kim *et al*.,2020).

According to PCR detection results, 45.7% (21 out of 46) of the specimens have HHV-8 genome, while 54.3 %(25 out of 46) specimens showed negative results for HHV-8 genome detection, and as indicated in table (4-4). The negative results are probably related to the absence of HHV-8-DNA in these samples or could be related its presence in the cells at different regions of that specimens. The presence of different types other than of HHV-8 which used in this study is another possibility.

HHV8-positive plasmablastic lymphoma, however, is a rare neoplastic lymphoprolipherative entity. The HHV8-infected cells have a pre-plasma cell phenotype and a plasmacytic/ plasmablastic morphology, characterized by a proliferation of larger plasmablastic cells with dense amphophilic cytoplasm and vesicular, often eccentrically placed nuclei containing one or two prominent nucleoli (Pádua *et al*.,2012).

Paner *et al*., (2003) suggested a possible association between HHV8-negative effusion-based lymphoma and HCV; however, a subsequent study demonstrated HCV infection in only 10% of patients with HHV8-negative effusion-based lymphoma (Rezk *et al*., 2013).The difference might be due to the peculiar characteristics of the referral centers, smaller case numbers, or geographic distribution.

Ohshima *et al* (2002) proposed that the genetic alteration of MYC may be involved in the pathogenesis of HHV8-negative effusion-based lymphoma.

Effusion or chronic serositis itself could create ideal body conditions for lymphomagenesis, similar to fibrin-associated diffuse large B-cell lymphoma and diffuse large B-cell lymphoma associated with chronic inflammation. A large proportion of HHV8-negative effusion-based lymphoma is associated with fluid overload caused by different oedematous disorders, such as liver cirrhosis or heart failure (Kim *et al*.,2020).

HHV-8 is therefore thought to play a major role in the pathogenesis of PEL and multicentric Castleman disease (Stingaciu *et al*.,2010).

However, the discovery of HHV-8 did make it possible to differentiate PEL from all other known types of lymphoma because of its association with infection by this novel herpes virus. Although most studies on PEL pathogenesis have focused on HHV-8 and HIV, other unidentified viruses might be involved in PEL. The diagnosis of PEL rests upon the pathologic analysis of involved tissue using morphologic, immunophenotypic, molecular, and virologic criteria. The diagnosis is usually made from a cytologic preparation of the involved effusion fluid, as this fluid usually lacks a solid component (Chen *et al*.,2007).

Viruses are seldom complete carcinogenesis and are essential but not sufficient factors even in those with viral carcinogenesis such as HPV, EBV, HTLV-1and HHV-8 related carcinogenesis (Brooks *et al.*,2010).

The most important of which are the great diversity of diseases included within the entity of NHL, each exhibiting a different rate of association with EBV, HHV-8 and the prevalence of the various diseases differs in different geographical regions and this may be described to genetic and environmental etiologic factors (Zhang *et al.*, 2010). In addition, the extent to which different types of NHL impair the immune

response, in particular those lead to the defective T-cell regulation was another effective factor. Moreover, some of these studies have investigated a restricted number of diseases and the number of cases in the other cohorts studies certainly influences the significance of these results and post transplantation lymphoproliferative diseases (Zhang *et al.*, 2010).

In this respect, the detected HHV-8 in this study is thought to have a synergistic effect in the pathogenesis of HL as well as NHL together along with many other etiological factors such as chemical, radiations, and genetic factors.

5.4. The Results of *IL-1R1rs14119620062* SNP .

Interleukin (IL)-1α and IL-1β are major proinflammatory cytokines that are synthesized during infection and modulating the potentially injurious effects of IL-1. The gene for IL-1ra is located on the long arm of the chromosome 2 on a 430 kb stretch of DNA. Intron 2 of the IL-1ra gene contains a variable number of identical tandem repeats (VNTR) of an 86 base pair length of DNA (Alrayes,2003).

The results of current results showed that DNA polymorphism distribution were DNA polymorphism distributions according to C\C; C\G; G\G C\T; T\T and G\T were 30%; 28% ;16%; 8%; 6% and 12%, respectively in patients with LY and 33.33%; 26.67%; 16.67%; 3.33%; 13.33% and 6.67% , respectively in AHC group. The difference in frequency of genotype distribution of the polymorphism between patients and controls groups was statistically significant Table (4-6). These results consistent with Sarani *et al*., (2021) who found the genotyping of IL-1A rs3783553, IL-1B rs3917356, rs16944, IL-1R1 rs10490571 and IL-1A rs3783550 polymorphism. The results showed that the CC genotype of rs3783550 as well Ins/del of rs3783553 increased the risk of NHL. In contrast the AG genotype of rs3917356 and AG also AG + AA genotype of rs10490571 decreased the risk of NHL. The result revealed that the CC genotype of rs3783550 and AG genotype of rs3917356 increased risk of HL.

While, these results disagreement with Alrayes, (2003) who found did not find any difference in genotype frequencies of IL-1RN between malignant lymphoma patients as a whole and control subjects**.**

In contrast, the data of Alrayes, (2003) study revealed that the IL-1RN2*/2* genotype was in 36.7% of HD cases compared to 17.46% in the control reference population, showing an association with increased risk of HD (OR=2.27 & 95% CI= 1.22-4.24).

On the other hand, Rollinson *et al*., (2003) reported a significant assoc - iation between gastric marginal zone lymphoma and inheritance of IL-1RN 2*/2* genotype.

Excess in risk of the malignant lymphoma have been described for patients with various conditions involving substantial immune dysfunction, particularly conditions where chronic antigenic stimulation is present (Machado *et al*., 2001). The consideration of polymorphisms involved in the immune response, in combination with those involved with the prevention of DNA damage, and further those involved in DNA repair, could allow the mechanisms underlying these associations to be explored further.

Effects of SNPs in IL-1R was identified as statistically significant by multiple logistic regression. IL-1R SNP is associated with imbalance in the system of innate immunity and, as a result, an increase in person organism sensitivity to the infections and malignancy risk.

5.5. Evaluation of Serum IL-1R concentration By ELISA Among Study Population

The mean of serum IL-1R concentration for AHC and patients with LY groups were 8.00 ±0.31pg./ml and 14.00 ±0.59 pg./ml, respectively. Statistically, significant difference ($p<0.05$) was found on comparing the mean of serum IL-1R concentration

among these study groups. It is postulated that the association between IL-1RN*2 allele with HD was due to impaired production of IL-1ra and the high IL-1β levels associated with Il-1RN*2 allele (Tountas *et al*., 1999).

Witkin *et al*., (2002) reported that it might be expected that people who were IL-1RN*2 homozygous (IL-1RN *2/*2) might have a genetic advantage in cancer prevention because this genotype is associated with low level of IL-1ra and elevated level of IL-1β (which has anti-neoplastic activity) with prolonged and more severe inflammatory reactions. These results with malignant lymphoma was consistent with the study done by Matsuo *et al*., (2001), who suggested that, the effects of IL-1 family gene polymorphisms in lymphoid malignancies seem to be limited.

5.6. Spearman's rho statistical testing to evaluate studied molecular markers in relation with HHV-8 infections in patient with Lymphoma

A strong positive relationship (with highly significant correlation) was found between HHV-8 and SNP *IL-1R1 rs1419620062* in HL and NHL (r = 0.968, P = 0.007). But, there is a non-relationship (non- significant correlation) between HHV-8 and *IL-1R1* concentration in Lymphoma (r = 0.175, P = 0. 8).

In addition, A strong positive relationship (with highly significant correlation) was found between HHV8 and SNPs of *IL-1R1 rs1419620062*according to ages patients who have HL & NHL (r=0.855, P= 0.001); (r=0.788, P= 0.009), respectively.

However, there are no significant correlations among HHV-8 and SNPs of *IL-1R1* concentration according to the HL & NHL of study population (and as illustrated in Table 4-7).

Understanding the risk factors associated with lymphoma is essential for identifying individuals who may be at higher risk and implementing appropriate

preventive measures and screening strategies. Age, sex, immunodeficiency, infections, autoimmune diseases, family history, genetic predisposition, and environmental factors all contribute to the development of lymphoma. By identifying and addressing these risk factors, healthcare professionals can work towards reducing the incidence and improving the outcomes of lymphoma patients. (Chiu& Hou.,2015).

Hodgkin lymphoma (HL), also known as Hodgkin's disease, is a type of lymphoma characterized by the presence of Reed-Sternberg cells. While the exact cause of Hodgkin lymphoma is unknown, there are several established risk factors that have been associated with its development. Understanding these risk factors is important for identifying individuals who may be at higher risk and for developing targeted prevention strategies. This discussion will highlight some of the key risk factors for Hodgkin lymphoma age; sex.

1. Age: Hodgkin lymphoma is most commonly diagnosed in two age groups: early adulthood (15-40 years) and late adulthood (over 55 years). The incidence of HL is relatively low in children and older adults.
2. Sex: Men are slightly more prone to developing Hodgkin lymphoma compared to women. The reasons behind this sex disparity are not fully understood.
3. Family History: Individuals with a family history of Hodgkin lymphoma have an increased risk of developing the disease. Several studies have reported a two to threefold higher risk in individuals with affected first-degree relatives.
4. Infectious Agents: Epstein-Barr virus (EBV), a common virus of the herpes family, has been strongly linked to the development of Hodgkin lymphoma. It is estimated that around 40% of Hodgkin lymphoma cases are associated with EBV infection. Other viral infections, such as HIV, human herpesvirus-8 (HHV-8), and human T-cell leukemia virus (HTLV-1), have also been implicated in HL development.

5. Immune System Disorders: Certain immune system disorders, such as autoimmune diseases and immunodeficiency disorders, increase the risk of developing Hodgkin lymphoma. Individuals with conditions like rheumatoid arthritis, systemic lupus erythematosus (SLE), and human immunodeficiency virus (HIV) infection have a higher incidence of HL.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The Following Conclusions are obtained from the Present Study:

1- The ratio of Female patients whom suffering from Hodgkin and Non-Hodgkin lymphoma was higher than their Male counterparts.

2- HHV-8 might be one of the most recently identified oncogenic viruses in Iraqi patients suffering from Lymphoma in the Iraqi population.

3- Our study indicated that *IL-1R rs1419620062* polymorphism may be associated with risky factor in the Iraqi patients with lymphoma. But, the exact role and effects of *rs1419620062* polymorphism in lymphoma is not fully identified. Interleukin-1R gene polymorphisms did consider a risk for HL and NHL Patients.

4- The significant correlation between the gene polymorphism of *IL-1R* with HHV-8 infection could indicate highly important role of these molecular factors in patients suffering from Lymphoma.

RECOMMENDATIONS

The recommendations of these study dependent on current results are:

1. HHV-8 could be added as another potential cause of Lymphoma.
2. Routine work uses of viral detection such as (HHV-8, Rubella, HCMV, HSV1&2 …etc) from blood for in centrals health especially in young age to prevent the pathogenesis; inflammation or carcinogenesis.
3. Further prospective studies are required with a large number of cases are needed to validate the results of the current study which may lead to a better understanding of the role of HHV-8 in patients with HL and NHL.
4. Studying the role of other Genetic biomarkers in deregulation of cell cycle pathway that play essential role in HL and NHL.
5. To study the sequence and the strains prevalent in Iraq and their differences from the global prevalence and importance, preparing traits against prevalence of HHV-8 in our country via proposing a vaccine which fits the Iraqi isolates is important.
6. To study the complete hole genome sequence and the detection the new genotypes and strains prevalent in Iraq and their differences from the global prevalence and importance in patients with HL and NHL.

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APPENDEIX

```
LOCUS       LC770967                480 bp    DNA     linear   HUM 17-JUN-2023
DEFINITION  Homo sapiens AJA2 IL1R1 gene for interleukin 1 receptor, type I,
            partial cds.
ACCESSION   LC770967
VERSION     LC770967.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
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            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 480)
  AUTHORS   Isam,Z.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-JUN-2023) to the DDBJ/EMBL/GenBank databases.
            Contact:Zahraa Isam
            Babylon University, Biotechnology; 40 Street, Babel, Hilla 51001,
            Iraq
REFERENCE   2
  AUTHORS   Al-Jawdhari,A.J. and Al-Alwany,S.H.
  TITLE     Immunogenetic of IL1R gene polymorphism and Huma Herpesvirus 8 in
            Patients with Lymphoma
  JOURNAL   Unpublished (2023)
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      121 taatgcttaa cttacctatt ttatttttatt ttagaaattc atcttactgc ctcagaatta
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      361 tatgacattt cacttttcca gaaaataaaa tagttccctg gacaatagaa tgtatctgca
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```

```
LOCUS       LC770968                480 bp    DNA     linear   HUM 17-JUN-2023
DEFINITION  Homo sapiens AJA4 IL1R1 gene for interleukin 1 receptor, type I,
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ACCESSION   LC770968
VERSION     LC770968.1
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  ORGANISM  Homo sapiens
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            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 480)
  AUTHORS   Isam,Z.
  TITLE     Direct Submission
  JOURNAL   Submitted (12-JUN-2023) to the DDBJ/EMBL/GenBank databases.
            Contact:Zahraa Isam
            Babylon University, Biotechnology; 40 Street, Babel, Hilla 51001,
            Iraq
REFERENCE   2
  AUTHORS   Al-Jawdhari,A.J. and Al-Alwany,S.H.
  TITLE     Immunogenetic of IL1R gene polymorphism and Huma Herpesvirus 8 in
            Patients with Lymphoma
  JOURNAL   Unpublished (2023)
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      121 taatgcttaa cttacctatt ttatttttatt ttagaaattc atcttactgc ctcagaatta
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```

الخلاصة

سرطان الغدد اللمفاوية هو سرطان شائع في الدم. ويعد ضعف الجهاز المناعي أحد اهم العوامل الرئيسية لمخاطر الاصابة باللمفوما. فيروس الحلأ البشري ٨ (HHV-8) يحفز تكاثر الخلايا اللمفاوية عن طريق تنشيط اشارات مسار مستقبل الحركي الخلوي-٦، جنبًا إلى جنب مـــع عدة آليات تنظيمية أخرى. يرتبط مضـــاد مستقبل الحركي الخلوي -١ (IL١RN) بمستقبل الحركي الخلوي IL-1 ويقوم بتعديل مجموعة من الاستجابات المناعية المرتبطة ب IL-1. تم تحديد تغاير الاشكال الوراثية في مجموعة الجينات الخاصة بالحركي الخلوي -١ (IL-1) كمساهمين مهمين في تنظيم الالتهاب. تهدف هذه الدراسة إلى تحديد النسبة المئوية لفيروس الحلأ البشري ٨ (HHV-8) وما إذا كانت تعدد الاشكال في موضع مستقبل الحركي الخلوي -١ (IL-1ra) تعدل خطر لحدوث سرطان اللمفوما الخبيثة في مجموعة من عينات المجتمع العراقي.

تضمنت الدراسة الحالات المختاره بصوره عشوائية **٢٠٠** عينة من الدم (**٢٥** حالة لمفوما هودجكين و**٧٥** حالة لمفوما اللاهودجكين و **١٠٠** حالة كمجموعة سيطرة ظاهريًا اصحاء) (٧٢ ذكرًا و **١٢٨** أنثى) تم جمعها من مستشفيات تعليمية مختلفة في مناطق الفرات الأوسط ومحافظة بغداد في العراق. وتتفاوت أعمار المجموعات المدروسة من **١٥** إلى **٨٠** سنة. وقد أجريت الدراسة في قسم علم الأحياء المجهرية، كلية العلوم، جامعة بابل خلال الفترة بين تشرين الأول/أكتوبر ٢٠٢٢ وحزيران/يونيو ٢٠٢٣. تم جمع خمسة ميليلترات من عينة الدم (٢ ميليلتر في انبوبة تحتوي على مانع تخثر الدم EDTA و٣ ميليلترات في انبوبة تحتوي على مادة الهلام Gel tube لعزل المصل)، ثم تم الحصول على الجينوم الفيروسي والحمض النووي الإجمالي وتخزينها في درجة حرارة -٢٠س°/ -٨٠ س° حتى الاستخدام.

كان متوسط عمر المرضى الذين يعانون من لمفوما اللاهودجكين (٤٦,٤٢±١٢,٠٦ سنة) أعلى من متوسـط عمر المصـابين بلمفوما الهودجكين (٤٥,١٢±١٤,٤٩ سـنة). بينما كان متوسـط عمر محموعة السيطرة)AHC((٤٤,٩±١٢,٥١ سنة).

كانت (٦٤٪: ١٦) من حالات اللمفوما الهودجكين إناثًا، بينما كانت النسبة المتبقية (٣٦٪: ٩)ذكورًا. فيما يتعلق بالمرضى الذين يعانون من لمفوما اللاهودجكين، فقد كانت نسبة الإناث أيضًا أعلى (٦٤٪: ٤٨) من نسبة الذكور (٣٦٪: ٢٧) وكانت نسبة الاناث الى الذكور (١:١,٧٨).

في مجموعة لمفوما الهودجكين، كانت الفئة العمرية **الأكثر تأثرًا** هي من ٢١-٤٠ سنة وتمثل (٦٠%: ١٥) حالة .أما في اللمفوما اللاهودجكين، فقد كانت الفئة العمرية **الأكثر تأثرًا** في الفئة من ٦١-٨٠ سنة وشكلت (٤٠%: ٣٠).

تم اكتشاف ٤٦ عينة من لمفوما اللاهودجكين ايجابيه للجينوم الفيروسي في هذه الدراسة، مما يشكل ٦١,٣٪ من العدد الإجمالي للمرضى المصابين بالمفوما اللاهودجكين، في حين تم اكتشاف ١٦ عينة من لمفوما هودجكين ايجابيه للجينوم الفيروسي في هذه الدراسة، مما يشكل ٦٤٪ من العدد الإجمالي للمرضى المصابين بالمفوما الهودجكين.

وفقًا لتقنية تفاعل البلمرة المتسلسل (PCR)، كانت نتائج ٤٣,٧٪ (٧ من أصل ١٦) من عينات لمفوما الهودجكين إيجابية لكشف جين K1-HHV-8. بينما بالنسبة للمجموعة اللاهودجكين فقد كانت ٤٥,٧٪ (٢١ من أصل ٤٦) من العينات تحمل جين K1-HHV-8.

أظهرت نتائج التغاير الوراثي لمستقبل الحركي الخلوي-١ IL-1R1rs 2234650 أن توزيع التعدد الاشكال للحمض النووي وفقاً لـ A\C;A\A وC\C كان ٦٠%; ٤% و ٣٦% على التوالي في المرضى الذين يعانون من اللمفوما و ٥٣,٣٣% ; ٠,٠٠% و٤٦,٦٧% على التوالي في مجموعة الاصحاء. وكان الفرق في تواتر التوزيع الجيني لتعدد الأشكال بين المرضى والاصحاء كبيرا من الناحية الإحصائية.

كان متوسط تركيز المصل للحركي الخلوي -١ IL-1R بالنسبة للمصل في مجموعة الاصحاء والمرضى الذين لديهم مرض اللمفوما هو ٨+٣١,٠ و ١٤ + ٠,٥٩ بيكوغرام /مل على التوالي ،احصائيا وجد فرق معنوي عند مقارنة المصل بين مجاميع الدراسة عند مستوى معنوية ٠,٠٥.

نحن نستنتج من هذه النتائج : بالرغم من الأعداد الصغيرة نسبياً التي تضمنتها دراستنا، تشير النتائج الحالية إلى احتمال أن تؤدي الاصابة بفيروس الحلا البشري نمط-٨ والتغاير الوراثي لمستقبل الحركي الخلوي-١ دوراً في بيولوجيا ورم الخلايا اللمفاوية وقد يسهم في تطورها.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم
قسم علوم الحياة

الترابط ما بين عدوى فيروس الحلا البشري نمط ٨ والتغاير الوراثي لجين مستقبل الحركي الخلوي الانترلوكين - ١ في المرضى المصابين بسرطان الغدد اللمفاوية

رسالة

مقدمة الى مجلس كلية العلوم في جامعة بابل، وهي جزء من متطلبات نيل درجة الماجستير في العلوم / علوم الحياة

من قبل
امير جاسم عباس عبيد
بكالوريوس علوم الحياة (٢٠١٢)

بأشراف
الأستاذ الدكتور
شاكر حماد محمد حسن العلواني
دكتوراه فايروسات طبية وجزيئية
جامعة بابل/كلية العلوم

١٤٤٥ هـ ٢٠٢٣ م