Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbala /College of Medicine

Department of Microbiology

Assessment of Nuclear Factor-kappa B in cardiovascular patients infected with Human Herpes virus 6

A thesis

Submitted to the Council of College of Medicine at University of Kerbala in Partial Fulfillment of the Requirements for the Master Science Degree in Medical Microbiology

By

Hawraa Haitham Mohammad Al-Zubaidy

B.Sc. College of Medical and Health Technology /kufa (2015)

Supervised By

Assist Proff. Dr.

Sawsan M Jabbar AL-Hasnawi

University of Kerbala

Collage of medicine

Prof.Dr.

Saif JabbarYasir Al-mayah

University of Kufa

Collage of medicine

2021 A.D

1443 A.H

بسم الله الرحمن الرحيم

﴿ نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ ۗ وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ ﴾

صدق الله العلي العظيم

سورة يوسف / آية 76

Certification

We certify this thesis was prepared under our supervision at the College of Medicine, University of Karbala, as a partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

Asst.Prof.Dr.

Sawsan M Jabbar AL-Hasnawi

PhD Immunology

Collage of medicine

University of Kerala

Proffessor Dr.

Saif Jabbar Yasir AL-Mayah

PhD Medical Virology

Collage of medicine

University of Kufa

Chair of microbiology department

In view of the available recommendation, I forward this thesis for debate by the examining committee

Head of medical microbiology department

Signature:

Assist. Prof. Sawsan M Jabbar AL-Hasnawi

MBCH.B/MSC &PHD Immunology

College of medicine / university of kerbala

Dedication

Iraq, my country.

To the martyrs' spirits

To my dear father.

To my dear mother

To my dear daughter (Fatima)

To my beloved husband

To my dear grandmother's soul

In addition, my brothers and sister

Martyrs Foundation / Najaf branch

and everyone assisted me and provided me with

Support

Hawraa

2021

Acknowledgements

First of all, I would like to commend and extend my gratitude to my Creator, who gave me the health and strength to complete this work.

I would also like to thank Dr. Saif Jabbar Al-Mayahi and Dr. Sawsan Muhammad Jabbar, the supervisors of this thesis, for their advice and keenness to complete this work in the best possible way and on time.

I would like to thank them once again for their advice and constant encouragement, and thank them for their great efforts to complete this study.

I thank the Department of Microbiology, Faculty of Medicine, University of Kerbala, and in particular the head of the Department of Microbiology, Dr. Sawsan Al-Hasnawi and all my respected professors for their great cooperation.

I extend my sincere thanks to Mr. Mahdi Abdul Ali Al-Faham and Mr. Nassar Kazem Al-Jourani for their great assistance to me in the ELISA technique.

I would also like to thank the staff of the Najaf Center for Cardiac Surgery and Catheter Intervention in Al-Sadr Medical City in Najaf, especially technologist Ismail, MSc microbiologists Falah Hassan, assist lab.Atheer, and all the laboratory workers for assist and guide them to collect samples from patients.My thanks to all the patients who participated in this study.

Also, my thanks to the Department of Microbiology at the Faculty of Medicine, University of Kufa, for their great cooperation.

Finally, all thanks and appreciation to everyone who helped and advised me to complete this thesis .***Hawraa2021***

Summary

The Human herpes virus-6A and Human herpes virus -6B establishes a persistent infection in the host that lasts a lifetime, which is referred to as latency .

The nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) is a protein complex that regulates transcription of DNA, production of cytokines and survival of a cell. The nuclear factor is required for the proper regulation of the immune response to infection. Inappropriate NF-kB regulation has been associated with cancer, inflammatory and autoimmune diseases, septic shock, viral infection and abnormal immune development.

Myocarditis was associated with HHV-6 infection, dilated cardiomyopathy idiopathic "left ventricle dysfunction in both immunocompromised and immunocompetent people.

These study was aimed to reveal the relationship between NF-κB and cardiovascular disease patients who are infected with HHV6 through sandwich Enzyme linked immunosorbent assay(ELISA) through detection of HHV6-Ag, anti-HHV6 IgM & anti-HHV6IgG in the serum of individuals with cardiovascular disorders.

The study is a cross-sectional study done at AL-Sader Teaching Medical City in Al-najaf governorate from the time between November, 2020 and May, 2021. A 200 patients with cardiovascular diseases were involved in study, there were 50 females and 150 males, and the patients' age averaged from 30-104 years. Laboratory tests were done by serological techniques, particularly sandwich ELISA for patient

serum samples.Descriptive analysis and statistics were performed on version 24 spss.

In this study, 60(30%) of the 200 patients with cardiovascular diseases were positive to HH6 Ag. A highly significant result for Anti-HHV6 IgM values were present among patients with positive HHV6-Ags with p-value< 0.00001, with highly significant mean levels of Anti-HHV6 IgM. Anti-HHV6 IgG positive values were present among patients with positive HHV6-Ags with p-value< 0.00001, with highly significant mean levels of Anti-HHV6 IgG. Highly significant results for positive NF-κB levels were found in patients with positive HHV6-Ags compared to those with negative HHV6-Ags with p-value< 0.00001. Highly significant association between positive results for both Anti-HHV6 IgM & Anti-HHV6 IgG with NF-κB. There was no significant association between different types of cardiovascular diseases in the study population and positivity of study markers with p-value > 0.05. For conclusion, a remarkable proportion of patients with cardiovascular diseases have HHV6 virus infection & increment in NF-KB level could be due to HH6 virus infection & inflammation of cardiac tissues.

List of contents

List of Figures

List of Tables

List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
AP-1	Activator protien-1
CAD	Coronary artery disorders
CAR	Coxsackie and adenovirus receptor
CBC	Complete blood count
CCR1	C-C chemokine receptor type 1
CCR2	C-C chemokine receptor type 2
CCR4	C-C chemokine receptor type 4
CCR5	C-C chemokine receptor type5
CCR6	C-C chemokine receptor type 6
CCR8	C-C chemokine receptor type 8
CD4	Cluster of differentiation 4
CD46	Cluster of designation46
CD8	Cluster of differentiation 8
COX-2	Cyclooxygenase-2
CSF	Cerebrospinal fluid
CTLs	Cytotoxic T lymphocyte cells
CVD	Cardiovascular disease
DAF	Decay-accelerating factor
DCM	Dilated cardiomyopathy
DNA	Deoxyribonucleic acid

DRL	Direct repeats Left
DRR	Direct repeats right
ds-DNA	Double stranded- DNA
E	Early
EBV	Epstein-Barr virus
Ecs	Endothelial cells
ELISA	Enzyme-linked immunosorbent assay
GM-CSF	Granulocyte-macrophage colony-stimulating factor
gp B	Glycoprotein B
gp H	Glycoprotein H
HBV	Hepatitis B virus
HCMV	Human Cytomegalovirus
HCV	Hepatitis C virus
HHV-6 Ab	Human herpesvirus 6 Antibody
HHV-6A	Human herpesvirus 6 strain A
HHV6-Ag	Human herpesvirus 6 –antigen
HHV-6B	Human herpesvirus 6 strain B
HHV-7	Human herpes virus-7
HIV	Human immunodeficiency virus
HRP	Horse radish peroxidase
HSCT	Hematopoietic stem cell transplantation

HSV-1	Herpes simplex virus-1
HSV-2	Herpes simplex virus-2
ICAM-1	Intracellular adhesion molecule-1
iciHH-6	Inherited chromosomally integrated HHV-6
ICTV	International Committee on Virus Taxonomy
IE	Immediate-early
IE1	immediate-early 1
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IkB	Inhibitor of NFkB
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-34	Interleukin- 34
IL-6	Interleukin-6
IL-8	Interleukin-8
INOS	Inducible nitric oxide synthase
L	Late
LCL	Lymphoblastoid cell lines
LDL	Low-density lipoprotein
MCP	Myocardiopathy

MI	Myocardial infarction
NEMO	Nuclear factor κB (NF-κB) essential modulator
NF-Kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
NIK	NFkB-inducing kinase
NK	Natural Killer cells
ORFs	Open reading frames
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
RANTES	Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted
SMCs	Smooth muscle cells
SOT	Solid organ transplantation
Th1	T helper 1
Th2	T helper 2
TLR	Toll like receptor
TLRs	Toll-like receptors
TMB	Tetramethylbenzidine
TNF	Tumor necrosis factor
TNFR	TNF-receptor
U- region	Unique region
VCAM-1	Vascular Cellular Adhesion Molecule 1
VZV	Varicella zoster virus

Chapter one

Introduction

and

Literature Review

1.1.Introduction

Both Human herpes virus 6A (HHV6 A) and Human herpes virus 6B are referred to as human herpesvirus 6 (HHV6).). These two strictly linked viruses are the human herpes viruses 6 A and B, which have humans as their major host (Adams *et al*., 2012).

Nearly all human groups appear to be infected with the human herpesviruses (HHV 6A and HHV 6B) (Jaworska *et al*.,2010).

When an extracellular HHV-6 virion comes into contact with human cells, it comes into contact with the human receptor protein cluster of differentiation 46 (CD46), which regulates the complement system. Because of alternative splicing, the CD46 protein has only one variable region. As a result, CD46 has at least fourteen isoforms, all of which bind HHV-6A.(Greenstone *et al. ,* 2002*)*

While the virus can infect of cell kinds, including Natural Killer (NK) cells, endometrial cells, and endothelial cells (ECs), it has a predilection for T-lymphocytes and macrophages, which is known as selective tropism (Caselli *et al.,* 2017; Rizzo *et al*., 2017).

The name "cardiovascular disease" refers to a group of diseases affecting the cardiovascular system and blood vessels. Angina and myocardial infarction (often referred to as a heart attack) are types of coronary artery disorders (CAD) (Mendis *et al*.,2011).

There was a connection between HHV-6 and myocarditis (Ashrafpoor *et al.*, 2013), dilated cardiomyopathy(DCM) (Tátrai *et al*., 2011), sinus tachycardia (Nishimoto *et al*., 2012), and "idiopathic" left ventricle dysfunction in both immunocompromised and immunocompetent people (Kühl *et al*., 2005). Despite an increase in records of HHV-6 myocarditis

in immunocompromised patients, the involvement of the virus in the pathology of acute chronic myocarditis is unknown (Hakacova *et al*.,2013).

The NF- κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is protein complex that regulates DNA transcription, cytokine production, and cell survival. NF- κB, the transcription factor found in all animal cell types, is helpful in the way cells respond to a variety of external influences, such as stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, oxidized LDL, and antigens from bacteria or viruses (Perkins, 2007).

The nuclear factor is necessary for the accurate regulation of the immune response to infection. Inappropriate NF- κB regulation is associated with cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and abnormal immune development. NF-B has also been implicated in synaptic plasticity and memory processes (Park and Youn, 2013). .

NF-κB, a nuclear factor that regulates the expression of proinflammatory cytokines, has been shown to activate genes involved in cardiac remodelling and heart failure pathogenesis in a number of investigations (Sorriento *et al*., 2015).

In the heart, NF κB is triggered in a variety of situations, including: acute ischemia and reperfusion (Li *et al*., 2001), unstable angina (Guro *et al*., 2000), and preconditioning (Valen, 2004). NFκB is activated in both cardiac myocytes (Siednienko *et al*., 2007) and peripheral white blood cells in patients with heart failure (Frantz *et al*., 2004). However, it is still unclear what role it plays in these pathological states. Numerous studies have demonstrated that NFκB protects the heart from acute hypoxia and

reperfusion injury by inhibiting the expression of BNIP-3 (Shaw *et al*., 2006).

On the other hand, activation of NFκB during cardiac remodeling is cytotoxic and promotes atrial fibrillation by inducing a persistent inflammatory response. As evidenced by these signals, NFκB is a highly complex process involving multiple components and a regulation process. In this complexity, timing and cellular context are critical variables affecting NF-κB (Gordon, 2011).

1.2. Aim of study

The main goal is to study the relationship between NF-κB(nuclear factor kappa-light-chain-enhancer of activated B cells) and cardiovascular disease patients that are infected with HHV6) by achieved the following objectives:

1.Detection of HHV6-Ag as well as anti HHV6 IgM and IgG in the serum of patients with cardiovascular diseases.

2.Evaluation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) levels in patients with cardiovascular diseases.

3.Study the relationship between above immunological parameters in cardiovascilar diseases cases.

1.3. Human herpes virus -6:

The Betaherpesvirinae subfamily's Roseovirus genus includes human herpesvirus 6 A and B. While HHV-6A causes roseola Infantum, HHV6B infects humans during their early life and has a prevalence rate of greater than 90%.Herpesviruses' genome, which lies latent in the host cell, is mainly stored in the cell as a circular episome, and it is found that HHV-6A and HHV-6B integrate their genome into telomeres. The virus genome is replicated in every nucleated cell in the body because HHV6A/B is integrated into the chromosomes of germ cells(Aimola *et al.*,2020).

1.3.1. History

The first location found was in the peripheral blood mononuclear cells (PBMCs) of people suffering from lymphoproliferative disorders to search for new lymphotropic human viruses (Salahuddin *et al*., 1986). HHV-6 was isolated in 1986 during attempts to find novel viruses in patients with lymphoproliferative diseases（Agut, 2011）.

Infection is typically self-limited in children, but HHV-6 encephalitis can occur in immunocompromised patients and is the most feared complication of HHV-6 disease. This was first described in 1994(Fida *et al*., 2019).HHV-6 encephalitis has been studied almost exclusively in HSCT(hematopoietic stem cell transplantation) and SOT (solid organ transplantation) recipients, in whom this clinical entity is most likely to occur（Ogata *et al.* 2015).

1.3.2.Classification

Herpesviridae is divided into three subfamilies: Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae. Herpes simplex virus-1(HSV-1), Herpes simplex virus-2(HSV-2), and Varicella zoster virus are all part of the Alphaherpesvirinae subfamily (V. zoster virus).

Human Cytomegalovirus (HCMV), human herpesvirus 6 (HHV-6A and HHV-6B), and human herpesvirus 7 (HHV-7) are all members of the Betaherpesvirinae subfamily, whereas the Gammaherpesvirinae subfamily includes: 1. Epstein-Barr virus EBV (HHV-4) and 2.human herpesvirus 8 (HHV-8) (Davison and Andrew,2010).

Shortly after the discovery of human herpesvirus 6 (HHV-6), two distinct variants, HHV-6A and HHV-6B, were identified. In 2012, the International Committee on Taxonomy of Viruses (ICTV) classified HHV-6A and HHV-6B as separate viruses (Ablashi *et al.*, 2013).

There are two HHV-6 strains (A and B), which are both categorized as different types of herpesviruses from one another(Pantry *et al.*, 2017; Ogata *et al.*, 2015). In seroprevalence research conducted in the United States and Japan, HHV-6B is the most prevalent strain of the virus. Only severely immunocompromised hosts have been shown to be infected with HHV-6A (Endo *et al.*,2014). It's unclear whether the two strains have clinically significant distinctions and they're treated the same for management purposes. The term HHV-6 should be used in its broadest sense, with the understanding that it mostly refers to HHV6-B (Endo *et al.*,2014).

1.3.3.Genome, Structure, and Content of human herpes virus-6A/B

The HHV-6A/B virus genome is 160 kb in length and is composed of double-stranded DNA with over (100) open reading frames (ORFs). "Unique region" (U) contains the majority of genes, which is encircled by 8-9 Kb of direct repeats (DRL and DRR) (Isegawa *et al*.,1999).

When measured as a percentage of the complete nucleotide sequence, HHV6 A and HHV6 B have a nucleotide sequence resemblance of roughly (90%), but this rises to approximately (95%) when measuring conserved core genes and falls to 70 percent near the genome's right end.Glycoprotein B, Glycoprotein H, and U94 are the three most varied genes that distinguish HHV6A and HHV6B in the immediate early 1(IE1) region (Achour *et al*., 2008).

The U region of HHV-6A/B contains the core genes that are maintained among all herpesviruses. Only betaherpesviruses and roseoloviruses share certain genes in the virus genome. The genes U83 and U94 are only found in HHV-6A/B. (Dominguez *et al*., 1999).

HHV-6 core, capsid, tegument, and the envelope are the four essential parts that HHV-6 shares with other herpesviruses (Kramarsky and Sander, 1992), as shown in figure 1. 1.

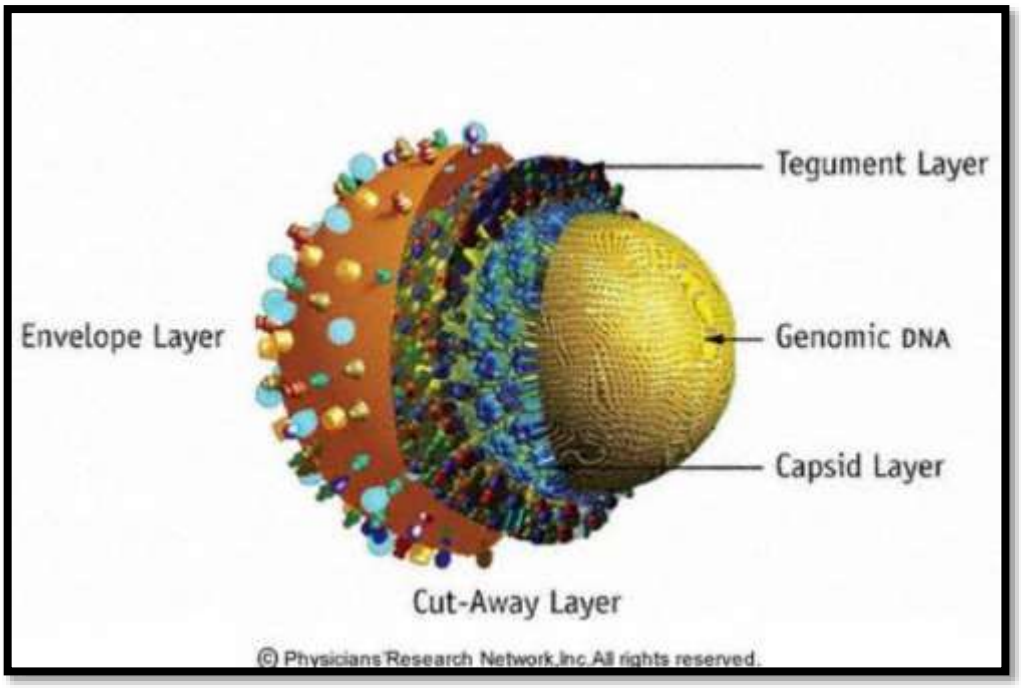


Figure 1-1: Human herpes virus-6 virion architecture (showing four fundamental elements: the core, capsid, tegument, and envelope adapted from(Kramarsky and Sander, 1992).

The bulk of genes are expressed in a nonrepetitive region between two identical long repeats (ca 8 Kb) named DRL and DRR in these two strains, (HHV-6A and HHV-6B). Bordering the two DRs are further repetitions (T1 and T2) made up of perfect telomere like repeats (TTAGGG) n in various copy numbers. The T1 area is longer than the T2 region and contains telomere-repeats that are defective (Wallaschek *et al*.,2016).

These telomere-like repeat allow for homologous recombination with the subtelomeric end of the telomere region7 (Arbuckle *et al*.,2010), and has been found in different chromosomes, but usually in a single copy per host (Arbuckle *et al*.,2010; Strenger *et al.*, 2013; Bell *et al*.,2014; Endo *et al.*, 2014 ; Sedlak *et al.*, 2014). While both T1 and T2 take part in the integration process, only T2 has been shown to be necessary(Wallaschek *et al.*,2016).

The integration in the telomere region could have negative effects on the host cell as it could interfere with its protective role against chromosome shortening or incorrect identification of the chromosome end as a double-strand break(Lazzerini-denchi *et al*., 2016). Huang *et al*. showed that while telomeres *in vitro* (lymphoblastoid cell lines (LCL) presenting HHV-6 integration were shorter than average, *in vivo* (sperm DNA) showed no different sign of erosion(Huang *et al*., 2014).

HHV-6's integrating influence on the function of the telomere and the host cell, on the other hand, is still unknown, and other parameters unrelated to chromosome shortening may be impacted (Kim *et al*., 2016). Furthermore, the full reactivation of integrated HHV-6 has been shown in vitro (Arbuckle *et al.*, 2013) and also in vivo (Gravel *et al.*, 2013). Integration happens in germinal cells on rare occasions, allowing them to penetrate the germ line. As a congenital disease, the virus can be passed down through generations and be found in each of the offspring cells (Hall *et al.*,2008).

Endogenous HH-6,named inherited chromosomally integrated HH-6 (iciHH-6), is a condition that affects 0.2–1% of the adult population globally (Leong *et al.*,2007). The name exogenous HHV-6 is given to the virus that is the most frequent non-congenital variant. The congenital form of a virus may have an intact genome, allowing the entire viral gene set to be expressed in each cell of the carrier (Arbuckle *et al.*,2011).

The incidence of iciHHV-6and its relationship to HHV-6-related illnesses are being investigated right now (Zhang *et al.*, 2016). The core of a mature virion contains tightly spaced double-stranded DNA in a spherical capsid. The capsid is an icosahedral protein shell that surrounds and protects the ds-DNA core and has a diameter of 1200–1300. Between

the capsid and the envelope is the tegument, which is a loosely distinct, asymmetrical layer of host and viral proteins. Certain proteins linked to the capsid in close proximity vary in thickness and placement around the capsid. The envelope is a lipid bilayer generated by the host that contains spikes of viral glycoproteins (Zhou *et al*., 1999).

Only 72.1 percent sequence similarity exists between the HHV-6A and HHV-6B glycoprotein-encoding genes that encode gQ (U97, 98, 99, and 100) (Isegawa *et al*.,1999). As a result, this glycoprotein may have a role in the differences between HHV-6A and HHV-6B infections.

Neutralizing antibodies recognize epitopes on gQ1, as do neutralizing antibodies to gB and gH, and it acts as a target for virus-specific neutralizing antibodies (Kawabata *et al*.,2011; Maeki and Mori,2012; Oyaizu *et al*.,2012).

The gH, gL, gQ1 and gQ2 complex are a key target for viral neutralizing antibodies (Maeki and Mori,2012).

1.3.4 . Mode of transmission

Asymptomatic nature of viral transfer with body fluids is considered to be the main route of transmission. Frequent HHV-6 findings in saliva and salivary gland tissue (Tanaka *et al*., 2012) suggest that the salivary glands are one of the HHV-6 persistence sites, and that saliva is a way of virus transmission, either to the child from the mother, or within children (Chen and Hudnall, 2006). It is important to note that all HHV-6 isolates from saliva are HHV-6B (Hall *et al.,* 2010).

HHV-6 DNA findings in umbilical cord blood in healthy newborns and lack of IgM, as well as in abortion fetus, are indicating to a possible hereditary transmission (Adams *et al.,* 1998). Vertical transmission frequency of HHV-6 is approximately 1-2%.

Apparently, at least in HHV-6B case, the most common transmission is through saliva. HHV-6 transmission occurs frequently in a horizontal way, usually the mother - child. It is shown that HHV-6 may be integrated into the cell genome. Chromasomaly integrated HHV-6 was first mentioned by Luppi et al (Luppi *et al.*, 1993), the work of three unrelated cases of patients with Hodgkin's disease, non-Hodgkin's B-cell lymphoma lines and multiple sclerosis. Daibata and co-authors (Daibata *et al*., 1999) described a family case showing that chromasomaly integrated HHV-6 genomic DNA is stably transferred in the gene level.
Integration into chromosomes can occure with both HHV-6 virus species, and some authors have found that virus DNA in serum/plasma is associated with white blood cell lysis, in which the genome of HHV-6 is integrated (Ward *et al*., 2005). *In vitro* experiments reveal that only one: either HHV-6A or HHV-6B can integrate, but not both at the same time.
Ward and co-authors (Ward *et al*., 2006) for the first time showed the presence of HHV-6 genomic sequence in hair follicle cells of the people with immune response disabilities. It is implied that the virus can be inherited and can be found in many cells of the body (Tanaka-Taya *et al*., 2004).

1.3.5.Cell tropism

The researches showed that CD4+ lymphocytes are the primary target cells for HHV-6, but CD8+ T cells, natural killer (NK) cells, macrophages, endothelial, epithelial, neural, and fibroblast cells are also susceptible to infection (Bolle *et al*., 2005).

HHV-6 is able to bind and infect human cells through CD46, which is a cellular receptor.During HHV-6 infection, surface CD46 was found to be down-regulated.Monoclonal antibodies that specifically target CD46 reduce both acute infection of HHV6 and fusion cells. Similarly,

soluble CD46 inhibited cell fusion. As soon as nonhuman cells were exposed to recombinant human CD46, they became sensitive to HHV-6 fusion and entrance. The utilization of a widely distributed immunoregulatory receptor provides a new viewpoint on the tropism and toxicity of HHV-6 (Santoro *et al*., 1999).

The ability of some viral chemokines to chemo-attract diverse cellular populations could possibly be connected to variations in cellular tropism. U83B, a chemokine belonging to the HHV-6B, is specific to the chemokine receptor type 2 (CCR2) and can attract cells possessing this receptor, like monocytic cells and some T cell subpopulations, whether in a latent or a lytic infection.The HHV-6A chemokine U83A, on the other hand, has a broader selectivity for monocytic CCR1, CCR4 ,CCR5, CCR6 and CCR8 chemokines (Clark *et al*.,2013).

U83 is one of the genes found in HHV-6A and HHV-6B but not in HHV-7, thus used to code for major differences between these viruses (Clark *et al*.,2013). HHV-6A's complex of gH, gL, gQ1 and gQ2 binds to CD46 (Tang *et al*.,2011).

Interestingly, despite comparable receptor expression levels on these cells, the efficiency of HHV6A and HHV6B entrance varies across distinct types of target cells. During the entry of the virus, the cellular factor gp96 interacts with the viral glycoprotein Q1 (gQ1), which is expressed on the cell surface (Ma *et al.*,2020). In addition to (gH, gL, gQ1 and gQ2), the gH, gL, gO complex was identified in the HHV6A envelope, which is essential for viral entry into human fibroblasts (Revello and Gerna,2010;Tang *et al*.,2010).

1.3.6. Replication cycle of human herpes virus-6

HHV-6 employs a viral ligand made of four glycoproteins, H, L, Q1, and Q2 (Yamanishi *et al*.,2013). HHV-6 enters into the cells via the gB and gH functions, fusing the envelope of the virus to the membrane of the cell by an unknown process. After that, it's possible that nucleocapsid travels to the nucleus by going through the cytoplasm and through the microtubule network. The nucleoplasm contains DNA from HHV-6.The IE-A locus has 2 genetic units, IE1 and IE2, which are arranged in chronological order, commencing with the immediate early (IE) genes from the IE-A locus (Tsao *et al*.,2009).Those genes are transcribed in the absence of denovo protein synthesis, followed by early (E) and late (L) gene transcription and expression.Phosphotransferases, uracil-DNA glycosylase, ribonic acid reductase, origin-binding protein, and other E proteins are produced as a result, having enzymatic activities linked to DNA metabolism and DNA synthesis (Tsao *et al*.,2009).

The replication of genomes is aided by the production of major DNA-binding proteins and helicase-primase complex activities. According to current theories, viral DNA replicates in a circular fashion(Tsao *et al*.,2009).

In the DRL and DRR regions of DNA, cleaving and packing signals are identified. These signals are subsequently employed to construct capsid precursors using the unique cleavage-packaging signals revealed in these locations (Yamanishi *et al*.,2013).Capsids leave the nucleus and create an intermediate envelope by budding through the inner part of the nuclear membrane. , which they then fuse with the outer part of the membrane to produce tegumentary forms in the cytoplasm. The trans-Golgi network acquires the final envelope containing viral glycoproteins, and mature virions are discharged through exocytosis. The

replication cycle, which lasts roughly 3 days, has an impact on the appearance and functioning of host cells(Yamanishi *et al*.,2013).

The infected cells that are involved in the process of virus production eventually perish as a result of apoptosis and/or necrosis. The replicative cycle of the virus is depicted in Figure 1.2.

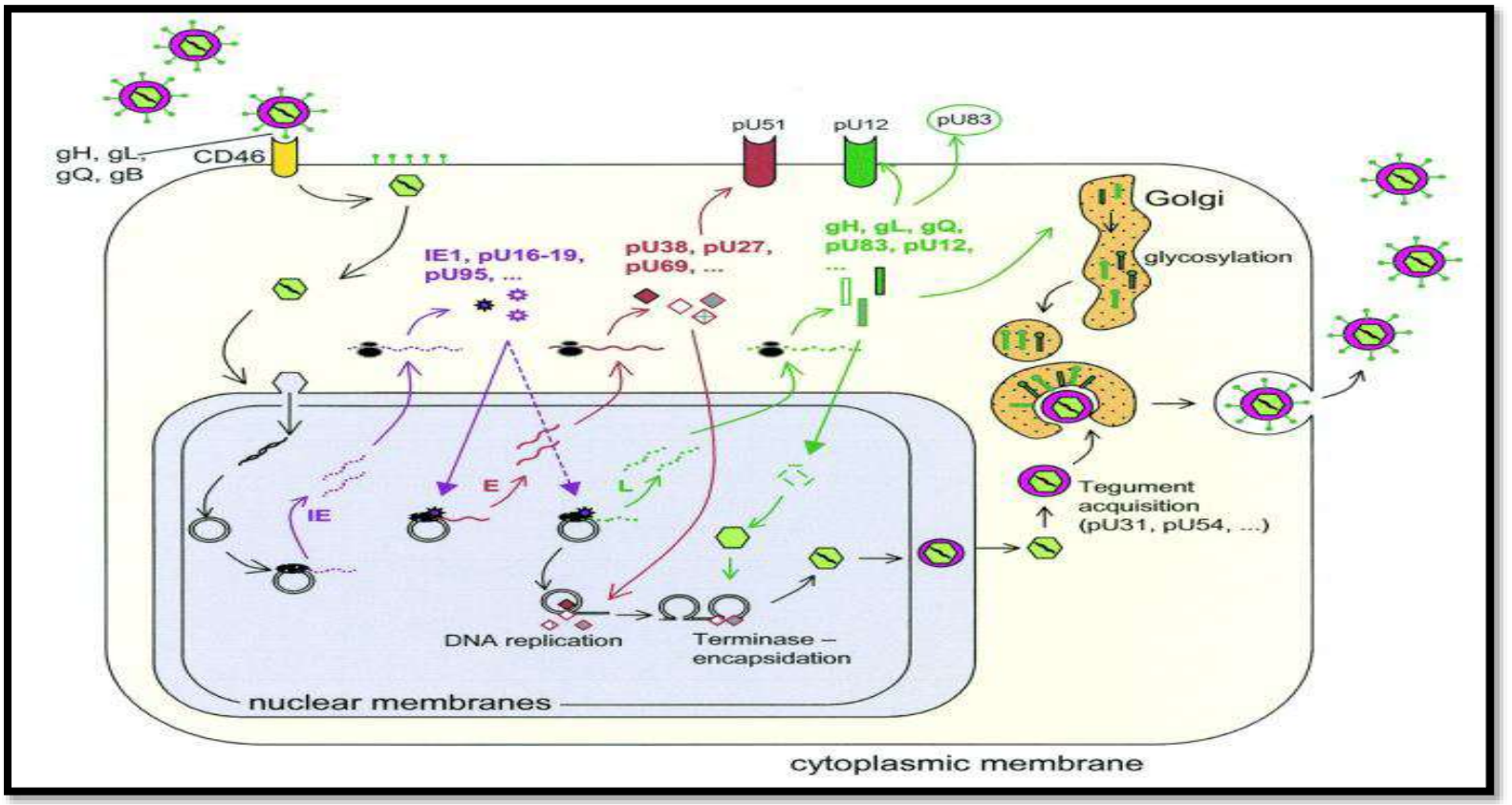


Figure 1.2. Scheme of HHV-6 replication in cells adapted from (Bolle *et al*., 2005).

1.3.7.Diseases attributable to HHV-6:

Children and babies are the most susceptible to primary HHV-6 infection. HHV-6 is the most frequent cause of fevers and seizures in infants 6 to 24 months of age .In immunocompetent people, acute HHV-6 infection is uncommon, while it can cause disease similar to mononucleosis with fever, lymphadenopathy, and hepatitis. (Hall *et al*., 1994).

HHV-6 infects many different types of cells and remains latent following infection, similar to other herpesviruses (Pantry and Medveczky,2017). HHV-6, unlike other herpesviruses, can be chromosomally integrated, which is the hypothesized method of vertical transmission, despite the fact that it occurs in less than 1% of the entire population who are infected for the first time (Endo *et al*.,2014).

HHV-6 encephalitis has only been described in one incidence of chromosomally integrated HHV-6A reactivation in an allogeneic HSCT recipient (Ogata *et al*.,2015). Chromosomally integrated HHV-6 (ciHHV-6) inherited from their parents is thought to affect 1% to 2% of the general population (Pantry and Medveczky,2017). When attempting to diagnose HHV-6 encephalitis, chromosomally integrated HHV-6 has the potential to impair a clinician's ability to interpret laboratory results (Ong *et al*.,2017).

1.3.7.1. Active infection

An active infection which describes original infections, endogenous reactivations, and foreign reinfections (Agut *et al*.,2011).They are more accessible to existing direct diagnosis tools, and they could be targets for specific antiviral treatment, which is currently aimed mostly at HHV-6 DNA replication, and can be connected to

concomitant disease more convincingly. The presence of detectable viremia is often regarded as a sign of a systemic active infection (Flamand *et al*., 2010).

Beta-herpesviruses stay latent after primary infection, but any immune system imbalance might promote virus activation (or reactivation) (Flamand *et al*., 2010).

Acute or chronic viral infection consider the outcomes of active viral infection. In both immunocompromised and immunocompetent people, severe encephalitis can result from an acute infection of HHV-6B or reactivation of a virus. In infants, HH6 B primary infection commonly causes status epilepticus and seizures. Chronic infection or low-level HHV-6 infection linked with subsets of people with multiple sclerosis (HHV-6A), cognitive dysfunction in transplant patients (HHV-6B), refractory temporal lobe epilepsy (HHV-6B), and some patients with chronic fatigue syndrome (HHV-6A) (Maeki and Mori, 2012).

1.3.7.2. Latent infection

Latency of HH6A and B, like many other herpesviruses, creates an infection in the host that lasts a lifetime (Agut and Gautheret, 2015).

Latent infection of HHV-6 A & HHV-6 B are established in a tiny percentage of somatic cells (and were originally discovered in peripheral blood mononuclear cells (PBMCs) (Wood and Royle, 2017).Macrophages and monocytes, progenitors of bone marrow and cells of the centeral nervous system in later studies, identified these as additional possible latent locations (Andre-Garnier *et al*., 2004).

HHV-6 remains dormant after primary infection except when the immune system is weak. This prolonged infection is usually a concern in

immunocompetent hosts.The occurrence of isolated cases of pulmonary failure in immunocompetent people who do not have other infections is thought to be due to HHV-6. But they are extremely rare, and there is no evidence that shows a cause-and-effect relationship (Merk *et al*., 2005). Few transcripts have been expressed when they are in latency(Kondo *et al*., 2002). The majority of herpesviruses retain their latent genome in the nucleus of the latent cell as a circular episome (Agut *et al.,* 2015).

1.3.7.3. Persistent infection

Persistent infections are those in which the virus survives the adaptive immune response and persists in the target cells of infected people. Persistence infections can have both silent (latent) and active (productive) stages, without killing or causing severe damage to the host cells. After a primary infection, HHV-6 can stay in the human body for the rest of one's life. In circumstances of compromised immunity, such as the use of immunosuppressive drugs or stress, the virus can reactivate. (Sampaio *et al*.,2011).

1.3.7.4. Reactivation of HHV-6A/B

Several years after primary infection, HHV6A/B could be found in the saliva of healthy people (Levy et al., 1990), demonstrating the reactivation of the virus to confirm community transmission. In both latently infected and iciHHV-6-infected people, HHV-6A/B reactivation is possible (Gravel *et al*., 2013; Endo *et al*., 2014).

Several investigations have indicated that reactivation of latent HHV6A/B in patients undergoing hematopoietic cell transplantation is associated with encephalopathy, graft rejection, and bone marrow suppression (Shimazu *et al.*, 2013).

1.3.8. Immune response to HHV-6 virus

1.3.8.1. Immune response after primary infection

During HHV6 infection, the innate immune response induces the generation of chemokines and cytokines that assist in the regulation of the immune system.

. Regulated upon activation normal T cell expressed and secreted (RANTES) is a proinflammatory β chemokine that draws monocytes and lymphocytes, inducing local response.

Interleukin-1 β (IL-1 β) induces inflammatory and interferon (IFN) responses and is required for B-lymphocyte immunological responses that are dependent on IL-6. . HHV-6 replication is inhibited in vitro by both IFN- α and IFN- β (Jaworska and flamand, 2010). TNF- α, which is usually an antiviral cytokine, may, however, promote monocyte development and thereby increase extracellular HHV-6 release (Niiya *et al*., 2006).

1.3.8.2. Humoral immunity

1. Antibody response

Adults have a high sero-prevalent rate of beta-herpesviruses, and almost all newborns have maternal antibodies to these viruses at birth. Antibody titers decrease dramatically from birth to 3 to 6 months of age. From 6 months after birth until the second year of life, when sero-prevalent approaches that of healthy adults, practically all HHV-6B primary infections occur. This is the period during which almost all HHV-6B primary infections occur (Magalhaes *et al*., 2011).

IgM antibodies occur 5–7 days after the onset of clinical symptoms, peak at 2–3 weeks, and then fade away by 2 months following

infection. The 101K antigen encoded by HHV-6 U11 is at one target of the IgM response (LaCroix *et al*., 2000).

After infection caused by HH6, the HHV6-specific neutralizing IgM response develops (LaCroix *et al*., 2000).

Antibodies to HHV-6 IgG normally arise 10 days to 2 weeks after the onset of clinical symptoms, grow in avidity with time, and remain detectable for many years (Braun *et al*., 1997). The avidity of the first IgG reaction is modest. Antibody avidity rises over time, making it a good marker for identifying new infections and distinguishing them from reactivations (Ward *et al*., 2001).

2.Neutralizing antibodies

HHV-6 neutralizing antibodies are detectable at birth, throughout and after the rash stage of primary infection, and not during the fever stage ,implying that maternal antibodies can prevent viral infection(Kawabata *et al*., 2011).

1.3.8.3. HHV-6's Role in Cellular Immunity

1.Cytokine production

An important issue in the organismal response to infectious agents is the balance between the Th1 and Th2 arms of the immune system. There have been many studies of the effect of HHV-6 infection on the ability of target cells to produce cytokines that affect this balance, with sometimes diametrically opposed results. As discussed in detail by Smith and colleagues ,this is probably due, at least in part, to the use of different cell types or cell populations in *ex vivo* conditions that do not fully represent *in vivo* regulatory circuits (Smith *et al*., 2003).

IL-12 plays a pivotal role in inducing Th1 responses, and IL-10 is one of the key effectors in shifting the balance toward Th2 responses. Under some conditions, HHV-6 infection of PBMC induces IL-10 expression, which inhibits IL-12 production (Arena *et al*., 1999).

Both HHV-6A and HHV-6B infections of monocytes transiently induce low levels of IL-12 production; simultaneously, these infections substantially restrict the level of IL-12 induction in response to IFN-γ and lipopolysaccharide; the sum is a net reduction in IL-12 production. From these results, it has been argued that HHV-6 infection might lead to inhibition of Th1-polarized immune responses (Smith *et al*., 2003).

2.Involvement of T lymphocytes

In healthy individuals, the glycoprotein membranes of HH6A and HH6B do not cause T-lymphocyte proliferation; rather, they decrease T-lymphocyte proliferation in response to mitogens or antigens (Horvat *et al*.,1993). Preparations Ag containing nucleocapsid proteins and tegument proteins of HH6A and HH6B induce T-lymphocyte proliferation responses in healthy seropositive children and adults (Soldan *et al*., 2000); the response peaks at 4 weeks after primary HHV-6 infection, compared to 1 to 2 weeks for other herpesvirus infections (Kumagai *et al*., 2006). The HHV-6B antigen elicited more responses from HHV-6 seropositive, healthy people than the HHV-6A antigen.

The cytotoxic T lymphocyte cells produced by the Th1 pathway, which is partially driven by IL-12. HHV-6 virions prevent macrophages from producing IL-12 in response to IFN- γ or lipopolysaccharides. While the cytotoxic T lymphocyte (CTL) response eventually develops, it's easy to conceive that delaying the response's formation for a brief period of time aids the infection's establishment (Smith *et al*., 2003).

HHV-6A roughly equally replicates in and kills CD4 and CD8 Tcells, but HH6B mostly replicates in and depletes CD4+ T-cells . HH6A and HHV6B both cause dramatic and widespread down-regulation of the CD46 molecule in both infected and non-infected cells, but only in infected lymphocytes, with some changes in this activity between these two viruses .After HHV6A and HHV6B infection, the CD4 promoter is active in infected cells, most likely due to direct CD4 promoter activation (Grivel *et al*., 2003).

3. Involvement of NK cells

In vitro cultured NK cell clones are infected and killed by HHV-6. Furthermore, HHV-6 causes NK cells to express the CD4 receptor, a helper T lymphocyte surface marker and the major cellular receptor for HIV-1, making NK cells susceptible to HIV infection. This has led to speculation that HHV-6 not only suppresses the immune system but also contributes to the progression of AIDS (Kumagai *et al*., 2006). HHV6 infection of PBMC, which includes a range of cell types, however, leads to increased NK activity and NK-mediated death of HHV-6 infected cells (Kumagai *et al*., 2006).

4. Involvement of stem cells

Exposure of bone marrow precursors to HHV-6 inhibited their ability to respond to growth factors such as granulocyte-macrophage colony-stimulating factor and interleukin-3, and also reduced the outgrowth of macrophages from bone marrow (Andre-Garnier *et al*., 2004).

This may be due to induction of IFN-α by HHV-6 (Jaworska *et al*., 2010). Nonetheless, the virus does cause dysfunction of blood monocytes and blocks their differentiation to macrophages (Smith *et al*., 2005).

5. Involvement of dendritic cells

CD4 and CD8 T lymphocytes depend on dendritic cells for antigen presentation. Immature dendritic cells promote HHV-6A or HHV-6B replication (Nordström and Eriksson, 2012). The majority of infected cells do not die and mature into mature forms, but they are functionally poor and unable to promote lymphocyte proliferation (Kakimoto *et al*., 2002).

6. Involvement of histocytes

Langerhans cell histiocytosis and hemophagocytic histiocytosis are characterized by dysregulated proliferation and migration of histiocytes (tissue macrophages).. Cases of hemophagocytic histiocytosis have been associated with HHV-6 activity following organ transplantation (Jeziorski *et al*., 2008).

1.3.9. HHV-6 virus Diagnosis

HHV-6, the causal agent in infants with roseola infantum signs and symptoms, is identified as human herpesvirus 6 (HHV6).Other causes of fever and rash should be ruled out because this diagnosis has several differential diagnoses. The disease bears a strong resemblance to mononucleosis in adults with HHV-6 disease who are not immunocompromised. Cytomegalovirus (CMV) or Epstein-Barr virus (EBV) infection should be ruled out.Because between 30% and 70% of those who undergo HSCT get reactivated with HHV-6, , as well as viremia, two to four weeks after the procedure, screening should not be routinely recommended but varies by institution (Pellett *et al*.,2019).

Risk factors for reactivation of HHV-6 in this group include steroid therapy, unrelated mismatched donor contribution, and umbilical cord blood transplantation. This puts the onus on the clinician to evaluate

all pertinent diagnostic clues. If an HSCT recipient is suspected of having HHV-6 encephalitis, only clinical laboratories or reference laboratories can perform targeted tests (such as PCR of serum or CSF) to confirm this. This test should be performed on those who have been diagnosed with HHV-6 encephalitis (Pellett *et al*.,2019). In the case of transplantation, a complete blood count (CBC) may reveal variable degrees of cytopenia (anemia thrombocytopenia) and leukopenia. An analysis of the blood (CBC) may show leukopenia with relative leukocytosis in an active infection. When considering treatment for HHV-6 encephalitis, electrolytes and renal function tests should be assessed. Hepatitis or liver dysfunction may be detected with liver function tests (Pellett *et al*.,2019).

1.Culture

Isolation of HHV-6 in research labs is facilitated by the availability of labor-intensive procedures that can take anywhere from 5 to 21 days: normal peripheral cell culture and shell vial testing culture (Caserta *et al*.,2010).

2.Serology

Within the first four to six weeks of a primary infection, symptoms such as IgG-negative to IgG-positive seroconversion and the existence of IgM to HHV-6 appear. Antibodies should not be employed for diagnosis because of their high seroprevalence and cross-reactivity in older children and adults (Zerr *et al*.,2005).

Although the IgM antibodies detected after the first week of infection have been depleted in 1 month, the IgG antibodies appeared later and have remained for life.These IgM and IgG antibodies can react with a variety of viral proteins. The U11 gene, for example, is one of the most important antigens (Agut *et al*.,2015).

HHV-6A and HHV-6B are common, life long infections that, unfortunately, are fairly common and thus poorly indicative and seropositivity has become extremely common. The presence of IgM and rapid rises in IgG antibodies don't always correlate with acute infection but may indicate reactivation of the virus(Agut *et al*., 2015)

3-Polymerase chain reaction assay

Using PCR assays for HHV-6 is useful for rapid diagnosis of primary or reactivated infections .It is also possible to detect co-infections with numerous herpesviruses, with quantitative results allowing for virus load monitoring during antiviral medication(Engelman *et al*.,2008).

1.3.10.Treatment / Management

There is currently no authorized medication specifically for the treatment of HHV-6, and no vaccination is available (De Bolle *et al*., 2005) .

Antiviral prophylaxis for HHV-6 infection is not needed in most cases. Instead, early antiviral treatment is recommended, especially for HHV-6 encephalitis. The preferred initial treatment options for HHV-6 are intravenous ganciclovir and foscarnet,with treatment lasting 3 to 4 weeks (Prichard and Whitley,2014).Treatment with ganciclovir show to be beneficial for individuals having stem-cell transplantation, and it is the antiviral of choice (Prichard and Whitley,2014). In immunocompetent children, HHV-6 infections are self-limiting and without any therapy needed. Roseola infantum is usually treated with a supportive approach. For high-grade fever and those at risk of febrile seizures, antipyretics such as acetaminophen or ibuprofen are advised(Agut *et al*.,2015).

1.3.11. Nuclear factor-κB (NF-κb)

NFkB is located in the cytoplasm of every cell and transfers to the nucleus when activated. It can be activated by any sort of stress, smoking, viral infection, bacterial infection, inflammation, inflammatory factors, cytokines, free radicals, carcinogens, tumor promoters, and toxins like endotoxins (Serasanambati and Chilakapati,2016).

The majority of NF- κB proteins are found in the cytoplasm (Sun,2011). IκB α is the most important member of the IκB family.

Furthermore, the precursor proteins of NF- κB1 and NF-κB2, p105 and p100, serve as IκB-like proteins because their C-terminal part matches the structure of IκB and contains NF-κB inhibitory action (Beinke and Ley,2004).

NF- κB1 (also known as p50), NF- κB2 (also known as p52), RelA (also known as p65), RelB, and RelC are members of this family that mediating transcription of target genes by binding as hetero-or homo-dimers to a specific DNA sequence named the B enhancer. The IκB family and related proteins contain ankyrin repeats, which are used to identify inhibitory proteins (Sun *et al*.,2013).

NF-κB is a family of transcription factors that regulates numerous genes involved in immune and inflammatory response pathways. NF-κB controls the expression of many of these genes (Oeckinghaus and Ghosh,2009).

A total of around 400 genes are regulated by NFkB, including enzymes (such as COX-2 and INOS), cytokines (including TNF, IL-1, IL-6, IL-8, and chemokines), cell cycle regulatory molecules, adhesion molecules, viral proteins, and angiogenic factors . Modifications in the

NF κB pathway have been linked to a wide range of diseases in humans, including diseases like asthma, atherosclerosis, AIDS, diabetes, Alzheimer's disease, and cancer. The activation of NF κB transcription factors helps coordinate innate and adaptive immune responses via their effects on signaling pathways.The researchers found that one of the reasons cancer starts and gets worse is because NF- κB signaling is vital (Serasanambati and Chilakapati,2016).

1.3.11.1.NF κB regulatory mechanisms

The NF κB is well-known for its involvement in innate immunity, and it has recently grown a lot of attention because inflammatory mediators including cytokines and toll-like receptors activate it during bacterial infection (TLRs). The NF κB subunits p65, RelB, c-Rel, p50, and p52 make up the NFκB family dimer (Oeckinghaus and Ghosh, 2009).

Homodimer complexes and hetero-dimer complexes aggregate into 15-membered dimers inside the cell, each having a different function. Each of the NF κB subunits has a Rel homology domain, a 300-amino-acid conserved region. The components p65 (RelA gene) and p50 are both prevalent (NFκB1 gene). In general, p65/p50 heterodimers are thought to promote transcription, whereas p50/p50 homodimers are thought to repress transcription (Oeckinghaus and Ghosh,2009).

An inhibitor of NF κB, which is generally an inhibitory protein, binds NF κB subunit dimers in the cytoplasm (IkB). The IkB kinase (IKK) complex is made up of two catalytic subunits (IKK α and IKK β) plus a regulatory component (NEMO). When IKK is activated, it phosphorylates IkB, designating it for proteasomal degradation and the release of NF κB subunit dimers.Toll like receptor-4, TNF- α receptor ,

and IL-1R stimulation causes IKK phosphorylation (Gerondakis *et al*., 2014).

The adaptor protein MyD88 is responsible for this (Laird *et al*., 2009). The classic or canonical NFκB pathway is this one. Alternatively(non canonical), NF κB-inducing kinase (NIK) activates IKK, which subsequently phosphorylates and converts p100, the p52 precursor protein, into p52/RelB heterodimers, or non-canonical NF κB dimers, which translocate to the nucleus after being released from the IkB complex, They bind to specific DNA sequences in the promoter regions of a number of genes, where they bind to specific DNA sequences (Oeckinghaus and Ghosh, 2009). Inflammatory mediators such as cytokines, chemokines, and cell adhesion molecules are produced when NF κB is stimulated, resulting in a positive feedback loop (see fig.1.3).

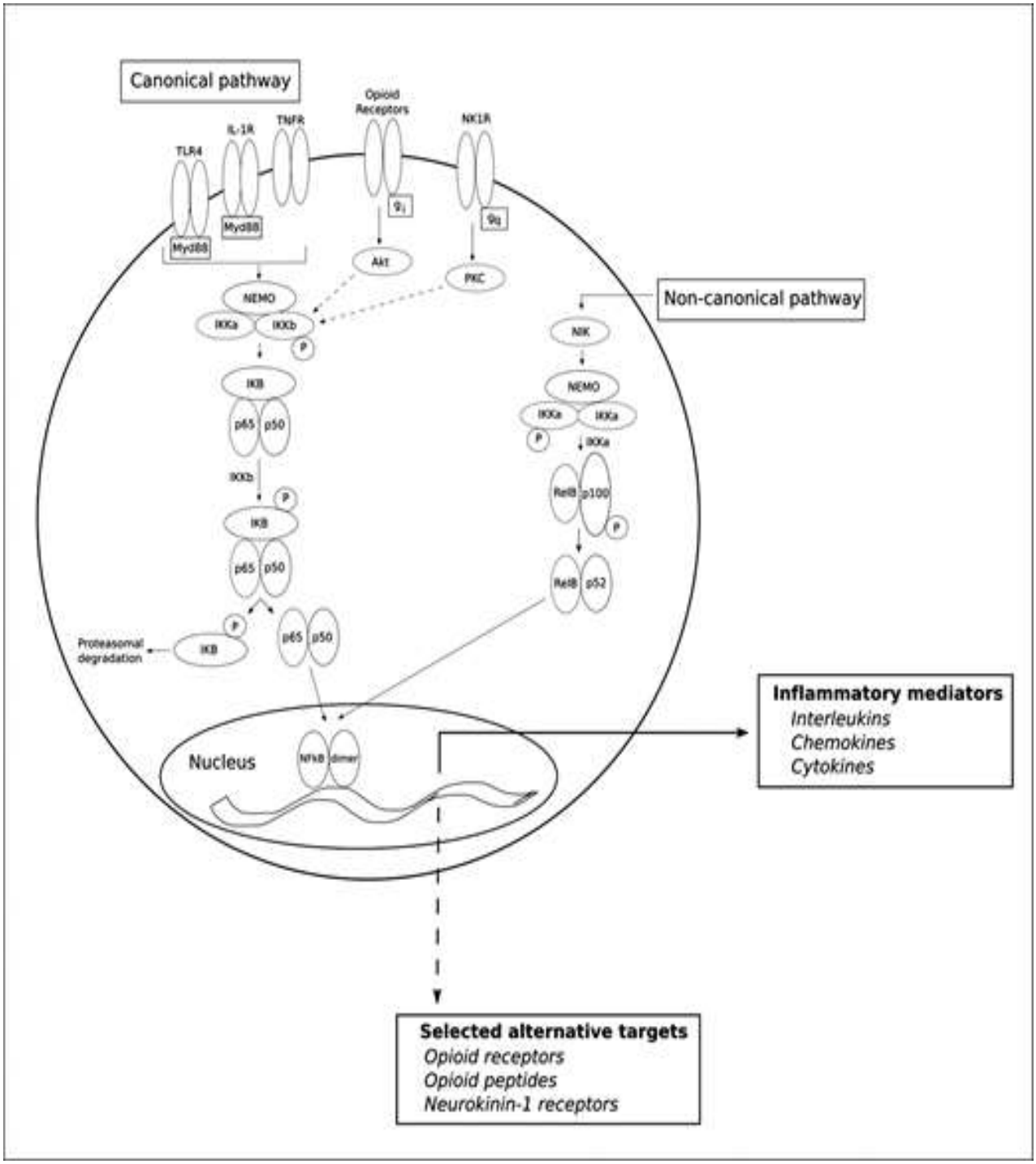


Fig.1-3: NFkB activation by the canonical and non-canonical pathwaysadapted from (Oeckinghaus and Ghosh,2009).

The activation of NFkB affects both inflammation mediators and specific alternative targets. To activate NFkB, both canonical and noncanonical pathways are used. NFkB dimers that have been released from the IkB complex go to the nucleus and attach to DNA sequences found in the promoter region of many different genes. The targeting genes are responsible for inflammatory responses(Oeckinghaus and Ghosh,2009).

1.3.11.2. NFkB and CVD

The impact of NFkB on cell survival and apoptosis could have effects on the development of the cardiovascular system. NFkB can have both good and harmful effects on the cardiovascular system in adult (Serasanambati andChilakapati,2016) .

Active NFkB (p65) is present in lesions of human aortic atherosclerotic. Nuclear NFkB was found in Ecs (epithelial cells) overlaying early lesions, as well as in SMCs (smooth muscle cells), macrophages, and T cells in much more progressive lesions (Tomita *et al*., 2003).

In healthy tissues, inactive nuclear p65 and p50 were found in SMCs, but active nuclear p65 and p50 were discovered in smooth muscle cells of human lesions(Tomita *et al*., 2003).

Active NF-κB p65, p50, and c-Rel were found in cells isolated from human atherosclerotic tissue, but not p52 or Rel-B. Activation and recruitment of inflammatory cells into ECs may be facilitated by NF κB signaling, while NF κB activity aids SMC proliferation. Nuclear factor kappa B (NFkB) is an important regulator of myocardial ischaemia and reperfusion responses. In response to ischaemia/reperfusion, proinflammatory cytokines (such as TNF α and IL-1) and endogenous

TLR ligands are released.Ischemia/reperfusion can also alter oxygen availability by activating NF κB (Atreya *et al*., 2008).

1.3.11.3.NF κB with HHV-6

The virus produces three types of viral proteins using the cellular transcription and translation machinery :immediate-early, early, and late protiens. Within a few hours of infection, HHV-6 IE proteins (immediate-early (IE) proteins) are produced and influence other genes expression.Expression of early genes depends on production of IE protein, and the genes make largely proteins related to DNA metabolism and replication. With a few exceptions, DNA polymerase activity of viruses stinguishes early proteins and late proteins temporally, hence named leaky transcripts (Oster *et al*.,2002). Despite the fact that late proteins are commonly glycosylated and function as parts of mature virus particles, the U83 gene, which encodes chemokines, is generated later in time. (Zou *et al*., 1999).

After the virus enters the cell, IE (immediate early) gene transcription occurs within a few minutes. Virion-associated proteins (like tegument proteins) are necessary for its expression, and it is independent from de novo protein synthesis.Despite the fact that the intermediate repeat sequence R3 is placed upstream of the IE-A region and has numerous potential binding sites for cellular transcription factors (for example PEA3, NF-kB, and AP-2), it has been suggested that it affects the IE-A gene's transcription (Martin *et al*.,1991).

The R3 region of HHV6 B interacts with NF-kB, that increasing the promoter activity of U95.Although the R3 sequence's repetitive organization differs between these two variants,The A variant R3 has a

greater number of NF-kB binding sites, and expression of HHV-6A U95 is likely to be regulated similarly (Takemoto *et al*., 2001).

As a result, while HHV-6A R3 may play a role in transcriptional control across the IE-A region, HHV-6B R3 is unlikely to do so (Takemoto *et al*.,2001).

It has been demonstrated that IE-A gene transcription may be begun by numerous promoters According to the stage of infection, resulting in many transcripts of varying sizes and kinetics (Gravel *et al*.,2002). Despite efforts to temporally plot HH6 transcripts (Oster *et al*.,2002), the mechanisms behind transcriptional control for just a limited early or late genes of HHV-6 have been studied in depth. Variance splicing, is usage of various transcription places, and post transcriptional alterations all contribute to the complex temporal expression patterns of early and late HH6 proteins (Taniguchi *et al*., 2000;Kondo *et al*., 2002).

Aside from that, they could differ amongst HHV-6 variations and be depending on the cell line or viral replication strategy that is being used. For the finding of numerous transcripts, such as those encoding late proteins, the researchers employed sensitive real-time PCR and found the results as early as one hour after infection (Oster *et al*.,2002).

1.4.Cardiovascular disease & HHV-6 virus

CVD refers to any condition that affects the heart and blood vessels.Angina and myocardial infarction are examples of coronary artery disorders (CAD) (often known as a heart attack), are examples of CVD (Mendis *et al*.,2011).Stroke, and other cardiovascular diseases include heart failure, hypertension, rheumatic heart disease, cardiomyopathy, irregular heart rhythms, congenital heart disease, valvular heart disease,

carditis, aortic aneurysms, peripheral artery disease, thromboembolic illness, and venous thrombosis (Abubakar *et al*.,2015).

A patients with diabetes mellitus are more likely to have a higher risk of cardiovascular disease due to " obesity, hypertension, and dyslipidemia.CVD risk is increased by independent biological mechanisms related to diabetes mellitus. To help patients avoid long-term problems with their hearts and blood vessels, physician should focus on CV risk factors in patients with diabetes (Leon and Maddox, 2015).

Acute leukemia (AL) patients are more likely to suffer from congestive heart failure (CHF) than other cancer patients. Acute leukemia might put patients at risk for cardiovascular problems before they have chemotherapy because they may release a huge amount of cytokines or leukemic cells could infiltrate the heart (Assuncao *et al*., 2016).

Enteroviral or adenoviral viruses are able to travel outside the heart to several locations in the body, including the reticuloendothelial system, where they can become a reservoir for infection outside the heart. After attaching to tight junction proteins, such as the coxsackie and adenovirus receptor (CAR), the viral sequences may travel to the heart,where they may infect cardiomyocytes, and thereby enter the central nervous system (DAF, CD55).

Patients who suffer from viral heart disease can be categorized according to their level of systolic function, diastolic function, and left ventricular dysfunction as having normal systolic function, abnormal diastolic function, segmental or compensated left ventricular dysfunction, or acute systolic left ventricular compromise (Pauschinger *et al*.,2006).

Cardiac structures destroyed and subsequent myocardial lesions influence the kind and severity of myocardial compromise. A partial

reversible disruption of the components needed for force generation and transmission or an irreversible loss of contract tissue may lead to systolic dysfunction and degeneration after cardiomyocyte infection (e.g., entero- and adenoviruses) (Schultheiss,2011).

Several studies have found HHV-6-specific DNA in the vascular endothelium in vivo, implying that the virus causes endothelial cell destruction (Caruso *et al*.,2003). Endothelial cells and cardiac myocytes have been postulated as potential reservoirs for viral latency and reactivation (Rotola *et al*.,2000).According to another study (Tang *et al*.,2013), CD134 is an HHV-6B receptor.

The widespread distribution of CD46 on a variety of cells explains HHV-6's extensive tissue tropism and dispersion in people whose infected blood cells come into direct contact with the vascular system.If herpesvirus-infected endothelium does not cause reactive inflammation, the underlying tissue infection could come from the vascular cell layer. HHV-6 genomes have recently been discovered in postmortem explanted hearts (Yoshikawa *et al*.,2001) and in EMBs (Endomyocardial biopsy) of patients with dilated cardiomyopathy (DCM) and clinically suspected myocarditis (Pankuweit *et al*.,2010).

For well-documented cardiotropic viruses (coxsackievirus, adenovirus),the distribution of viral receptors in the myocardium is highly varied, with a significant increase in patients with dilated cardiomyopathy (Noutsias *et al*.,2001). CAR (coxsackie and adenovirus receptor) is a critical deterrent for both viruses' cellular uptake as well as the molecular pathogenesis of coxsackievirus and adenovirus-associated illnesses (Poller *et al*.,2002). Differentiation of porcine mesenchymal stem cells into cardiomyocytes demonstrated the expression of CD46 in terms of

herpesvirus receptors (Moscoso *et al*.,2005). For both CAR and CD46, the dynamics of viral receptors in the myocardium have been demonstrated. Endothelial cells express CAR and CD46 in DCM, suggesting that viruses that target these receptors may have an easier time entering heart cells following intravascular delivery (Toivonen *et al*.,2010). Although HHV-6 genomes have been found in several organs using sensitive PCR, direct imaging of HHV-6 viral particles or proteins in wounded or inflamed arteries has yet to be proven. Although the chromosomal integrated virus can not be eradicated, patients with symptomatic heart failure can benefit from antiviral medication that reduces ciHHV-6 transcriptional activity (Lassner *et al*.,2013).

Chapter Two

Materials

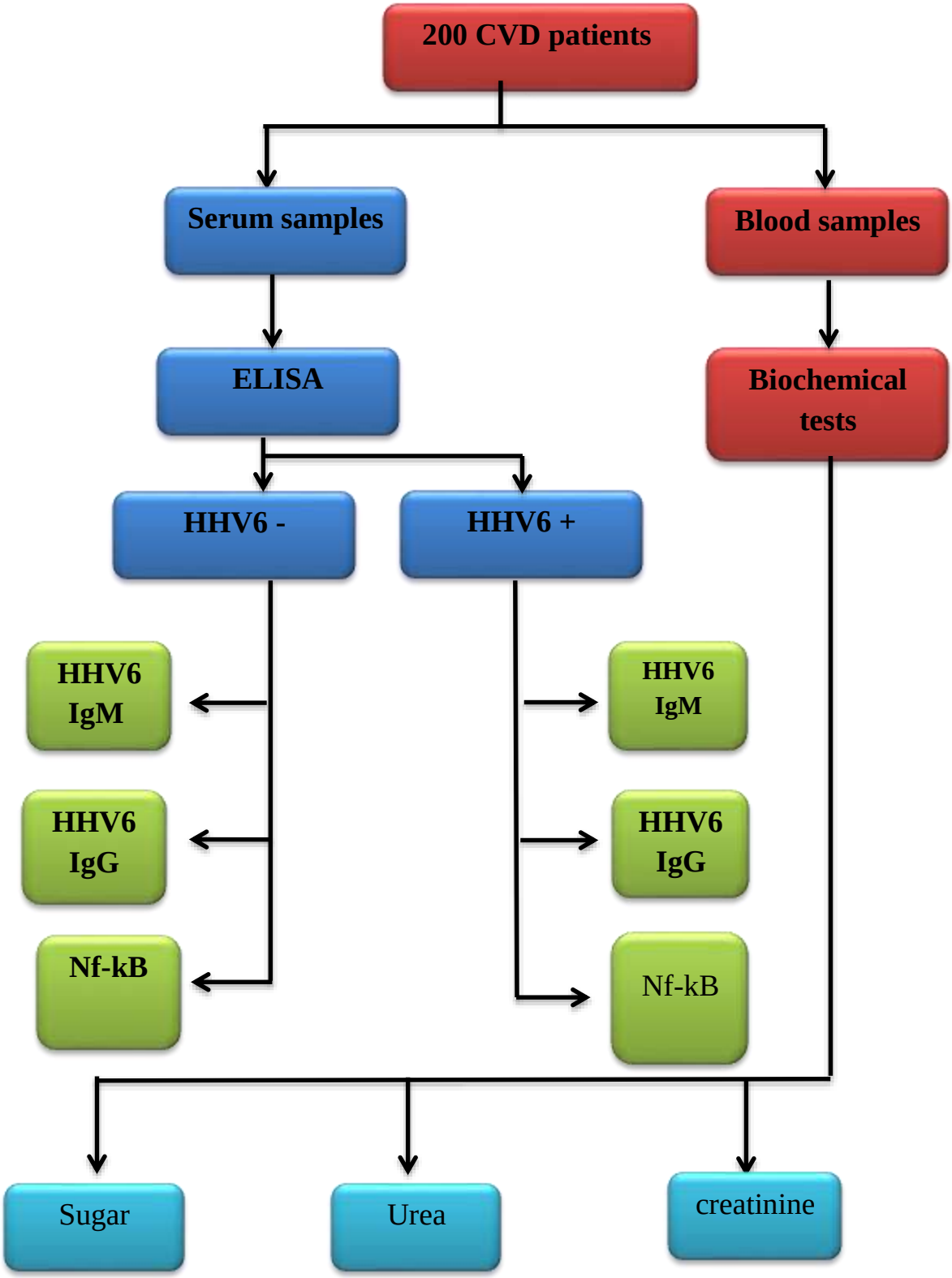
And

Methods

2.1.Study design

The study is a cross-sectional done at AL-Sader Teaching Medical City in Al-najaf governorate from the time between November, 2020 and May, 2021.

Study design : Cross sectional study



Figure(2.1): Study design(cross sectional study).

2.2. Subject groups

2.2.1.Patients

200 patients with cardiovascular diseases (clinically diagnosed according to cardiologist physicians) were enrolled in this study. Of 200 cases, there were 50 females and 150 males, and the patients' age averaged 30-104 years.

2.2.1.1.Included criteria:

1-Acute Coronary Syndrome (ACS): Ischemic Heart Disease

2-Heart Failure: Congestive Heart Failure.

3-Angina: Stable Angina and Unstable Angina.

4-Myocardial Infarction (MI).

5-Myocardiopathy (MCP).

2.2.1.2.Exclusion Criteria:

1.Valvular Heart Disease.

2.Congenital heart diseases.

3.Rheumatic heart disease.

4.Structural cardiac diseases.

5.Cardiac arrhythmia.

6-Patients with covid-19.

7.This study did not include patients who had HCV or HBV.

2.3. Ethical Approval:

Samples of blood were taken from the individuals enrolled in this study after obtaining the oral and written consent of them. Kerbala College of Medicine's ethical committee has approved the study protocol.

2.4. Samples Collection:

Blood samples are clotted at room temperature for roughly an hour before being used to make serum samples. Following that, centrifugation for 15 min at 3000 rpm, the serum was separated into new plain tubes for immunological tests (HHV-6 Ag, HHV-6 IgM, IgG, and NF-kappa) and stored at (-20 °C) till the time of analysis.

2.5 Materials

2.5.1. Equipment and Instruments:

The study's equipment and instruments are recorded in Table 2.1.

Table 2.1.: Equipment and Instruments

Types of Equipment		Model's Name (Origin)
1.	70% alcohol for sterilization	China
2.	Centrifuge (High Speed)	Hettich / Germany
3.	Computer	USA
4.	Cotton	PRC (China)
5.	Deep Freezer	Hitachi/Japan
6.	Disposable glass gel serum tubes	Unimedica / Iraq
7.	Disposable gloves	Provi, Switzerland
8.	Disposable pipette tips	Bio-Hit / Finland
9.	Disposable plastic EDTA tubes	AFCO / Jorden
10.	Disposable sterile syringes 5ml	LUER Lock/USA
11.	ELISA automated washer	BioTek/USA
12.	Elisa Kit	SUNLONG/China
13.	ELISA Printer	Epson/ Japan
14.	ELISA Reader	BioTek/USA
15.	Filter paper	China
16.	Fujifilm/biochemestery	Japan
17.	Incubater	Binder/USA
18.	Micropipette :10 to100 (µl)	Germany
19.	Micropipette :10 to1000 (µl)	
20.	Micropipette :2to20 (µl)	
21.	Multi-channel Micropipette 0-250(µl)	Germany
22.	Piptte Tip Rack	China
23.	Plastic Rack	PRC (China)
24.	Refrigerator	Concord/Lebanon
25.	Sterile Eppendrof Tubes	China
26.	Tourniquet	China

2.5.2.ELISA kits & contents:

HHV-6 Ag, HHV-6 IgM and HHV-6IgG antibodies, and NF Kappa were detected in serum samples from patients using commercial ELISA kits (SUNLONG(CHINA)) see in Appendix.

2.6. Method

2.6.1. ELISA Detection of HHV-6 Antigen

2.6.1.1. Principle of the assay:

In this ELISA kit, the procedure was Sandwich-ELISA. This kit includes a Micro-elisa strip-plate that has been precoated with a precise antibody for HHV6 samples or standards are placed in the relevant Microelisa stripplate wells and mixed with the specified antibody.then, every Microelisa stripplate well was treated with a HRP-conjugated HHV6-specific antibody for.The parts that were no longer required were rinsed away.The Substrate solution TMB was poured into every well. After the stop solution, the wells that contained HHV6 and HRP-conjugated HHV6 antibodies exhibited blue before turning yellow.By spectrophotometer, the optical density (OD) was calculated at a 450 nm wavelength. The optical density value is related to the concentration of HHV6. Compute HHV6 concentration in specimens by comparing the samples' optical density to a standard curve.

2.6.1.2.The procedure

1.Standards Dilution

The standard was first diluted in small tubes, then pipetted 50ul from each tube into a well of a microplate (each tube uses two wells, for a total of ten wells).

Table(2-2):Standards dilution steps(ELISA kit for HHV6)

120 ng/L	No.1 Standard	300µl Original Standard + 150µl diluent standard.
80 ng/L	No.2 Standard	300µl No.1 Standard + 150µl diluent standard.
40 ng/L	No.3 Standard	150 µl No.2 Standard + 150µl diluent standard.
20 ng/L	No.4 Standard	150 µl No.3 Standard + 150µl diluent standard.
10 ng/L	No.5 Standard	150µl No.4 Standard + 150µl diluent standard.

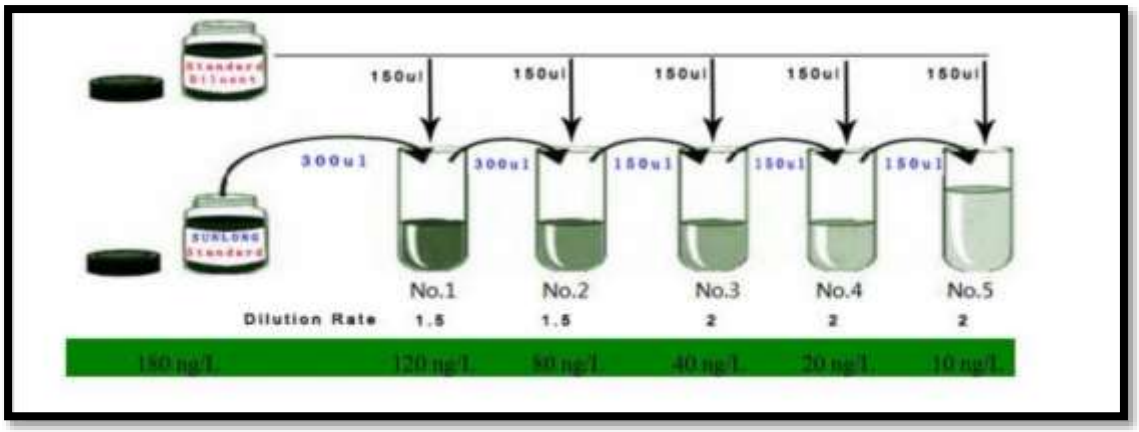


Figure2-2 : Standards dilution steps(ELISA kit for HHV6

2.As a blank control, a well on the Microelisa stripplate was left empty. The sample dilution buffer (40µl) and sample (10µll were placed in wells (dilution factor is 5).Without hitting the well wall, samples were loaded onto the bottom. To combine, the well was gently shaken.

3.Incubation: after the closure plate membrane is added for sealing, it is incubated for 30 min at 37°C.

4. Dilution: diluted the concentrated washing buffer with distilled water (30 times for a 96T and 20 times for a 48T).

5.The membrane on the closure plate was carefully peeled off, aspirate, and replaced with the washing solution for wash.After 30 seconds of relaxing, the wash solution was dumped.The washing process was repeated 5 times.

6. Every well except the well of blank control received 50 µ l HRP-Conjugate reagent.

7.Step 3's instructions was followed for incubation.

8.Step 5's instructions was followed for washing

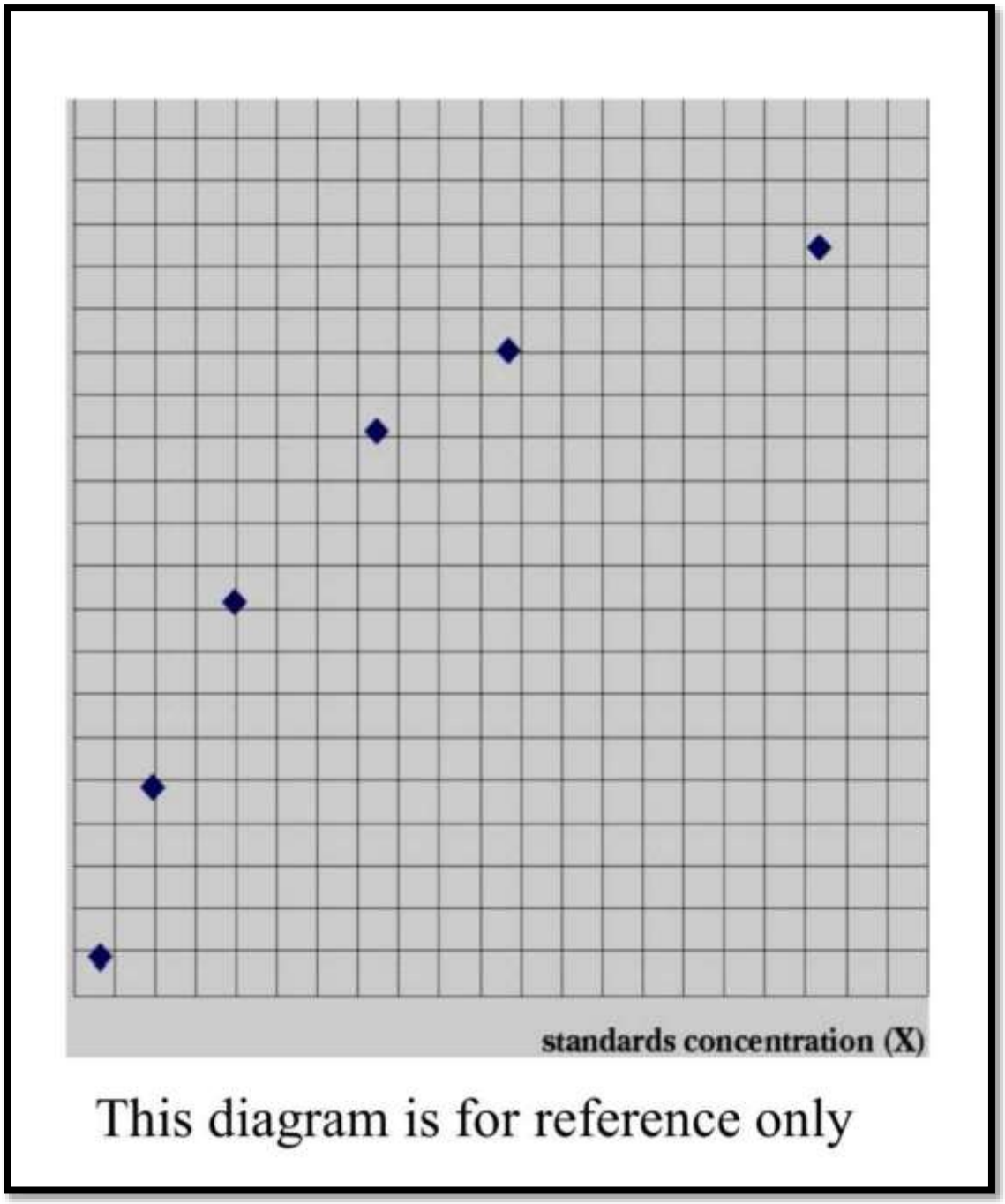
9.50 µl each of Chromogen Solution (A) and (B) were added, gently shaken, then at 37°C for 15 minutes. During the coloring process, light was avoided. useful for coloring.

10.To finish the reaction, in each well, stop solution (50 µl) was added. wells have been changed in color from blue to yellow.

11. absorption A Microtiter Plate Reader was used to read the O.D. at wavelength (450nm). The control of blank well's OD value was locate to zero. After adding the stop solution, the test was finished in 15 min.

2.6.1.3. Results Calculation

The log scale axes (x) and (y) were used to plot the known concentrations of HHV6 Ag, and it is the corresponding reading optical density. The HHV6 Ag concentrations of the samples were measured by graphing the sample's (O.D.) on the (Y) axis. Multiplying the dilution factor yielded the original concentration.



This diagram is for reference only

2.6.2. Anti-HHV-6 ELISA (IgM)

2.6.2.1. The principle

In this ELISA kit, the procedure was Sandwich-ELISA. This kit includes a Microelisa stripplate that has been pre-coated with a definite antigen for HHV6-IgM samples or standards are mixed with the specific antigen and put in the appropriate Microelisa stripplate wells.Then, all well of microelisa stripplate was treated with a HRP conjugated specific antigen for HHV6-IgM.The parts that were no longer required were rinsed away. The Substrate solution TMB was poured into every well. After the stop solution, the wells that contained HHV6-IgM that bound to HRP-conjugated HHV6 antigen exhibited a blue color before turning yellow.

By spectrophotometer, (optical density (OD)) was assayed at a (450) nm wavelength. The amount of (OD) is related to the HHV6-IgM concentration. The standard curve is the way that computes HHV6-IgM concentration in the samples and the OD value.

2.6.1.2.The procedure

1.Standards Dilution

The standard was first diluted in small tubes, then pipetted 50ul from each tube into a microplate well (each tube uses two wells for a total of ten wells).

Table(2-3):Standards dilution steps (ELISA kit for IgM)

60 ng/L	No.1 Standard	300µl Original Standard + 150µl diluent standard
40 ng/L	No.2 Standard	300µl No.1 Standard + 150µl diluent standard
20 ng/L	No.3 Standard	150µl No.2 Standard +150µl diluent standard
10 ng/L	No.4 Standard	150µl No.3 Standard + 150µl diluent standard
5 ng/L	No.5 Standard	150µl No.4 Standard + 150µl diluent standard

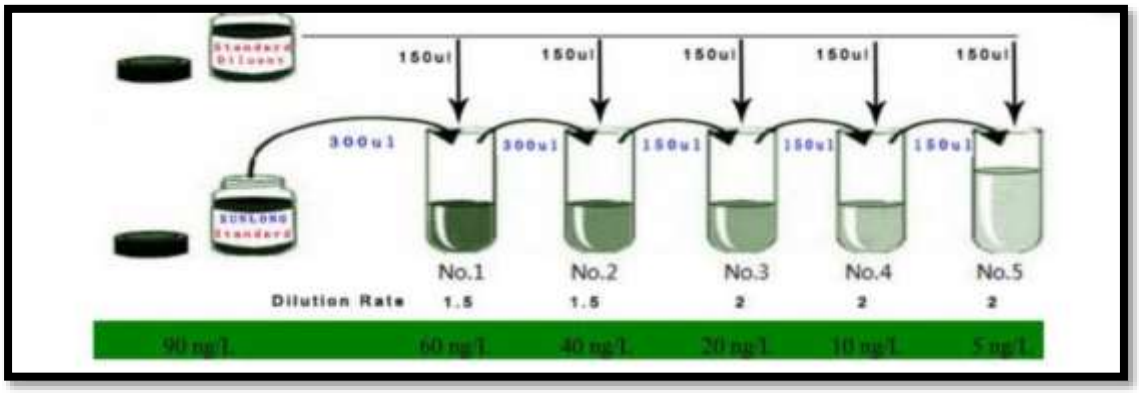


Figure 2-3: Standards dilution steps (ELISA kit for IgM)

2.As a blank control, a well on the Microelisa stripplate was left empty.40µl from sample dilution buffer and 10µl 0f sample were placed in wells (dilution factor is 5). Without hitting the well wall, samples were loaded onto the bottom. To combine, the well was gently shaken.

3.Incubation: after a closure plate membrane is added for sealing, incubate at 37°C for 30 minutes.

4. Dilution: diluted the concentrated washing buffer with distilled water (30 times for a 96T and 20 times for a 48T).

5. The membrane on the closure plate was peeled off carefully, aspirated, and washed with wash solution. The wash solution was discharged after 30 seconds of relaxation. performed process of washing 5 times .

6. Except for the well of blank control, 50 µl of HRP-Conjugate reagent was put into every wells.

7.Step 3 instructions were followed for incubation.

8.Step 5 instructions were followed for washing

9.50 µl from each solution having Chromogen A and B were added, gently shaken, then incubated (37°C for 15 minutes). During the coloring process, the use of light was avoided. useful for coloring

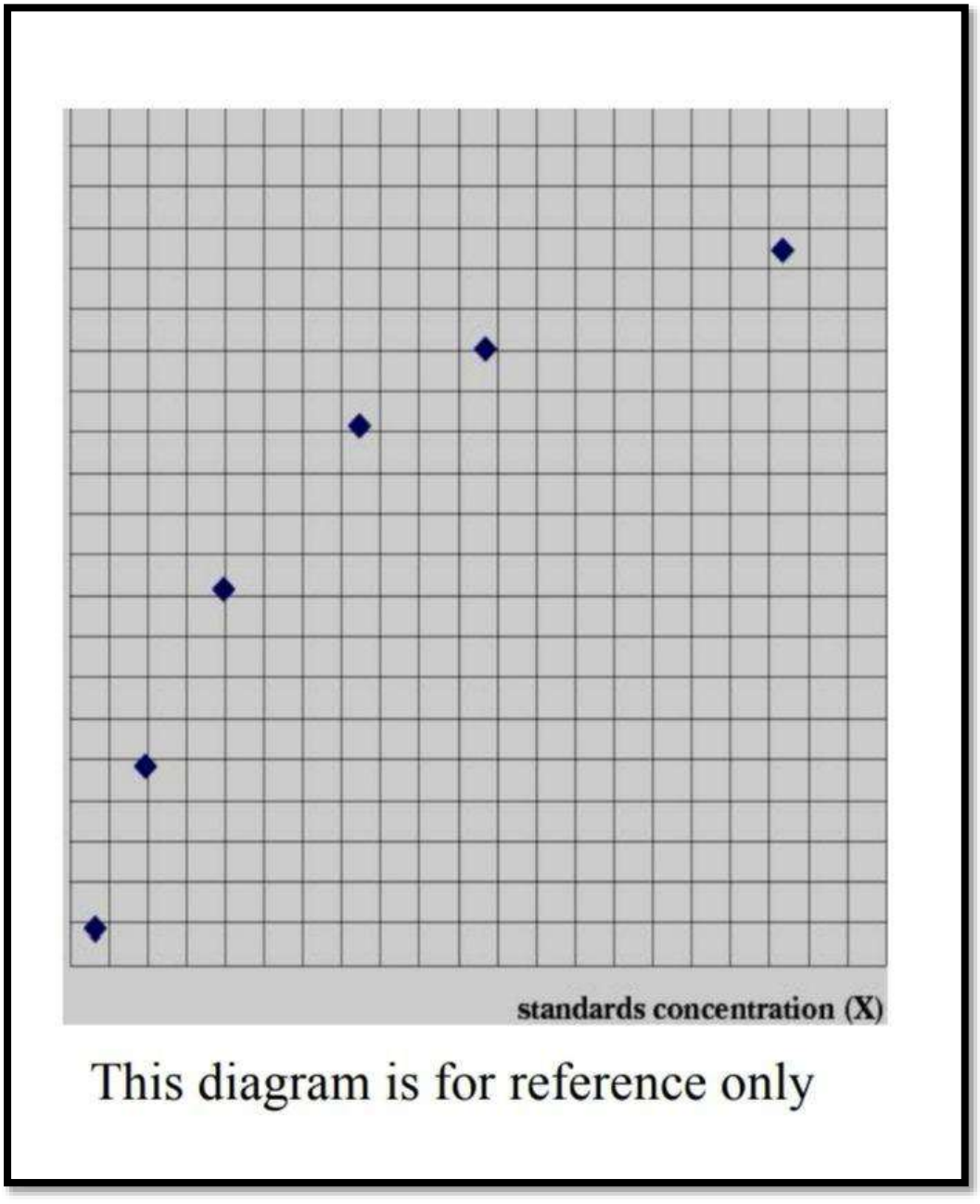
10.To finish the reaction, in each well, a stop solution (50 µl was added. wells have been changed from blue to yellow.

11.Absorption a microtiter plate reader was used to read the optical density. wavelength at 450nm.The optical density of the well of blank was established to zero. After the stop solution was added, the test finished in 15 min.

2.6.1.3.Results Calculation

The log scale axes (x) and (y) were used to plot the known concentrations of anti HHV6 IgM Standard, as well as the corresponding reading OD. The Anti HHV6 IgM, concentrations of the samples were measured by graphing the sample's (O.D.) on the (Y) axis.

Multiplying the dilution factor yielded the original concentration.



2.6.3.Anti-HHV-6 ELISA (IgG)

2.6.3.1. Principle

In this ELISA kit, the procedure was Sandwich-ELISA. This kit includes a Micro-elisa strip-plate that has been precoated with a precise antigen specific to IgG HHV-6 samples or standards are placed in the relevant wells of microelisa stripplated and mixed with the specific antibody. Later, every single microelisa stripplate well was treated with a HRP conjugated antigen specific for HHV6-IgG.The parts that were no longer required were rinsed away.The Substrate solution TMB was poured into every well. After the stop solution, the wells that contained HHV6-IgG and HRP-conjugated HHV6 antigen exhibited blue before turning yellow. By spectrophotometer, at a wavelength of (450) nm, theoptical density (OD) was considered. The rate of OD is related to the HHV6-IgG concentration. Compute HHV6-IgG concentration in the specimens by comparing the samples' OD to the typical curve.

2.6.3.2.Procedure

1.Standards Dilution

The standard was first diluted in small tubes, then pipetted 50ul from each tube into a microplate well (each tube uses two wells, for a total of ten wells).

Table(2-4):Standards dilution steps (ELISA kit for IgG)

150 ng/L	No.1 Standard	300µl Original Standard + 150µl diluent standard
100 ng/L	No.2 Standard	300µl No.1 Standard + 150µl diluent standard
50 ng/L	No.3 Standard	150µl No.2 Standard + 150µl diluent standard
25 ng/L	No.4 Standard	150µl No.3 Standard + 150µl diluent standard
12.5 ng/L	No.5 Standard	150µl No.4 Standard + 150µl diluent standard

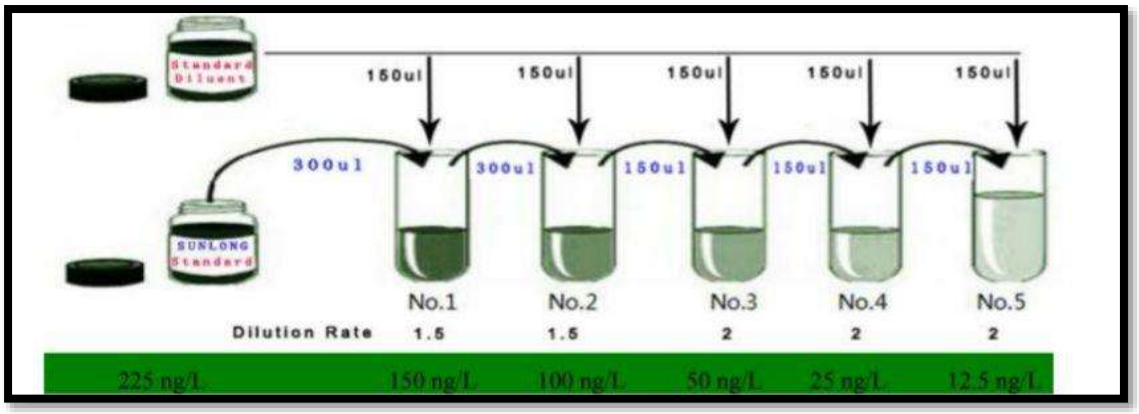


Figure 2-4: Standards dilution steps (ELISA kit for IgG)

2.As a blank control, a well on the Microelisa stripplate was left empty. The sample dilution buffer (40µl) and the sample (10µl) were placed in wells (dilution factor is 5). Without hitting the well wall, samples were loaded onto the bottom. To combine, the well was gently shaken.

3.Incubation: after closing the plate membrane for sealing, incubated at 37°C for 30 min.

4. Dilution: diluted the concentrated washing buffer with distilled water (30 times for a 96T and 20 times for a 48T).

5.The membrane on the closure plate was carefully peeled off, aspirated, and replaced with wash solution for washing. Following resting for 30 seconds, the solution of washing was discarded. A process of washing was carried out five times.

6.All wells with the exception of the blank control received 50 µl from HRP conjugated reagent.

7.Step 3 instructions were followed for incubation.

8.Step 5 instructions were followed for washing.

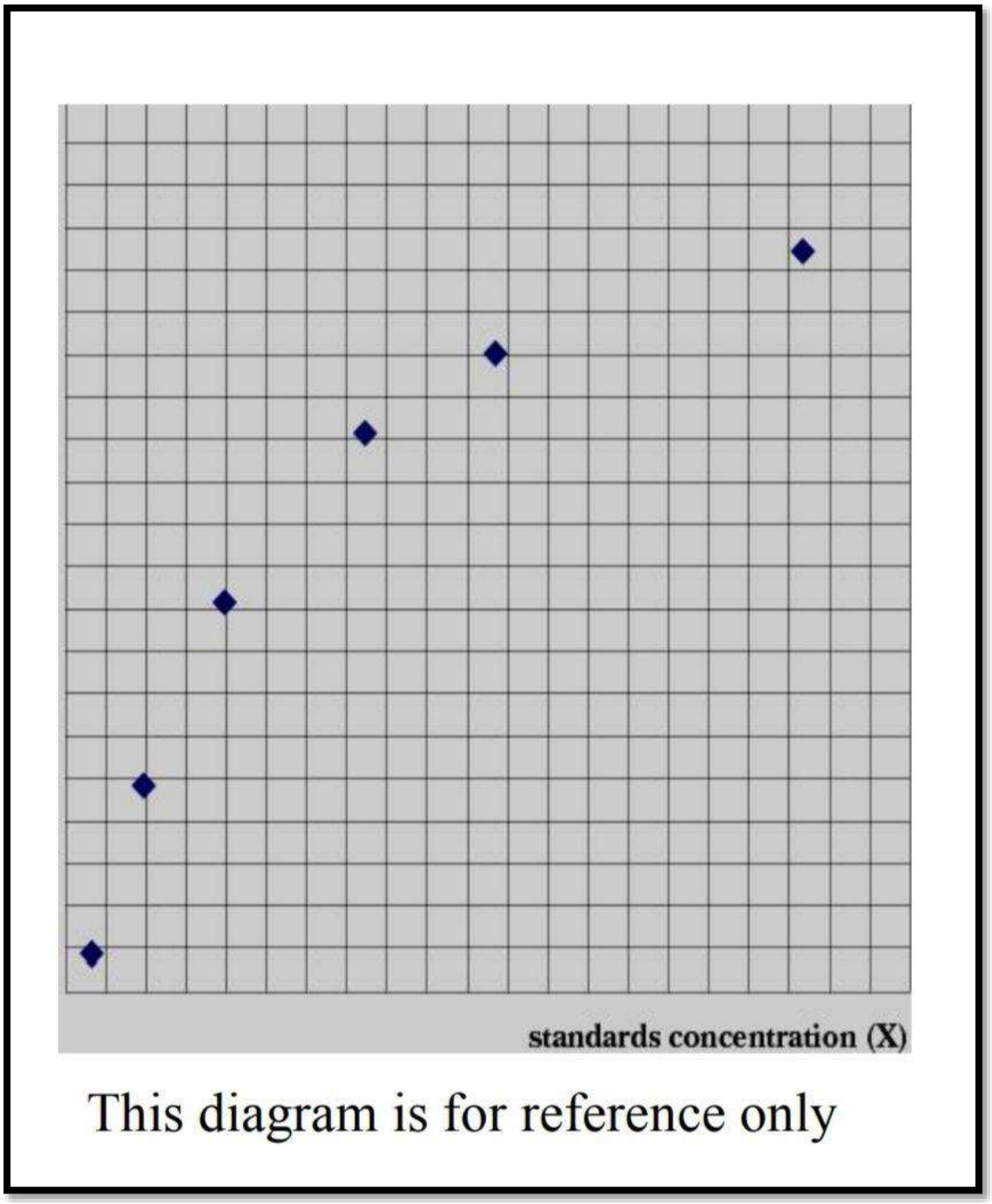
9.50µl from each Chromogen A and B solution were genitive. gently shaken, and incubated (37°C for 15 min). During the coloring process, the use of light was avoided. useful for coloring.

10.To finish the reaction, in each well, a stop solution (50 µl) was added. The wells have been changed blue color to yellow color.

11.Absorption:the reader of microtiter plate used to reading the O.D. wavelength at450nm.Optical density of well the blank control value was put zero. After adding stop solution, the test was finished in 15 min.

2.6.3.3. Results Calculation

The log scale axes (x) and (y) were used to plot the known concentrations of anti HHV6 IgG standard, as well as the corresponding reading OD. The Anti HHV6 IgG concentrations of the samples were measured by graphing the sample's (O.D.) on the (Y) axis.Multiplying the dilution factor yielded the original concentration.



2.6.4. Human Nuclear Factor Kappa B, NF-Kb ELISA

To assay Nf-kappa level in Human serum.

2.6.4.1. Principle:

In this ELISA kit, the procedure was sandwich-ELISA. This kit consists of a microelisa stripplate that has been precoated with a specific antibody that is specific to NF-Kb. The specific antibody is then added to the appropriate Microelisa stripplate wells containing samples or standards, then mixed with each others. Next, each microelisa stripplate well was treated with HRP-conjugated antibodies that were unique. The parts that were no longer required were rinsed away. The Substrate solution TMB was poured into every well. After the stop solution, the wells that contained NFKb and HRP-conjugated NFKb antibodies exhibited blue before turning yellow.

The wavelength of the spectrophotometer is 450 nm, at which OD was calculated. The value of OD is correlated with the concentration of NFKb. Calculate the concentration of NFKb in the specimen through comparison of the optical density of the sample to the standard curve.

2.6.4.2 The procedure:

1. Standards dilution

The standard was first diluted in small tubes, then pipetted 50ul from each tube into a well of a microplate (each tube uses two wells, for a total of ten wells).

Table (2-5):Standards dilution steps (ELISA kit for NFkb)

3.6 ng/L	No.1 Standard	300µl Original Standard + 150µl diluent standard
2.4 ng/L	No.2 Standard	300µl No.1 Standard + 150µl diluent standard
1.2 ng/L	No.3 Standard	150µl No.2 Standard + 150µl diluent standard
0.6 ng/L	No.4 Standard	150µl No.3 Standard + 150µl diluent standard
0.3 ng/L	No.5 Standard	150µl No.4 Standard + 150µl diluent standard

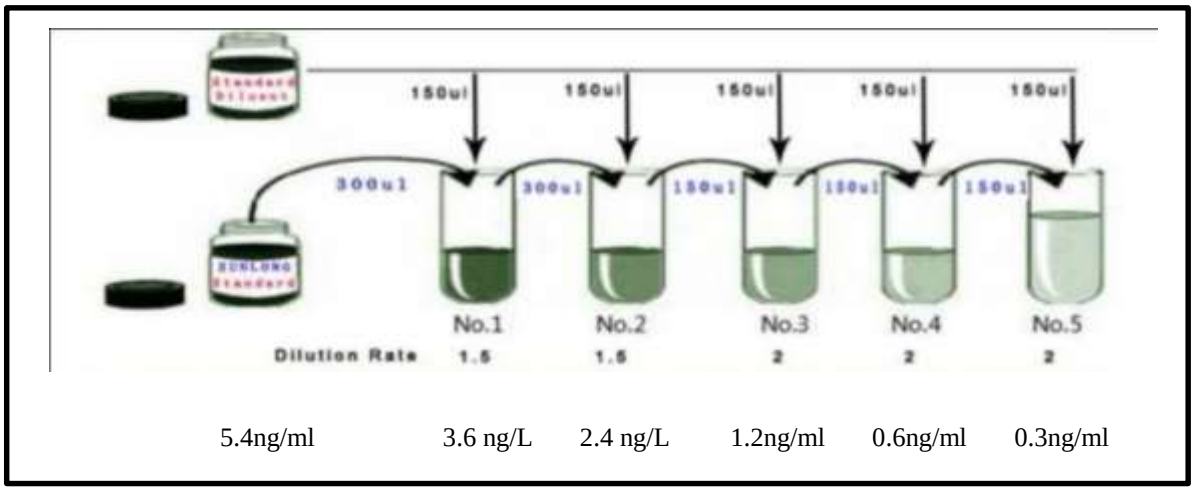


Figure (2-5): Standards dilution steps (ELISA kit for NFkb)

2.As a blank control, a well on the Microelisa stripplate was left empty.The sample dilution buffer (40µl) and sample (10µl) were placed in wells (dilution factor is 5). Without hitting the well wall, samples were loaded onto the bottom.To combine, the well was gently shaken.

3.Incubation: after the closure plate membrane is sealed, at 37°C and for 30 min incubation.

4. Dilution: diluted the concentrated washing buffer with distilled water (30 times for a 96T and 20 times for a 48T).

5. To clean the plate, we removed the membrane on the closure plate and rinsed it out before washing it with wash solution. The washing fluid spilled after 30 seconds of resting. Five sets of washing were performed.

6. put 50 µl of HRP-Conjugate reagent in each well except the blank control well.

7.Step 3 'instructions were followed for incubation.

8.Step 5's instructions were followed for washing

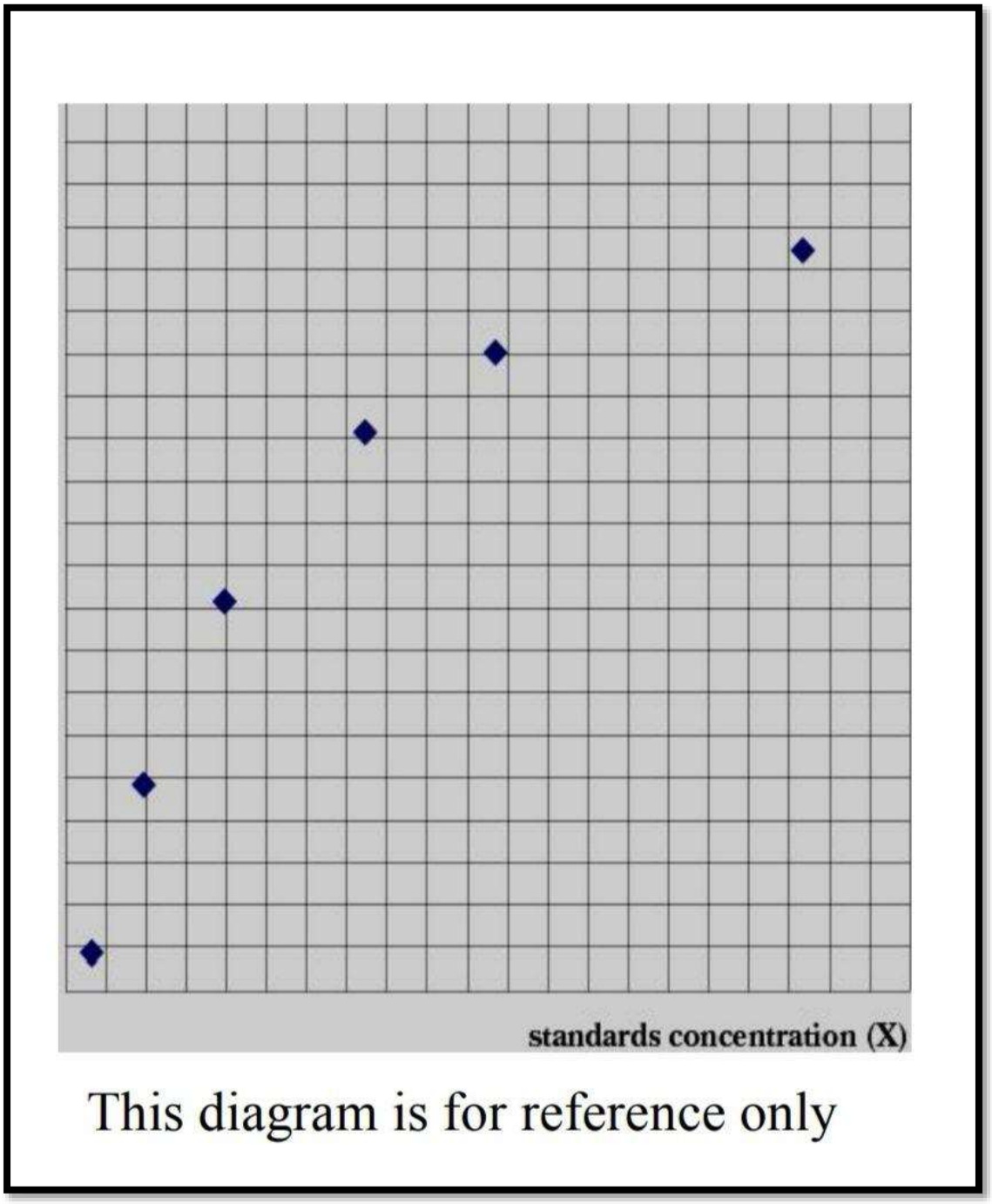
9. 5µl of each Chromogen Solution (A) and (B) were added, gently shaken, then incubated at 37°C for 15 minutes. During the coloring process, the use of light was avoided. useful for coloring.

10.To finish the reaction, in each well, 50 µl of stop solution was added. wells have been changed in color from blue to yellow.

11.absorption A Microtiter Plate Reader was used to read the O.D. at a wavelength of 450nm. The well blank control OD value was set to zero. After adding the stop solution, the test was finished in 15 min.

2.6.4.3. Results Calculation

The log scale axes (x) and (y) were used to plot the known concentrations from the NF Standard, as well as the corresponding reading OD. The NF concentrations of the samples were measured by graphing the sample's (O.D.) on the (Y) axis.Multiplying the dilution factor yielded the original concentration.



2.7.Statistical Analysis:

The package for the social sciences version 24 was used to enter, manage, and analyze data, with variables presented as mean, standard deviation, frequencies and percentage accordingly. All variables of scale (continuous) type were checked for normal statistical distribution. The Chi square test is used to evaluate the connotation between categorical variables. To assess laboratory parameters and variables . All statistical procedures and tests were applied at a level of significance of 0.05 to be significant. Using MS Word and Excel software version 2016, the results are presented in tables and figures with an explain paragraph.

Chapter three

Results

3. Results:

3.1. Demographic data of study populations

3.1.1. Past-medical history of study population

The findings of a study of the medical records of 200 patients with cardiovascular diseases revealed that 40 patients (20%) suffer from diabetes millitus(DM), while 62 cases (31%) suffer from high blood pressure (HT), as well as data contained.

According to the current study, 77 patients (38.5 %) had diabetes and high blood pressure combined, and only two cases of leukemia were observed, while the remaining 19 patients with cardiovascular diseases (9.5 %) did not have any other diseasesas shown in the table (3.1)

Table 3.1.Past-medical history of study population

Variable	Frequency	Percent (%)	Valid %	Cumulative %
DM	40	20.0	20.0	20.0
HT	62	31.0	31.0	51.0
HT, DM	77	38.5	38.5	89.5
Leukemia	2	1.0	1.0	90.5
CVD only	19	9.5	9.5	100.0
Total	200	100.0	100.0	

DM: Diabetes Mellitus, HT: Hypertension.

3.1.2. History of smoking of study population

According to the medical histories of the 200 patients with cardiovascular disease who smoked, the patients were classified into three groups:

The first group consisted of nonsmokers, with 94 patients out of 200 totaling 47%, while the second group consisted of smokers, with 93 patients totaling 46.5 %, and the third group, with 13 patients totaling 6.5 %, were formerly smokers who had stopped smoking shown in the (table 3.2).

Table 3.2. History of smoking of study population

	Frequency	Percent(%)	Valid (%)	Cumulative (%)
NO	94	47.0	47.0	47.0
Previous Smoker	13	6.5	6.5	53.5
Smoker	93	46.5	46.5	100.0
Total	200	100.0	100.0	

3.2. Distribution of patients and disease

3.2.1. Total number of patients with cardiovascular diseases

The current study comprised 200 samples of cardiovascular disease patients, with the results showing that 191 patients (95.5 %) were from ischemic heart diseases.

Only 8 patients (4%) suffered from heart failure, and only 1 case (0.5%) complained of myocardiomyopathy shown in Figure (3.1)

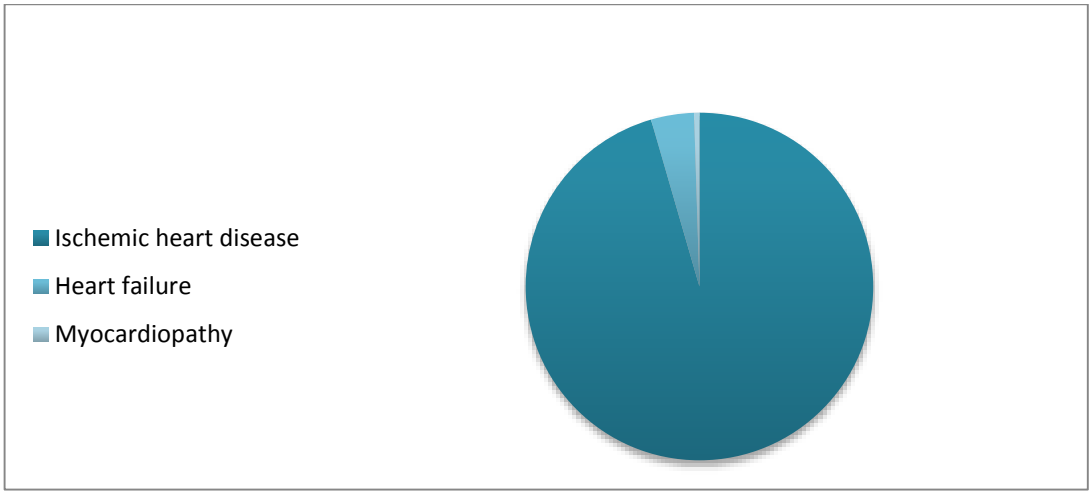


Figure (3.1): Disterbution of patients with cardiovascular diseases

3.2.2: Distribution of Total number of patients with cardiovascular diseases according to age group

Figure 3.2 shows the distribution of patients with cardiovascular disease according to age groups. Patients with cardiovascular disease were distributed into four age groups, and the results of the distribution were as follows:

The first age group (30-44) years old had 28 cases of ischemic heart disease out of 29 cases (14%), with only one case of cardiomyopathy (0.5%).

The second age group (45-59) years old had 80 patients, with 78 cases of ischemic heart disease (39%) and only two cases of heart failure (1%).

The third age group (60-74) years old had 72 cases, with 68 cases of ischemic heart disease (34%) and only 4 cases of heart failure (2%).

As for the last group, which is 75 years old and above, it included 19 cases, 17 of which were ischemic heart disease (8.5%) and only two cases were heart failure (1%) as shown in (Figure 3.2).

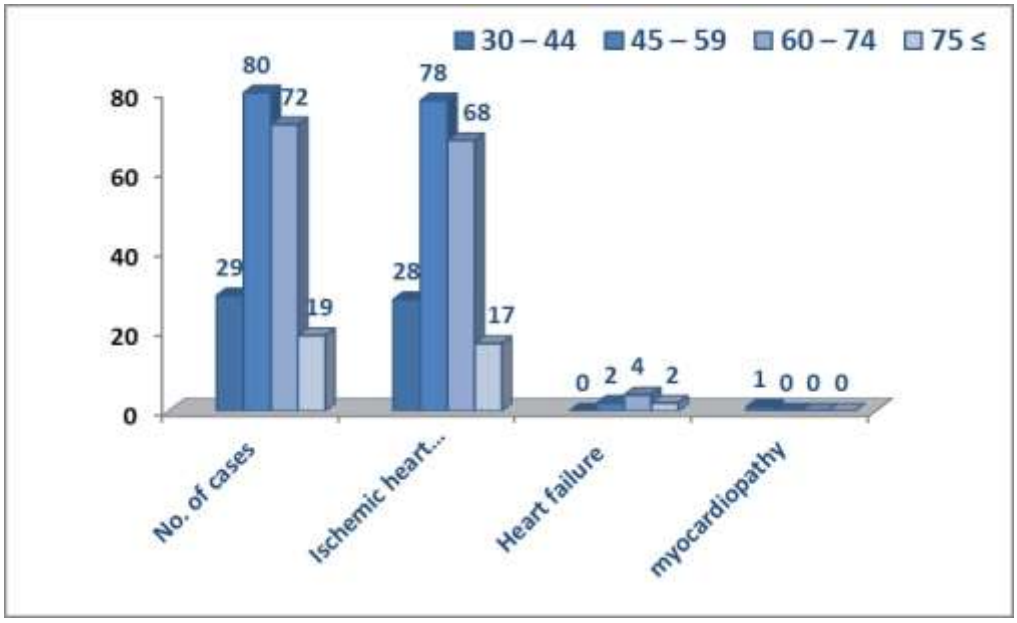


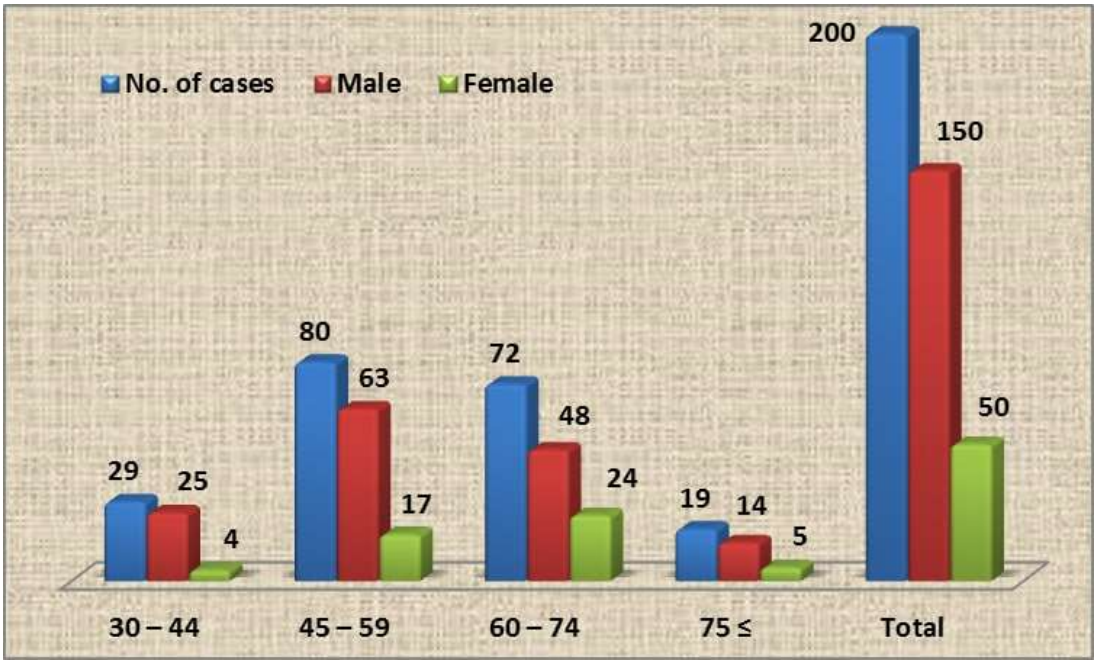
Figure 3.2: Distribution of total number of patients with cardiovascular diseases according to age group.

3.2.3:Sex distribution of cardiovascular disease in overall patients according to age groups

Cardiovascular patients included in the current study were divided into males and females, where the total number of males was 150 cases (75%) of the total number of 200 cases, while the total number of female cases was 50 cases out of 200 cases (25%), where males and females were distributed across age groups.

The Male distribution was as follows: 25 (12.5%) cases in the category (30-44) out of 29 cases, while the category (45-59) had 63 (31.5%) cases out of 80 and the group (60 -74) had 48 cases (24%) out of 72, while the last category ≤ 75 contained 14 (7%) out of 19 cases.

While the results of females were distributed as follows: 4 cases (2%) for the age group (30-44), 17 cases (8.5%) for the group (45-59) and 24 cases (12%) for the category (60-74),and the last category ≤ 75 was only 5 cases (2.5%)shown in (Figure 3.3).



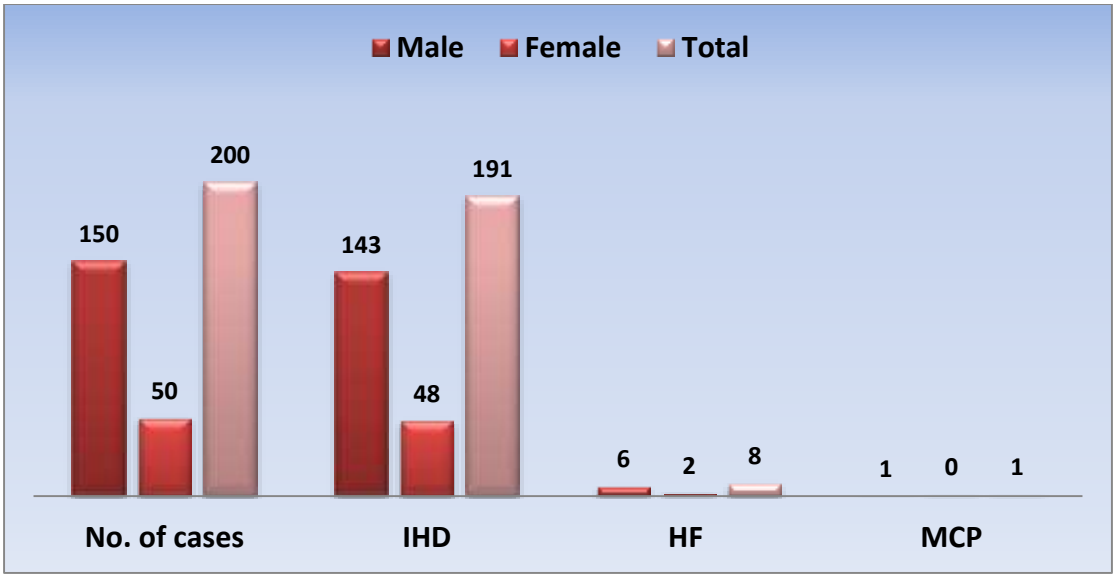
Figure(3.3): Sex distribution of cardiovascular disease in overall patients according to age groups

3.2.4 : Total patient sex distribution according to cardiovascular disease types.

When cardiovascular diseases types were distributed according to sex between males and females, the males included:

143 cases (71.5%) of ischemic heart disease, while there were 6 cases (3%) of heart failure and only one case (0.5%) of cardiomyopathy out of the total number of 150 patients.

For females,the results were: 48 cases (24%) of ischemic heart disease and only two cases of (1%) heart failure, out of a total of 50 patientsas shown in (Figure 3.4).



IHD: ischemic heart disease,HF :heart failure,MCP:myocardiopathy

Figure(3.4):Total patient sex distribution according to cardiovascular disease types.

3.3: Immunological parameters distribution among cardiovascular disease types

3.3.1: Distribution of immunological parameters according cardiovascular diseases types in HHV6-Ags positve cases

Immunological techniques were used to investigate immunological parameters such as the antigen of human herpes virus 6, Anti-HHV6 IgM & Anti-HHV6 IgG,in addition to the NF-κB factor.

The human herpes virus Ag was positive in 60 cases (30%) of these cases, 55 cases (27.5%) with ischemic heart disease, 4 cases (2%) with heart failure and only one case (0.5%) of all cases had cardiomyopathy.

The study also showed that the total cases with positive Anti-HHV6 IgM were 60 (30%), including 56 cases (28%) of ischemic heart

disease, 3 cases (1.5%) of heart failure, and only one case (0.5%) of cardiomyopathy.

Anti-HHV6 IgG positivity was recorded in 60 case (30%), these included 56 cases (28%) with ischemic heart disease, 3 cases (1.5%) with heart failure, and only one case (0.5%) with cardiomyopathy.

The study also included an investigation of the NF-κB, which revealed a total of 57 cases (28.5%), including 53 cases (26.5%) of ischemic heart disease, 3 cases (1.5%) of heart failure, and only one case (0.5%) of cardiomyopathy as shown in (Table 3.3)

Table 3.3: Distribution of immunological parameters according to cardiovascular disease types in HHV6-Ags positive cases

Parameters	No. of cases	IHD	HF	MCP
HHV6-Ags	60	55	4	1
IgM	60	56	3	1
IgG	60	56	3	1
NF-κB	57	53	3	1

3.3.2: Distribution of immunological parameters according to cardiovascular diseases types in HHV6-Ags negative cases

When patients with cardiovascular diseases were distributed according to negative HHV6-Ags, the results were as follows: zero positive cases for HHV6-Ags.

The study also showed that the total positive cases for Anti-HHV6 IgM are 3 (1.5%),all of them have IHD. Anti-HHV6 IgG revealed the presence of 20 (10%) positive cases, which were distributed among 17

cases (8.5%) with ischemic heart disease and 3 cases (1.5%) with heart failure.

The NF-κB result was 19 positive cases 16 of them (8%) with ischemic heart disease and 3 cases (1.5%) with heart failure.

Collectively,60/200 cases were positive for HHV6-Ags, 140/200 cases were negative for HHV6-Ags, 63/200 cases were positive for Anti-HHV6 IgM, 80/200 cases were positive for Anti-HHV6 IgG and lastly, 76/200 cases were positive for NF-κB shown in the (Table 3.4).

Table 3.4: Distribution of immunological parameters according to cardiovascular disease types in HHV6-Ags negative cases

Parameters	No. of cases	IHD	HF	MCP
HHV6-Ags	0	0	0	0
IgM	3	3	0	0
IgG	20	17	3	0
NF-κB	19	16	3	0

3.3.3: Anti-HHV6 IgM seropositivity & mean among study groups:

In the current study, it was observed through the results obtained, as well as the study of the correlation between the investigation of anti-HHV6 IgM and the diagnosis of viral antigen (HHV-6Ag):

Among the 63 positive samples for Anti-HHV6 IgM testing, there were 60 cases (30%) that gave a positive result on the HHV6-Ags test, while only 3 cases (1.5%) gave a positive result 0n the Anti-HHV6 IgM test. In regards to the remaining samples, the study found that 0 (0%) of the negative samples for the Anti-HHV6 IgM test had a result from the HHV6-Ags test, and 137 cases (68.5%) were negative for both Anti-HHV6 IgM and the presence of HHV6-Ags in the samples.

Table 3.5 also shows that highly significant results for Anti-HHV6 IgM values were present among patients with positive HHV6-Ags with p-value< 0.00001.

Also, patients with positive HHV6-Ags had highly significant mean levels of Anti-HHV6 IgM as shown in (Table 3.5).

Table 3.5: Anti-HHV6 IgM seropositivity & mean among study groups

Parameter	Anti-HHV6 IgM +ve	Anti-HHV6 IgM –ve	Total	P-value
HHV6-Ags +ve	60 (30%)	0 (0%)	60 (30%)	**< 0.00001***
HHV6-Ags –ve	3(1.5%)	137 (68.5%)	140 (70%)	
Total	63(31.5%)	137 (68.5%)	200 (100%)	
Mean± SD **(ng/L)**	1.05±0.77	0.18±0.12		**< 0.00001***

Chi-square test, Student T-test: +ve: positive, -ve: negative, N: number, SD: standard deviation, HHV6: human herpes virus 6, Ag: antigen, P: probability, *: highly significant, Significant at p < 0.05

3.3.4. Anti-HHV6 IgG seropositivity & mean among study groups

Concerning the study of the correlation between the results of the HHV6-Ags test and the Anti-HHV6 IgG test, it was discovered that out of the total number of positive samples for the Anti-HHV6 IgG test, 80 (40%), there were 60 positive cases for the HHV6-Ags test (30%) and 20 negative cases for the HHV6-Ags test (10%).

Out of the total number of negative samples for the Anti-HHV6 IgG test, the findings showed 0 positive cases (0%) for the HHV6-Ags assay and 120 (60%) negative cases for the HHV6-Ags test.

In addition to the above, Table 3.6. shows that highly significant results for Anti-HHV6 IgG positive values were present among patients with positive HHV6-Ags with p-value< 0.00001. Also, patients with positive HHV6-Ags had highly significant mean levels of Anti-HHV6 IgG as shown in (Table 3.6).

Table 3.6. Anti-HHV6 IgG seropositivity & mean among study groups

Parameter	Anti-HHV6 IgG +ve	Anti-HHV6 IgG –ve	Total	P-value
HHV6-Ags +ve	60 (30%)	0 (0%)	60 (30%)	**< 0.00001***
HHV6-Ags –ve	20 (10%)	120 (60%)	140 (70%)	
Total	80 (40%)	120 (60%)	200 (100%)	
Mean± SD **(ng/L)**	0.63±0.28	0.15±0.07		**< 0.00001***

Chi-square test, Student T-test: +ve: positive, -ve: negative, N: number, SD: standard deviation, HHV6: human herpes virus 6, Ag: antigen, P: probability, *: highly significant, Significant at p < 0.05

3.3.5. NF-κB level among study groups

The association between the findings of the factor study (NF-κB) and the antigen was also investigated.

It was found that out of the total number of positive samples for HHV6-Ags test 60, there were 57 positive cases for the NF-κB test (28.5%) and 3 negative cases for the NF-κB test (1.5%) among positive cases for HHV6-Ags testing,

While the remaining negative cases for HHV6-Ags testing yielded 19 positive cases for NF-κB (9.5%) and 124 (62%) negative cases.

Highly significant results for positive NF-κB levels were found in patients with positive HHV6-Ags compared to those with negative

HHV6-Ags with p-value< 0.00001 as demonstrated in table 3.7. Also, patients with positive HHV6-Ags had higher mean levels of NF-κB as shown in **(Table 3.7).**

Table 3.7. NF-kappa-B seropositivity & mean among study groups

Parameter	NF-κB +ve	NF-κB -ve	Total	P-value
HHV6-Ags +ve	57 (28.5%)	3 (1.5%)	60 (30%)	< **0.00001***
HHV6-Ags –ve	19 (9.5%)	121 (60.5%)	140 (70%)	
Total	76(38%)	124 (62%)	200 (100%)	
Mean± SD **(ng/L)**	0.76±0.43	0.27±0.11		< **0.00001***

Chi-square test, Student T-test,: +ve: positive, -ve: negative, N: number, SD: standard deviation, HHV6: human herpes virus 6, Ag: antigen, NF: nuclear factor, P: probability, *: highly significant, Significant at p < 0.05

3.3.6. Association between NF-κB Anti-HHV6 IgM and Anti-HHV6 IgG

The table 3.8 included a statement of the relationship between the presence of NF-κB,Anti-HHV6 IgM and in patients with cardiovascular disease:

The results showed that out of the total number of samples positive for Anti-HHV6 IgM examination of 63 cases, 57 cases were positive for the NF-κB (28.5%) and only 6 (3%) gave a negative result.

Only the findings of the investigation into NF-κB yielded 19 positive results (9.5 %) and 118 negative results (59%) out of the total number of negative results for the Anti-HHV6 IgM test.

The results of the search for the statement of the relationship between this table and the presence of NF-κB and Anti-HHV6 IgG in cardiovascular

patients revealed that among the total samples positive for Anti-HHV6 IgG examination of 64 cases,

There were 57 cases positive for NF-κB (28.5%) and of the remaining samples, only 7 (3.5%) gave a negative result.

Only the findings of the study for NF-κB ,19 gave a positive result (9.5%) and 117 (58.5%) a negative result out of the total number of negative results for the Anti-HHV6 IgG test.

Highly significant association between positive results for both Anti-HHV6 IgM & Anti-HHV6 IgG with NF-κB as shown in table 3.8.

Table 3.8: Association of NF-κB & Anti-HHV6 IgM and Anti-HHV6 IgG

Parameter	NF-κB +ve	NF-κB -ve	Total	P-value
Anti-HHV6 IgM +ve	57(28.5%)	6 (3%)	63 (31.5%)	**<0.0001***
Anti-HHV6 IgM –ve	19 (9.5%)	118 (59%)	137 (68.5%)	
Total	76 (38%)	124 (62%)	200 (100%)	
Anti-HHV6 IgG +	57 (28.5%)	7 (3.5%)	64 (32%)	**<0.0001***
Anti-HHV6 IgG –ve	19 (9.5%)	117 (58.5)	136 (68%)	
Total	76 (38%)	124 (62%)	200 (100%)	

Chi-square test: +ve: positive, -ve: negative, N: number, SD: standard deviation, HHV6: human herpes virus 6, NF-κB: nuclear factor, P: probability, *: highly significant, Significant at p < 0.05

3.3.7. Association between type of cardiovascular diseases and study markers:

The study involved the analysis of immunological biomarkers in three categories of total cardiovascular diseases including 200 patients: 191 cases of ischemic heart disease, 8 cases of heart failure, and only one case of cardiomyopathology.

According to the findings from the investigation of immune markers were distributed to the three types of cardiovascular diseases, with the study revealing that the positive results of the HHV6-Ag test were 55 cases in ischemic heart diseases patients and 136 negative cases, and only 4 positive cases in heart failure patients, with the remaining 4 cases giving a negative result ,whereas myocardiomyopathy had just one positive case.

The findings of the Anti-HHV6 IgM examination were distributed as follows:

Only 59 ischemic heart disease patients are positive, while the remaining 132 are negative; only 3 heart failure patients are positive, whereas the remaining 5 are negative; and only one myocardiomyopathy is positive.

The findings of the Anti-HHV6 IgG test were distributed as follows:

The ischemic heart disease patients revealed 73 cases are positive, whereas the remaining 118 are negative; only 6 heart failure patients are positive, while the remaining 2 are negative; and only one myocardiomyopathy patient is positive.

In addition to the above mentioned findings, the study also included an analysis of the NF-κB in cardiovascular patients, with the findings revealing the following, based on its distribution among the three categories of heart patients:

In ischemic heart disease patients, 69 cases are positive and the remaining 122 cases are negative; in heart failure patients, only 6 cases are positive and the remaining 2 cases are negative; and lastly, only one case in myocardiomyopathy disease.

Accordingly, the table 3.9. has been shown no significant association between different types of cardiovascular diseases in study population and positivity of study markers with p-value> 0.05.

Table 3.9: Association between types of cardiovascular diseases and study markers

Markers / CVD	IHD	HF	MCP	Total	P- value
HHV6-Ags +ve	55	4	1	60	0.2
HHV6-Ags -ve	136	4	0	140	
Anti-HHV6 IgM +ve	59	3	1	63	0.8
Anti-HHV6 IgM –ve	132	5	0	137	
Anti-HHV6 IgG +ve	73	6	1	80	0.1
Anti-HHV6 IgG -ve	118	2	0	120	
NF-κB +ve	69	6	1	76	0.2
NF-κB -ve	122	2	0	124	

Chi-square test: +ve: positive, -ve: negative, N: number, HH6: human herpes 6, Ag: antigen, NF-κB: nuclear factor, P: probability, Significant at p < 0.05

Chapter Four

Discussion

4. Discussion

4.1. Study populations' demographic and clinical information

The existence of risk factors, such as chronic diseases like diabetes and high blood pressure, is one of the most prominent risk factors linked with cardiovascular diseases, and the pathological condition of patients with cardiovascular diseases is worsened by the presence of these two factors.

Fuchs *et al*., (2020)mention that, preventing age-related increases in blood pressure would reduce the vascular complications commonly associated with aging, and when combined with thorough treatment of established hypertension, it would reduce a major percentage of the community incidence of BP-related cardiovascular disease.

This study included 200 patients. As shown in table 3.1, among these patients, it was found that 40 (20 %) patients suffer from diabetes, while it was found that there were 62 (31 %) patients suffering from hypertension. The study also documented the presence of 77 (38.5%) patients with both diabetes and hypertension. The remaining of patients with cardiovascular diseases, which is 19 patients out of 200 patients, were not suffering from such chronic diseases.

The present study is in correspondence with studies done by Fuchs *et al*.,(2020) & Wu *et al*.,(2015), which give insight into determining the appropriate blood pressure for older people's survival, as well as expanding awareness of the effects of hypertension on mortality risks in older adults and women. In older people, hypertension was linked to an increased risk of death from all causes, cardiovascular, and extensive cardiovascular disease.

In the present study, the patients with cardiovascular disease were organized into three groups based on their histories of smoking, as in table 3.2:

In the first group, the patients were non-smokers (94 patients, 47%), while in the second group, the patients were smokers (93 patients, 46.5%) and in the third group, which had only 13 patients, all of them former smokers (6.5%).

Gupta *et al*., (2019); Anand *et al*., (2017) and Siddiqi *et al*., (2015) who refered to Smoking and years of smoking have harmful effects on the heart and cause a significant increase in heart problems for each year of continued smoking. Cigarettes and other tobacco products are considered to be equally dangerous because they all contain nicotine, and causes cardiovascular problems than those who smoke less or who started recently.

Catlin *et al*., (2009) study involved cigarette smoke is made up of multiple particles, and each of those particles contains nicotine, a dangerously addictive substance that increases heart rate, hypertension, and heart contractility.

Csordas *et al*.,(2013) concluded that Heart disease is caused by total aerosol residue (tar), which influences vascular inflammation, endothelial damage (blood vessel lining), blood clot formation, and HDL cholesterol reduction levels.

These findings correspond with other studies, such as the study done by Gallucci *et al*., (2020). Smoking has long been shown to cause problems with endothelial function, even though the specific mechanisms by which this occurs are not yet well understood. Smoking triggers

oxidative processes and has negative effects on platelet function, fibrinolysis, inflammation, and blood vessel function.

Similar data, such as Carter *et al*.,(2015), recorded by other articles, showed that smokers had mortality rates two to three times higher than nonsmokers.

4.2. Distribution of patients with cardiovascular diseases

According to (WHO, 2019) , CVDs are the largest cause of death worldwide, killing an estimated 17.9 million people every year. Coronary heart disease, cerebrovascular illness, rheumatic heart disease, and other disorders are disorders of the heart and blood vessel known as CVDs. Heart attacks and strokes are responsible for more than four out of every five CVD deaths, with one-third of these deaths occurring before the age of 70.

According to figure 3.1 in this study, of the 191 patients, 95.5% were patients with ischemic heart disease, only 4% of the patients suffered from heart failure, while 1 case with cardiomyopathy was reported. Ischemic heart disease involving angina (stable and non-stable) and myocardial infraction (MI). This distribution goes with global epidemiology of CVD as mentioned by WHO in which Ischemic heart disease is more common

Curtis *et al*., (2018) say that age has a crucial influence in the development of cardiovascular complications in older individuals, making it more susceptible to cardiovascular disease (CVD).

Patients with cardiovascular disease were divided into four age groups, as shown in figures 3.2. as follows:

There were 28 cases of ischemic heart disease out of 29 cases (14%) in the first age group (30-44) years, with just one case of cardiomyopathy (0.5 %).

There were 80 patients in the second age group (45-59 years old), with 78 cases of ischemic heart disease (39%) and just two cases of heart failure (1 %).There were 72 patients in the third age group (60-74) with 68 cases of ischemic heart disease (34%) and only 4 occurrences of heart failure (2 %).

The last group, consisting of those aged 75 and over, included 19 instances, 17 of which were ischemic heart disease (8.5 %), and only two had heart failure (1%).

In the current study, patients with cardiovascular diseases were divided into two groups: males and females. The total number of males was 150 cases (75 percent) of the total number of 200 cases, while the total number of females was 50 cases (25%) of the total number of 200 cases. The male and female patients were divided into four age groups, with the males and females being evenly distributed.

The following distribution of males: Females were represented by 25 cases in the category (30-44) out of 29 cases, representing 12.5 percent of the total number of cases in this category, while the category (45-59) contained 63 cases out of 80 cases (31.5 percent), and the group (60-74) contained 48 cases out of 72 (24 percent).

While the last category, 75, contained 14 (7%) out of 19 cases, with the following distribution of females: 4 cases (2%), 17 cases (8.5%), and 24 (12%) and 5 (2.5%) as shown in Figure (3.3.).

The above findings are in agreement with the American Heart Association (AHA): Yazdanya and Newman, (2009). The report found that 40% of males and females between 40 and 59 years old have CVD, while those aged 60–79 have an incidence of 75%, and those more than 80 years old have a CVD incidence of 86%.

Andersson and Vasan's review in (2018) found that CVD rates are rising among younger adults, and this increase is likely a result of a high incidence of obesity and a lack of exercise and good food.

Study done by Benjamin *et al*. (2019), showed over the age of 60 to 79 years,the CVD rate was 77.2% for males and 78.2% for females, and adults who are over 80 years old were reported to have 89.3% of men and 91.8% of women suffering from CVD.

It's proven by Benjamin *et al*. (2019)that older males are more likely to develop coronary heart disease (CHD) than females of the same age, and, furthermore, among patients ages 60-79, 11.5% of men who have a myocardial infarction (MI) are diagnosed, while only 4.2% of women who have a heart attack are diagnosed.

In the Gao *et al*., (2019) study, the occurrence of CVD in female was shown to generally be lower than in male, but female still has an even worse prognosis and higher mortality rates after an acute cardiovascular infection.

According to a study by Villa *et al*., (2015) females are somewhat protected against cardiovascular disorders before menopause, but their risk for cardiac disease rises after menopause. The reduction of sexual hormones has been proven to have a key role in the development of CVD in both males and females as they become older.

When cardiovascular diseases were distributed between males and females based on gender according to Figure 3.4, out of a total of 150 patients, there were 143 cases (71.5%) of ischemic heart disease, 6 cases (3%) of heart failure, and only one case (0.5%) of cardiomyopathy in the men's group.

Out of a total of 50 patients, 48 cases (24 %) of ischemic heart disease and just two cases (2%) of heart failure were found among females.

A Pimple *et al*.,(2018) showed that women with stable ischemic heart disease were more likely than males to experience angina symptoms, according to a study of 950 individuals. Furthermore, under the influence of emotional stress, women were twice as likely to suffer ischemia in this group of patients. In the male population, no such change was found.

Women with Ischaemic heart disease are more likely to experience angina under stress than men. In this line, Pimple *et al*., (2018) suggests that females have a higher probability of suffering from angina than males with Ischaemic heart disease. Mental stress is causing CAD, and this role is known as mental stress induced myocardial ischemia (MSIMI).

4.3: Distribution of immunological parameters among cardiovascular diseases

Immunological parameters such as the antigen of human herpes virus 6, Anti-HHV6 IgM & Anti-HHV6 IgG, and the NF-κB factor were investigated using immunological techniques.

The human herpes virus Ag was found to be positive in 60 cases (30%). The study also revealed that there were 60 patients (30%) with positive Anti-HHV6 IgM, with 56 cases (28%) of ischemic heart disease, 3 cases (1.5%) of heart failure, and only one case (0.5%) of cardiomyopathy.

Anti-HHV6 IgG positive was found in 60 patients (30%), with 56 cases (28%) having ischemic heart disease, three cases (1.5%) having heart failure, and only one case (0.5%) having cardiomyopathy.

The NF-κB was also investigated, and 57 patients (28.5%) were found to be positive, with 53 cases (26.5%) of ischemic heart disease, 3 cases (1.5%) of heart failure, and only one case (0.5%) of cardiomyopathy as shown in table 3.3.

Caruso *et al*., (2002) who showed infection with HHV-6 induces prolonged cardiac damage due to direct cytopathic effects or the production of low-grade inflammation with the release of cytokines that change cell signaling pathways or modify extracellular matrix components also influence components of the extracellular matrix.

Rotola *et al*., (2000) explained that HH6 has tropism for CD4+T cells and CD8+ T cells, B cells, and NKcells, which are among the major reservoirs for viral latency and reactivation of the virus.

Nam Leong *et al*.,(2007) study showed that HHV-6 can insert its own genome into human telomeres of the chromosomes, making HHV-6 transmittable through germline.

Kühl *et al*.,2015 study showed that of the 1656 cardiac tissues of patients suffering from cardiovascular disease, 273 of them (16.5%) had HHV-6 identified by polymerase chain reaction.

Ashrafpoor *et al*., (2013) , Tatrai *et al*., (2011) and Kühl *et al*.,(2005) who showed in both immunocompromised and immunocompetent individuals, HHV-6 infection has been shown to cause cardiac worsening and complications, such as myocarditis, DCM, and sinus tachycardia (Nishimoto *et al*., 2012).

Simpson *et al*., (2016) who showed that blood PCR tests revealed that 80 percent of 21 immunocompetent babies with myocarditis were positive for a cardiotropic virus (of which 21 two were HH6 positive) compared to only 4% of 22 healthy controls.

Comar *et al*.,(2009) study showed that in an analysis of tissue samples from children with DCM or congenital cardiac disorder, 43% of them were infected with HHV-6.

In cases of immune suppression or ciHHV-6, the serologic profile may be unusual. Antibodies to four human betaherpesviruses, namely, HHV-6A, HHV-6B, HHV-7, and HCMV, are known to be cross-reactive.

Toyabe *et al*., (2002) study reported the baby was aged 9-month-old and had active HHV6 infection revealed that the patient had elevated IgM and IgG antibodies to HHV-6 and viral DNA was found in the patient's peripheral blood and in the mononuclear cells of their blood.

Baldwin and Albert , (2001) was reported that NF-κB has been shown to be involved in a wide range of cellular processes related to autoimmunity and inflammation, and it is regulate several genes related to atherosclerosis. Several human illnesses and abnormal conditions, such as asthma, atherosclerosis, stroke, and IBD (inflammatory bowel disease), are associated with the improper activation of NF-κB.

Research by Fiordelisi *et al*., (2019) was found that inflammation is the most important component in cardiovascular disorders. Atherosclerosis is absolutely dependent on it, and it raises the risk of a heart attack or stroke. NF-κB is involved in the development and progression of both inflammation and cardiac and vascular destruction.

Jones *et al*.,(2005) explained that in animal research has been done on NF-κB inhibition in Cardiovascular diseases. Blocking the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in rodents diminishes myocardial infarct size after ischemic/reperfusion injury.

Research by Sorriento *et al*., (2015) discovered that NF-κB, a known inflammatory cytokine regulator, activates genes associated with multiple heart diseases such as cardiac remodeling and heart failure. Many cardiac conditions, such as during acute ischemia and reperfusion, activate NF-κB.

Valen *et al*., (2000); Li *et al*., (2001); Valen and Guro, (2004); Siednienko *et al*., (2007) who showed that linked NF-κB to unstable angina inflammation, HF related inflammation, including.

Kumar *et al*., (2013) concluded that infections with β–herpes viruses causes pattern recognition receptors to activate and interferon regulatory factors to stimulate the production of inflammatory cytokines, chemokines, and type I interferons to be produced as a result of the activation of NF-κB and other signaling pathways.

From the author's point of view, the elevation of serum levels of NF-kB could be attributed to HHV6 virus infection in addition to the inflammatory process of heart tissues.

Tousoulis *et al*.,(2016) evidence demonstrates NF-κB–dependent mechanisms seem to be behind chronic diseases that have consistently high CK levels. Quercetin treatment reduces the expression of the IkBα gene, and therefore reduces the amount of IL-1β in the blood.

Studies by Vicentini *et al*., (2011) and Panicker *et al*., (2010) have shown that quercetin's potential benefits in protecting against atherosclerosis reduce inflammation , due to the inhibition of NF-κB and AP-1 activation by quercetin.

Fan *et al*., (2015) and Intayoung *et al*., (2016) both demonstrated that TNF-α can induce NF-κB activation, which induces transcription of various proteins, involved in survival of cells, including anti-apoptotic factors. It has been proven that an anti-inflammatory effect can be achieved by inhibiting the NF-κB and AP-1 pathways.

Yan *et al*., (2015) and Youn *et al*., (2014) show there has been evidence of how the leukocyte adhesion mechanism in inflammatory lesions activates NF-κB and AP-1(activator protein-1), causing ICAM-1(intracellular adhesion molecule-1) and VCAM-1 (Vascular Cellular Adhesion Molecule 1) to be expressed.

In the study by Hasegawa *et al*.,(2012) showed that the activation of NF-κB leads to inflammation in metabolic and age-related diseases. An examination performed showed that inhibiting endothelial NF-κB activity extended the lifespan of mice and improved obesity-related endothelial insulin resistance. Transgenic mice with endothelium-specific overexpression of IkBα were protected against glucose intolerance in fatty tissue and skeletal muscle because of its inhibitory nature.

The Tas *et al*., (2009) who discovered that NF-κB, as well as being a powerful mediator of age-induced myocardial inflammation and fibrosis, also induces changes in the heart. Thus, in order to minimize hypertrophy and remodeling of the heart, NF-κB should be suppressed using short hairpin p65 RNA. This should be done by direct gene delivery.

The present data reveals a total of 140 cases were negative to HHV6-Ags. Of these 140 cases, three cases have positive Anti-HHV6 IgM , all of them have IHD. Anti-HHV6 IgG testing confirmed the presence of 20 positive cases (10%), with 17 (8.5%) having ischemic heart disease and 3 (1.5%) having heart failure. Table 3.4

A positive NF-κB result was found in 19 cases (9.5%), 16 of which (8%) had ischemic heart disease and 3 instances (1.5%) had heart failure.

Recent research notes that the presence of IgM and IgG antibodies to the human herpes virus decreases in the absence of the viral antigen, as well as in the presence of NF-κB. The explanation for this seropositivity could be cross-reaction or in the latent phase for IgG antibodies.

While looking at the correlation between the results of the HHV6-Ags test and the Anti-HHV6 IgG test, it was discovered that out of the total number of positive samples for the Anti-HHV6 IgG test, there were 60 positive cases for the HHV6-Ags test (30 percent) and 20 negative cases for the HHV6-Ags test (10 percent).

Positive instances for the HHV6-Ags assay were found in 0 percent of the total number of negative samples for the Anti-HHV6 IgG test, while negative cases for the HHV6-Ags test were found in 120 cases of the total number of negative samples for the Anti-HHV6 IgG test.

Moreover, Table 3.6 demonstrates that patients with positive HHV6-Ags had very significant results for Anti-HHV6 IgG positivity, with a p-value less than 0.00001, as demonstrated by the results of the study. Additionally, patients who tested positive for HHV6-Ags had significantly higher mean levels of Anti-HHV6 IgG than the control group. The p-value is < .00001. The result is significant at $p < .05$.

The findings of this current study demonstrate that the differences between the two groups are highly significant when analyze the results using the chi-square test, which compares groups based on their differing presence of viral antigens.

This first group has noticeably higher levels of antibodies IgM and IgG, as well as NF-κB than the second group.

As compared to the second group of the current , which did not include any viral antigens, there was a significant drop in the proportions of these immunological markers in this group as a result of the absence of viral antigens in this group.

We find that there are no significant differences between the immune factors when comparing them within a single group, and that the link between them is a direct one; for instance they rise simultaneously, which may imply that the immune factors are related to virus generation.

The presence of the viral antigen, on the other hand, causes the factors to interact with one another, and the viral antigen may have a direct or indirect influence on the other immune factors. Same holds true for those who do not have the viral antigen present in their blood.

We discover that the quantities of the immunological factors are lower and that they are also more closely related to one another.

When the results of the Freitas *et al*., (2003) seroprevalence study are subdivided by gender, females 60.6% and males 55.7%, people have similar rates. Seventy-seven (23.8%) patients were anti HH6-IgM and anti HH6-IgG positive, with positivity rates of 29.7% for females and 17.7% for males ($p = 0.0007$). Active infections (IgM+ and/or IgG+ high levels of specific antibodies plus detection of viral DNA) were diagnosed in 20/77 (20.0 percent) and 8/43 (18.6%) of the 120 people with high HHV-6 antibody levels. In the IgM+ and IgG+ groups, the rates of HHV-6 DNA detection were similar in female and male patients ($p > 0.05$): 20.4 percent versus 35.7 percent and 25.0 percent compared to 13.0 percent, respectively. For patients whose serum samples were IgM+, HHV-6 DNA was discovered at rates ranging from 7.7% (females aged five years) to 80.0 percent (females aged 41-50 years and males aged 11-20 years old).

Serological studies by Agut *et al*., (2015) in individuals with primary infection demonstrate the development of specific IgM antibodies in the first week and then elimination after a month, but IgG antibodies are found later than IgM antibodies but survive indefinitely, .In people who are already seropositive for HHV-6, reactivation or reinfection with the virus can happen.

The study by Flamand *et al*., (2010) found that the temporary presence of Ig-specific antibodies provides important confirmation of the first infection of a naive subject by HHV-6.

The association between the results of the HHV6-Ags test and the Anti-HHV6 IgG test was declared that the total number of positive samples for the Anti-HHV6 IgG test was 80 (40%), 60 were positive for

the HHV6-Ags test (30%), and 20 were negative for the HHV6-Ags test (10%).

Agut *et al*., (2015) who showed that large increases in IgG and the presence of IgM antibodies may not indicate an acute primary infection, as it is possible due to reactivations of the virus.

Research conducted by Becerra *et al*., (2014) discovered that there was a correlation between a certain anti-HHV6 maternally derived IgG and protection of infants from HHV6 infection. The anti-HHV6 IgG levels are significant because they dropped off after five months.

Anti-HHV-6 IgG has been used as a marker of both past and latent infection, and various studies have come up with varied results.

Study done by Bhattarakosol *et al*.,(2001) explained the prevalence of anti-HH6 IgG in healthy Thai children between the ages of 0 and 12 months was 88.10% as measured using an ELISA technique.

Study by Politou *et al*., (2014) showed in Greece, 78.7% of blood donors were found to be HH6 positive, and there was no gender or age group difference in the seroprevalence.

Study by Hasan *et al*., (2019) showed almost half of apparently healthy two-year-old children in Diyala had anti-HHV6 IgG antibodies, and family members with primary HHV6 infections were more likely to have anti-HHV6 IgG antibodies if they had siblings with a primary HHV-6 infection.

Additionally, the relationship between the findings of the research NF-kB and the antigen was studied.In the study, it was discovered that, among the total number of positive samples for HHV6-Ags testing 60, there were 57 positive cases for the NF-kB test (28.5%) and 3 negative

cases for the NF-kB test (1.5%) among positive cases for HHV6-Ags testing.

While the remaining negative cases for HHV6-Ags testing yielded 19 positive cases for the NF-B test (9.5%) and 124 (62%)

A highly substantial relationship was found between positive findings for both Anti-HHV6 IgM and Anti-HHV6 IgG and NF-B, as seen in table 3.8.

Study by Fiordelisi *et al*., (2019**)** showed that inflammation is crucial to the functioning of the cardiovascular system. Atherosclerosis is absolutely dependent on it, and it increases the likelihood of a heart attack or stroke. NF-κB is involved in the development and progression of both inflammation and cardiac and vascular damage.

Hayden *et al*., (2008) study showed that NF-kB can be activated during active viral replication and can cause inflammatory factors like IL-1, IL-6, P53, CD95, and Bcl-XS to become expressed. Despite this, HHV-6B does not cause an immune system response overactive.

In study by Zandi *et al*.,(1997), it was found that the levels of phosphorylation of TAK-1, IKKα/β, and IκB-α in HHV-6-infected DCs appeared to be decline when compared to DCs infected with mock. When the IKK complex is activated, phosphorylation of IκB causes the NF-κB complex to activate, thereby resulting in the production of inflammatory cytokines.

Mack *et al*., (2008) found that infection with HHV-6 impairs the degradation of IκB-α and inhibits NF-κB activation. Moreover, it was found that inoculation with inactivated HHV-6 does not induce

impairment of TLR signaling. In other hands, infection with HHV-6 and inoculation with inactivated HHV-6 do not induce any significant difference in cytokine production by DCs stimulated with TLR ligands.

Conclusions And Recommendations

Conclusion And Recommendation

Conclusions

The resent study conclude the following:

1. A remarkable proportion of patients with cardiovascular diseases have HHV6 infection.
2. There are not significant differences between the parameters in single group (the positive antigen group or in negative antigen group), i.e. between HHV6 and between IgM, IgG and NF-kB but there were highly significant differences between the parameters between the positive antigen group and the negative antigen group.
3. Activation of HHV6 virus infection could be responsible for cardiovascular diseases or worsening of pre-existence disease as many patients had increment in both HHV6 IgG & IgM levels together.
4. The males were more affected by cardiovascular diseases than females and it is associated with increase age .
5. The HHV-6 antigen negative results does not role out HHV-6virus infection as still a small proportion of patients with negative results have positive IgG which could indicate previous infection.
6. The increment of NF-KB level may be due to HHV6 virus infection & inflammation of cardiac tissues.
7. There is relationship between cardiovascular diseases and HHV6 infection.

Recommendations

1- Concentration on possible role of NF-kB as an early diagnostic biomarker in patients with heart disease infected by HHV-6virus
2- In case of Patient with cardiovascular diseases unresponsive to treatment, physicians are recommended to exclude HHV6 viral infection as it can alter the protocol of treatment.
3- study of viral load and its relation with severity of cardiovascular diseases or in case of unresponsive to treatment is highly recommended.
4- A long Follow-up study for patients with positive HHV6 virus infection & cardiovascular diseases is recommended.
5- Genetic Assessment study for HHV6 viral infection is recommended.

6- The need for other studies to study the relationship between other cardio-trophic viruses and cardiovascular diseases.
7- Study the relationship between the virus and other immune factors in patients with heart disease.
8- Studying the relationship between viruses and the NF-kB factor in other diseases.
9- Study of the polymorphism analysis of the genes responsible for NF-kB in heart patients.

Reference

Ablashi, D., Agut, H., Alvarez-Lafuente, R., Clark, D.A., Dewhurst, S., DiLuca, D., Flamand, L., Frenkel, N., Gallo, R., Gompels, U.A. and Höllsberg, P., 2014. Classification of HHV-6A and HHV-6B as distinct viruses. *Archives of virology*, *159*(5),863-870.

Abubakar, I.I., Tillmann, T. and Banerjee, A., 2015. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, *385*(9963),117-171.

Achour, A., Malet, I., Le Gal, F., Dehée, A., Gautheret-Dejean, A., Bonnafous, P. and Agut, H., 2008. Variability of gB and gH genes of human herpesvirus-6 among clinical specimens. *Journal of medical virology*, *80*(7), pp.1211-1221.

Adams, M.J. and Carstens, E.B., 2012. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2012). *Archives of virology*, *157*(7), pp.1411-1422.

Adams, O., Krempe, C., Kögler, G., Wernet, P. and Scheid, A., 1998. Congenital infections with human herpesvirus 6. *Journal of Infectious Diseases*, *178*(2), pp.544-546.

Agut, H., 2011. Deciphering the clinical impact of acute human herpesvirus 6 (HHV-6) infections. *Journal of clinical virology*, *52*(3), pp.164-171.

Agut, H., Bonnafous, P. and Gautheret-Dejean, A., 2015. Laboratory and clinical aspects of human herpesvirus 6 infections. *Clinical microbiology reviews*, *28*(2), pp.313-335.

Aimola, G., Beythien, G., Aswad, A. and Kaufer, B.B., 2020. Current understanding of human herpesvirus 6 (HHV-6) chromosomal integration. *Antiviral research*, *176*, p.104720.

Anand, A. and Sk, M.I.K., 2017. The risk of hypertension and other chronic diseases: comparing smokeless tobacco with smoking. *Frontiers in public health*, 5, p.255.

Andersson, C. and Vasan, R.S., 2018. Epidemiology of cardiovascular disease in young individuals. *Nature Reviews Cardiology*, 15(4), pp.230-240.

Andre-Garnier, E., Milpied, N., Boutolleau, D., Saiagh, S., Billaudel, S. and Imbert-Marcille, B.M., 2004. Reactivation of human herpesvirus 6 during ex vivo expansion of circulating CD34+ haematopoietic stem cells. *Journal of general virology*, *85*(11), pp.3333-3336.

Arbuckle, J.H. and Medveczky, P.G., 2011. The molecular biology of human herpesvirus-6 latency and telomere integration. *Microbes and infection*, *13*(8-9), pp.731-741.

Arbuckle, J.H., Medveczky, M.M., Luka, J., Hadley, S.H., Luegmayr, A., Ablashi, D., Lund, T.C., Tolar, J., De Meirleir, K., Montoya, J.G. and Komaroff, A.L., 2010. The latent human herpesvirus-6A genome specifically integrates in telomeres of human chromosomes in vivo and in vitro. *Proceedings of the National Academy of Sciences*, *107*(12), pp.5563-5568.

Arbuckle, J.H., Pantry, S.N., Medveczky, M.M., Prichett, J., Loomis, K.S., Ablashi, D. and Medveczky, P.G., 2013. Mapping the telomere integrated genome of human herpesvirus 6A and 6B. *Virology*, *442*(1), pp.3-11.

Arena, A., Liberto, M.C., Iannello, D., Capozza, A.B. and Foca, A., 1999. Altered cytokine production after human herpes virus type 6 infection. *The new microbiologica*, *22*(4), pp.293-300.

Ashrafpoor, G., Andréoletti, L., Bruneval, P., Macron, L., Azarine, A., Lepillier, A., Danchin, N., Mousseaux, E. and Redheuil, A., 2013. Fulminant human herpesvirus 6 myocarditis in an immunocompetent adult: role of cardiac magnetic resonance in a multidisciplinary approach. *Circulation*, *128*(23), pp.e445-e447.

Assuncao, B.M., Tan, T.C., Cheng, K.H., Yucel, E., Handschumacher, M.D. and Scherrer-Crosbie, M., 2016. Acute Leukemia is Associated With Cardiac Dysfunction Before Chemotherapy. *Circulation*, 134(suppl_1), pp.A20407-A20407.

Atreya I, Atreya R and Neurath MF. (2008). NFkB in inflammatory bowel disease. Journal of Internal Medicine, 263(6), 591-596.

Baldwin, A.S., 2001. Series introduction: the transcription factor NF-κB and human disease. The Journal of clinical investigation, 107(1), pp.3-6.

Becerra, A., Gibson, L., Stern, L.J. and Calvo-Calle, J.M., 2014. Immune response to HHV-6 and implications for immunotherapy. *Current opinion in virology*, 9, pp.154-161.

Beinke, S. and Ley, S.C., 2004. Functions of NF-κB1 and NF-κB2 in immune cell biology. *Biochemical Journal*, *382*(2), pp.393-409.

Bell, A.J., Gallagher, A., Mottram, T., Lake, A., Kane, E.V., Lightfoot, T., Roman, E. and Jarrett, R.F., 2014. Germ-line transmitted, chromosomally integrated HHV-6 and classical Hodgkin lymphoma. *PLoS One*, *9*(11), p.e112642.

Benjamin, E.J., Muntner, P., Alonso, A., Bittencourt, M.S., Callaway, C.W., Carson, A.P., Chamberlain, A.M., Chang, A.R., Cheng, S., Das, S.R. and Delling, F.N., 2019. Heart disease and stroke statistics—2019 update: a report from the American Heart Association. *Circulation*, 139(10), pp.e56-e528.

Bhattarakosol, P., Pancharoen, C., Mekmullica, J. and Bhattarakosol, P., 2001. Seroprevalence of anti-human herpesvirus-6 IgG antibody in children of Bangkok, Thailand. *Southeast Asian journal of tropical medicine and public health*, 32(1), pp.143-147

Braun, D.K., Dominguez, G. and Pellett, P.E., 1997. Human herpesvirus 6. *Clinical microbiology reviews*, *10*(3), pp.521-567.

Carter, B.D., Abnet, C.C., Feskanich, D., Freedman, N.D., Hartge, P., Lewis, C.E., Ockene, J.K., Prentice, R.L., Speizer, F.E., Thun, M.J. and Jacobs, E.J., 2015. Smoking and mortality—beyond established causes. *New England journal of medicine*, *372*(7), pp.631-640.

Caruso, A., Favilli, F., Rotola, A., Comar, M., Horejsh, D., Alessandri, G., Grassi, M., Di Luca, D. and Fiorentini, S., 2003. Human herpesvirus-6 modulates RANTES production in primary human endothelial cell cultures. *Journal of medical virology*, *70*(3), pp.451-458.

Caruso, A., Rotola, A., Comar, M., Favilli, F., Galvan, M., Tosetti, M., Campello, C., Caselli, E., Alessandri, G., Grassi, M. and Garrafa, E., 2002. HHV-6 infects human aortic and heart microvascular endothelial cells, increasing their ability to secrete proinflammatory chemokines. *Journal of medical virology*, 67(4), pp.528-533

Caselli, E., Bortolotti, D., Marci, R., Rotola, A., Gentili, V., Soffritti, I., D'Accolti, M., Lo Monte, G., Sicolo, M., Barao, I. and Di Luca, D., 2017.

HHV-6A infection of endometrial epithelial cells induces increased endometrial NK Cell-mediated cytotoxicity. *Frontiers in microbiology*, *8*, p.2525.

Caserta, M.T., Hall, C.B., Schnabel, K., Lofthus, G., Marino, A., Shelley, L., Yoo, C., Carnahan, J., Anderson, L. and Wang, H., 2010. Diagnostic assays for active infection with human herpesvirus 6 (HHV-6). *Journal of Clinical Virology*, *48*(1), pp.55-57.

Catlin, M.C., Deng, R., Martinez, R.S., Sharma, R. and Grossblatt, N., 2009. Secondhand smoke exposure and cardiovascular effects: making sense of the evidence. Washington (DC): Institute of Medicine of the National Academies

Chen, T. and Hudnall, S.D., 2006. Anatomical mapping of human herpesvirus reservoirs of infection. *Modern Pathology*, *19*(5), pp.726-737.

Clark, D.J., Catusse, J., Stacey, A., Borrow, P. and Gompels, U.A., 2013. Activation of CCR2+ human proinflammatory monocytes by human herpesvirus-6B chemokine N-terminal peptide. *Journal of General Virology*, *94*(7), pp.1624-1635.

Comar, M., D'Agaro, P., Campello, C., Poli, A., Breinholt, J.P., Towbin, J.A. and Vatta, M., 2009. Human herpes virus 6 in archival cardiac tissues from children with idiopathic dilated cardiomyopathy or congenital heart disease. *Journal of clinical pathology*, 62(1), pp.80-83.

Csordas, A. and Bernhard, D., 2013.The biology behind the atherothrombotic effects of cigarette smoke. *Nature Reviews Cardiology*, 10(4), pp.219-230.

Curtis, A.B., Karki, R., Hattoum, A. and Sharma, U.C., 2018. Arrhythmias in patients≥ 80 years of age: pathophysiology, management, and outcomes. *Journal of the American College of Cardiology*, 71(18), pp.2041-2057.

Daibata, M., Taguchi, T., Nemoto, Y., Taguchi, H. and Miyoshi, I., 1999. Inheritance of chromosomally integrated human herpesvirus 6 DNA. *Blood, The Journal of the American Society of Hematology*, *94*(5), pp.1545-1549.

Davison, A.J., 2010. Herpesvirus systematics. *Veterinary microbiology*, *143*(1), pp.52-69.

De Bolle, L., Naesens, L. and De Clercq, E., 2005. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clinical microbiology reviews*, *18*(1), pp.217-245.

Dominguez, G., Dambaugh, T.R., Stamey, F.R., Dewhurst, S., Inoue, N. and Pellett, P.E., 1999. Human herpesvirus 6B genome sequence: coding content and comparison with human herpesvirus 6A. *Journal of virology*, *73*(10), pp.8040-8052.

Endo, A., Watanabe, K., Ohye, T., Suzuki, K., Matsubara, T., Shimizu, N., Kurahashi, H., Yoshikawa, T., Katano, H., Inoue, N. and Imai, K., 2014. Molecular and virological evidence of viral activation from chromosomally integrated human herpesvirus 6A in a patient with X-linked severe combined immunodeficiency. *Clinical Infectious Diseases*, *59*(4), pp.545-548.

Engelmann, I., Petzold, D.R., Kosinska, A., Hepkema, B.G., Schulz, T.F. and Heim, A., 2008. Rapid quantitative PCR assays for the simultaneous detection of herpes simplex virus, varicella zoster virus, cytomegalovirus,

Epstein-Barr virus, and human herpesvirus 6 DNA in blood and other clinical specimens. *Journal of medical virology*, *80*(3), pp.467-477.

Fan, B., Dun, S.H., Gu, J.Q., Guo, Y. and Ikuyama, S., 2015. Pycnogenol attenuates the release of proinflammatory cytokines and expression of perilipin 2 in lipopolysaccharide-stimulated microglia in part via inhibition of NF-κB and AP-1 activation. *PLoS One*, 10(9), p.e0137837.

Fida, M., Hamdi, A.M., Bryson, A., Razonable, R.R. and Abu Saleh, O., 2019, July. Long-term outcomes of patients with human herpesvirus 6 encephalitis. In *Open forum infectious diseases* (Vol. 6, No. 7, p. ofz269). US: Oxford University Press.

Fiordelisi, A., Iaccarino, G., Morisco, C., Coscioni, E. and Sorriento, D., 2019. NFkappaB is a key player in the crosstalk between inflammation and cardiovascular diseases. *International journal of molecular sciences*, 20(7), p.1599.

Flamand, L., Komaroff, A.L., Arbuckle, J.H., Medveczky, P.G. and Ablashi, D.V., 2010. Review, part 1: Human herpesvirus-6-basic biology, diagnostic testing, and antiviral efficacy. *Journal of medical virology*, *82*(9), pp.1560-1568.

Frantz, S., Stoerk, S., Ok, S., Wagner, H., Angermann, C.E., Ertl, G. and Bauersachs, J., 2004. Effect of chronic heart failure on nuclear factor kappa B in peripheral leukocytes. *The American journal of cardiology*, *94*(5), pp.671-673.

Freitas, R.B., Freitas, M.R. and Linhares, A.C., 2003. Evidence of active herpesvirus 6 (variant-A) infection in patients with lymphadenopathy in Belém, Pará, Brazil. Revista do *Instituto de Medicina Tropical de São Paulo*, 45, pp.283-288.

Fuchs, F.D. and Whelton, P.K., 2020. High blood pressure and cardiovascular disease. Hypertension, 75(2), pp.285-292.

Gallucci, G., Tartarone, A., Lerose, R., Lalinga, A.V. and Capobianco, A.M., 2020. Cardiovascular risk of smoking and benefits of smoking cessation. *Journal of thoracic disease*, *12*(7), p.3866.

Gao, Z., Chen, Z., Sun, A. and Deng, X., 2019. Gender differences in cardiovascular disease. *Medicine in Novel Technology and Devices*, *4*, p.100025.

Gerondakis, S., Fulford, T.S., Messina, N.L. and Grumont, R.J., 2014. NF-κB control of T cell development. *Nature immunology*, *15*(1), pp.15-25.

Gordon, J.W., Shaw, J.A. and Kirshenbaum, L.A., 2011. Multiple facets of NF-κB in the heart: to be or not to NF-κB. *Circulation research*, *108*(9), pp.1122-1132.

Gravel, A., Gosselin, J. and Flamand, L., 2002. Human herpesvirus 6 immediate-early 1 protein is a sumoylated nuclear phosphoprotein colocalizing with promyelocytic leukemia protein-associated nuclear bodies. *Journal of Biological Chemistry*, *277*(22), pp.19679-19687.

Gravel, A., Hall, C.B. and Flamand, L., 2013. Sequence analysis of transplacentally acquired human herpesvirus 6 DNA is consistent with transmission of a chromosomally integrated reactivated virus. *The Journal of infectious diseases*, *207*(10), pp.1585-1589.

Gravel, A., Hall, C.B. and Flamand, L., 2013. Sequence analysis of transplacentally acquired human herpesvirus 6 DNA is consistent with transmission of a chromosomally integrated reactivated virus. *The Journal of infectious diseases*, *207*(10), pp.1585-1589.

Greenstone, H.L., Santoro, F., Lusso, P. and Berger, E.A., 2002. Human herpesvirus 6 and measles virus employ distinct CD46 domains for receptor function. *Journal of Biological Chemistry*, *277*(42), pp.3911239118.

Grivel, J.C., Santoro, F., Chen, S., Fagá, G., Malnati, M.S., Ito, Y., Margolis, L. and Lusso, P., 2003. Pathogenic effects of human herpesvirus 6 in human lymphoid tissue ex vivo. *Journal of virology*, *77*(15), pp.8280-8289.

Gupta, Ruchika, Sanjay Gupta, Shashi Sharma, Dhirendra N. Sinha, and Ravi Mehrotra. "Risk of coronary heart disease among smokeless tobacco users: results of systematic review and meta-analysis of global data." Nicotine and Tobacco Research 21, no. 1 (2019): 25-31

Guro, V., 2000. Unstable angina activates myocardial HSP 72, eNOS and transcription factors NFκB and AP-1. *Cardiovasc Res*, *47*, pp.49-56.

Hakacova, N., Klingel, K., Kandolf, R., Engdahl, E., Fogdell-Hahn, A. and Higgins, T., 2013. First therapeutic use of Artesunate in treatment of human herpesvirus 6B myocarditis in a child. *Journal of Clinical Virology*, *57*(2), pp.157-160.

Hall, C.B., Caserta, M.T., Schnabel, K., Shelley, L.M., Marino, A.S., Carnahan, J.A., Yoo, C., Lofthus, G.K. and McDermott, M.P., 2008. Chromosomal integration of human herpesvirus 6 is the major mode of congenital human herpesvirus 6 infection. *Pediatrics*, *122*(3), pp.513-520.

Hall, C.B., Caserta, M.T., Schnabel, K.C., Shelley, L.M., Carnahan, J.A., Marino, A.S., Yoo, C. and Lofthus, G.K., 2010. Transplacental congenital human herpesvirus 6 infection caused by maternal chromosomally integrated virus. *The Journal of infectious diseases*, *201*(4), pp.505-507.

Hall, C.B., Long, C.E., Schnabel, K.C., Caserta, M.T., McIntyre, K.M., Costanzo, M.A., Knott, A., Dewhurst, S., Insel, R.A. and Epstein, L.G., 1994. Human Herpesvirus-6 Infection in Children--A Prospective Study of Complications and Reactivation. *New England Journal of Medicine*, *331*(7), pp.432-438.

Hasan, A.S., Mehdi, S. and Noor, A.H., 2019. The Seropositivity Rate of Human Herpesvirus Type 6 among Infants in Diyala Province, Iraq. *Journal of Biosciences and Medicines*, 7(6), pp.129-137.

Hasegawa, Y., Saito, T., Ogihara, T., Ishigaki, Y., Yamada, T., Imai, J., Uno, K., Gao, J., Kaneko, K., Shimosawa, T. and Asano, T., 2012. Blockade of the nuclear factor-κB pathway in the endothelium prevents insulin resistance and prolongs life spans. *Circulation*, 125(9), pp.1122-1133.

Hayden, M.S. and Ghosh, S., 2008. Shared principles in NF-κB signaling. Cell, 132(3), pp.344-362.

Horvat, R.T., Parmely, M.J. and Chandran, B., 1993. Human herpesvirus 6 inhibits the proliferative responses of human peripheral blood mononuclear cells. *Journal of Infectious Diseases*, *167*(6), pp.1274-1280.

Hp, S., 2011. Kühl U. Cooper LT. The management of myocarditis. *Eur Heart J*, *32*(21), pp.2616-25.

Huang, Y., Hidalgo-Bravo, A., Zhang, E., Cotton, V.E., Mendez-Bermudez, A., Wig, G., Medina-Calzada, Z., Neumann, R., Jeffreys, A.J., Winney, B. and Wilson, J.F., 2014. Human telomeres that carry an integrated copy of human herpesvirus 6 are often short and unstable, facilitating release of the viral genome from the chromosome. *Nucleic acids research*, *42*(1), pp.315-327.

Intayoung, P., Limtrakul, P. and Yodkeeree, S., 2016. Antiinflammatory activities of crebanine by inhibition of NF-κB and AP-1 activation through suppressing MAPKs and Akt signaling in LPS-induced RAW 264.7 macrophages. *Biological and Pharmaceutical Bulletin*, pp.b15-00479.

Isegawa, Y., Mukai, T., Nakano, K., Kagawa, M., Chen, J., Mori, Y., Sunagawa, T., Kawanishi, K., Sashihara, J., Hata, A. and Zou, P., 1999. Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B. *Journal of virology*, *73*(10), pp.8053-8063.

Jaworska, J., Gravel, A. and Flamand, L., 2010. Divergent susceptibilities of human herpesvirus 6 variants to type I interferons. *Proceedings of the National Academy of Sciences*, *107*(18), pp.8369-8374.

Jeziorski, E., Senechal, B., Molina, T.J., Devez, F., Leruez-Ville, M., Morand, P., Glorion, C., Mansuy, L., Gaudelus, J., Debre, M. and Jaubert, F., 2008. Herpes-virus infection in patients with Langerhans cell histiocytosis: a case-controlled sero-epidemiological study, and in situ analysis. *PLoS One*, *3*(9), p.e3262.

Jones, W.K., Brown, M., Wilhide, M., He, S. and Ren, X., 2005. NF-κB in cardiovascular disease. *Cardiovascular toxicology*, 5(2), pp.183-201.

Kakimoto, M., Hasegawa, A., Fujita, S. and Yasukawa, M., 2002. Phenotypic and functional alterations of dendritic cells induced by human herpesvirus 6 infection. *Journal of virology*, *76*(20), pp.10338-10345.

Kawabata, A., Oyaizu, H., Maeki, T., Tang, H., Yamanishi, K. and Mori, Y., 2011. Analysis of a neutralizing antibody for human herpesvirus 6B reveals a role for glycoprotein Q1 in viral entry. *Journal of virology*, *85*(24), pp.12962-12971

Kim, W., Ludlow, A.T., Min, J., Robin, J.D., Stadler, G., Mender, I., Lai, T.P., Zhang, N., Wright, W.E. and Shay, J.W., 2016. Regulation of the human telomerase gene TERT by telomere position effect—over long distances (TPE-OLD): implications for aging and cancer. *PLoS biology*, *14*(12), p.e2000016.

Kondo, K., Shimada, K., Sashihara, J., Tanaka-Taya, K. and Yamanishi, K., 2002. Identification of human herpesvirus 6 latency-associated transcripts. *Journal of virology*, *76*(8), pp.4145-4151.

Kramarsky, B. and Sander, C., 1992. Electron microscopy of human herpesvirus-6 (HHV-6). In *Perspectives in Medical Virology* (Vol. 4, pp. 59-68). Elsevier.

Kühl, U., Lassner, D., Wallaschek, N., Gross, U.M., Krueger, G.R., Seeberg, B., Kaufer, B.B., Escher, F., Poller, W. and Schultheiss, H.P., 2015. Chromosomally integrated human herpesvirus 6 in heart failure: prevalence and treatment. *European journal of heart failure*, 17(1), pp.9-19.

Kühl, U., Pauschinger, M., Seeberg, B., Lassner, D., Noutsias, M., Poller, W. and Schultheiss, H.P., 2005. Viral persistence in the myocardium is associated with progressive cardiac dysfunction. *Circulation*, 112(13), pp.1965-1970.

Kühl,U., Pauschinger, M., Noutsias, M., Seeberg, B., Bock, T., Lassner, D., Poller, W., Kandolf, R. and Schultheiss, H.P., 2005. High prevalence of viral genomes and multiple viral infections in the myocardium of adults with “idiopathic” left ventricular dysfunction. *Circulation*, *111*(7), pp.887-93.

Kumagai, T., Yoshikawa, T., Yoshida, M., Okui, T., Ihira, M., Nagata, N., Yano, S., Shiraki, K., Yamada, M., Ichihara, K. and Asano, Y., 2006. Time course characteristics of human herpesvirus 6 specific cellular immune response and natural killer cell activity in patients with exanthema subitum. *Journal of medical virology*, *78*(6), pp.792-799.

Kumar, S., Ingle, H., Prasad, D.V.R. and Kumar, H., 2013. Recognition of bacterial infection by innate immune sensors. *Critical Reviews in Microbiology*, *39*(3), pp.229-246.

LaCroix, S., Stewart, J.A., Thouless, M.E. and Black, J.B., 2000. An immunoblot assay for detection of immunoglobulin M antibody to human herpesvirus 6. *Clinical Diagnostic Laboratory Immunology*, *7*(5), pp.823-827.

Laird, M.H., Rhee, S.H., Perkins, D.J., Medvedev, A.E., Piao, W., Fenton, M.J. and Vogel, S.N., 2009. TLR4/MyD88/PI3K interactions regulate TLR4 signaling. *Journal of leukocyte biology*, *85*(6), pp.966-977.

Lassner, D., Siegismund, C.S., Stehr, J., Rohde, M., Escher, F. and Tsch, C., 2013. Recent advances in molecular diagnostics and treatment of heart muscle diseases.

Lazzerini-Denchi, E. and Sfeir, A., 2016. Stop pulling my strings—what telomeres taught us about the DNA damage response. *Nature reviews Molecular cell biology*, *17*(6), p.364.

Leon, B.M. and Maddox, T.M., 2015. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. *World journal of diabetes*, *6*(13), p.1246.

Levy, J.A., Ferro, F., Greenspan, D. and Lennette, E.T., 1990. Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population. *The Lancet*, *335*(8697), pp.1047-1050.

Li, C., Kao, R.L., Ha, T., Kelley, J., Browder, I.W. and Williams, D.L., 2001. Early activation of IKKβ during in vivo myocardial ischemia. *American Journal of Physiology-Heart and Circulatory Physiology*, *280*(3), pp.H1264-H1271.

Luppi, M., Marasca, R., Barozzi, P., Ferrari, S., Ceccherini-Nelli, L., Batoni, G., Merelli, E. and Torelli, G., 1993. Three cases of human herpesvirus-6 latent infection: Integration of viral genome in peripheral blood mononuclear cell DNA. *Journal of medical virology*, *40*(1), pp.44-52.

Ma, J., Jia, J., Jiang, X., Xu, M., Guo, J., Tang, T., Xu, X., Wu, Z., Hu, B., Yao, K. and Li, L., 2020. gp96 is critical for both human herpesvirus 6A (HHV-6A) and HHV-6B infections. *Journal of virology*, *94*(13), pp.e00311-20.

Mack, C., Sickmann, A., Lembo, D. and Brune, W., 2008. Inhibition of proinflammatory and innate immune signaling pathways by a cytomegalovirus RIP1-interacting protein. *Proceedings of the National Academy of Sciences*, 105(8), pp.3094-3099.

Maeki, T. and Mori, Y., 2012. Features of human herpesvirus-6A and-6B entry. *Advances in virology*, *2012*.

Magalhães, I.D.M., Martins, R.V.N., Vianna, R.O., Moysés, N., Afonso, L.A., Oliveira, S.A. and Cavalcanti, S.M.B., 2011. Detection of human herpesvirus 7 infection in young children presenting with exanthema subitum. *Memórias do Instituto Oswaldo Cruz*, *106*(3), pp.371-373.

Martin, M.E., Nicholas, J.O.H.N., Thomson, B.J., Newman, C.A.R.O.L. and Honess, R.W., 1991. Identification of a transactivating function mapping to the putative immediate-early locus of human herpesvirus 6. *Journal of Virology*, *65*(10), pp.5381-5390.

Mendis, S., Puska, P., Norrving, B. and World Health Organization, 2011. *Global atlas on cardiovascular disease prevention and control*. World Health Organization.

Merk, J., Schmid, F.X., Fleck, M., Schwarz, S., Lehane, C., Boehm, S., Salzberger, B. and Birnbaum, D.E., 2005. Fatal pulmonary failure attributable to viral pneumonia with human herpes virus 6 (HHV6) in a young immunocompetent woman. *Journal of intensive care medicine*, *20*(5), pp.302-306.

Moscoso, I., Centeno, A., Lopez, E., Rodriguez-Barbosa, J.I., Santamarina, I., Filgueira, P., Sanchez, M.J., Domınguez-Perles, R., Penuelas-Rivas, G. and Domenech, N., 2005, January. Differentiation "in vitro" of primary and immortalized porcine mesenchymal stem cells into cardiomyocytes for cell transplantation. In *Transplantation proceedings* (Vol. 37, No. 1, pp. 481-482). Elsevier.

Nam Leong, H., Tuke, P.W., Tedder, R.S., Khanom, A.B., Eglin, R.P., Atkinson, C.E., Ward, K.N., Griffiths, P.D. and Clark, D.A., 2007. The prevalence of chromosomally integrated human herpesvirus 6 genomes in the blood of UK blood donors. *Journal of medical virology*, *79*(1), pp.45-51.

Nam Leong, H., Tuke, P.W., Tedder, R.S., Khanom, A.B., Eglin, R.P., Atkinson, C.E., Ward, K.N., Griffiths, P.D. and Clark, D.A., 2007. The prevalence of chromosomally integrated human herpesvirus 6 genomes in

the blood of UK blood donors. *Journal of medical virology*, *79*(1), pp.45-51.

Niiya, H., Lei, J., Guo, Y., Azuma, T., Yakushijin, Y., Sakai, I., Hato, T., Tohyama, M., Hashimoto, K. and Yasukawa, M., 2006. Human herpesvirus 6 impairs differentiation of monocytes to dendritic cells. *Experimental hematology*, *34*(5), pp.642-653.

Nishimoto, M., Nakamae, H., Hayashi, Y., Koh, H., Nakane, T., Yoshida, M., Bingo, M., Okamura, H., Aimoto, M., Nanno, S. and Yoshimura, T., 2012. Prolonged sinus tachycardia caused by human herpesvirus 6 (HHV6) encephalomyelitis after allogeneic bone marrow transplantation. *Internal Medicine*, *51*(10), pp.1265-1267.

Nishimoto, M., Nakamae, H., Hayashi, Y., Koh, H., Nakane, T., Yoshida, M., Bingo, M., Okamura, H., Aimoto, M., Nanno, S. and Yoshimura, T., 2012. Prolonged sinus tachycardia caused by human herpesvirus 6 (HHV6) encephalomyelitis after allogeneic bone marrow transplantation. *Internal Medicine*, 51(10), pp.1265-1267.

Nordström, I. and Eriksson, K., 2012. HHV-6B induces IFN-lambda1 responses in cord plasmacytoid dendritic cells through TLR9. *PLoS One*, *7*(6), p.e38683.

Noutsias, M., Fechner, H., de Jonge, H., Wang, X., Dekkers, D., Houtsmuller, A.B., Pauschinger, M., Bergelson, J., Warraich, R., Yacoub, M. and Hetzer, R., 2001. Human coxsackie-adenovirus receptor is colocalized with integrins αvβ3 and αvβ5 on the cardiomyocyte sarcolemma and upregulated in dilated cardiomyopathy: implications for cardiotropic viral infections. *Circulation*, *104*(3), pp.275-280.

Oeckinghaus, A. and Ghosh, S., 2009. The NF-κB family of transcription factors and its regulation. *Cold Spring Harbor perspectives in biology*, *1*(4), p.a000034.

Ogata, M., Fukuda, T. and Teshima, T., 2015. Human herpesvirus-6 encephalitis after allogeneic hematopoietic cell transplantation: what we do and do not know. *Bone marrow transplantation*, *50*(8), pp.1030-1036.

Ong, D.S., Bonten, M.J., Spitoni, C., Verduyn Lunel, F.M., Frencken, J.F., Horn, J., Schultz, M.J., van der Poll, T., Klein Klouwenberg, P.M., Cremer, O.L. and Molecular Diagnosis and Risk Stratification of Sepsis Consortium, 2017. Epidemiology of multiple herpes viremia in previously immunocompetent patients with septic shock. *Clinical Infectious Diseases*, *64*(9), pp.1204-1210.

Øster, B. and Höllsberg, P., 2002. Viral gene expression patterns in human herpesvirus 6B-infected T cells. *Journal of virology*, *76*(15), pp.7578-7586.

Oyaizu, H., Tang, H., Ota, M., Takenaka, N., Ozono, K., Yamanishi, K. and Mori, Y., 2012. Complementation of the function of glycoprotein H of human herpesvirus 6 variant A by glycoprotein H of variant B in the virus life cycle. *Journal of virology*, *86*(16), pp.8492-8498.

Panicker, S.R., Sreenivas, P., Babu, M.S., Karunagaran, D. and Kartha, C.C., 2010. Quercetin attenuates monocyte chemoattractant protein-1 gene expression in glucose primed aortic endothelial cells through NF-κB and AP-1. *Pharmacological Research*, 62(4), pp.328-336.

Pankuweit, S. and Maisch, B., 2010. The heart in viral infections. *Der Internist*, *51*(7), pp.836-843.

Pantry, S.N. and Medveczky, P.G., 2017. Latency, integration, and reactivation of human herpesvirus-6. *Viruses*, *9*(7), p.194.

Park, H.J. and Youn, H.S., 2013. Mercury induces the expression of cyclooxygenase-2 and inducible nitric oxide synthase. *Toxicology and industrial health*, *29*(2), pp.169-174.

Pauschinger, M., Noutsias, M., Lassner, D., Schultheiss, H.P. and Kuehl, U., 2006. Inflammation, ECG changes and pericardial effusion. *Clinical research in cardiology*, *95*(11), pp.569-583.

Pellett Madan, R., Hand, J. and AST Infectious Diseases Community of Practice, 2019. Human herpesvirus 6, 7, and 8 in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clinical transplantation*, *33*(9), p.e13518.

Perkins, N.D., 2007. Integrating cell-signalling pathways with NF-κB and IKK function. *Nature reviews Molecular cell biology*, *8*(1), pp.49-62.

Pimple, P., Hammadah, M., Wilmot, K., Ramadan, R., Al Mheid, I., Levantsevych, O., Sullivan, S., Garcia, E.V., Nye, J., Shah, A.J. and Ward, L., 2018. Chest pain and mental stress–induced myocardial ischemia: sex differences. *The American journal of medicine*, *131*(5), pp.540-547.

Politou, M., Koutras, D., Kaparos, G., Valsami, S., Pittaras, T., Logothetis, E., Panayiotakopoulos, G. and Kouskouni, E., 2014. Seroprevalence of HHV-6 and HHV-8 among blood donors in Greece. *Virology journal*, 11(1), pp.1-4.

Poller, W.C., Fechner, H., Noutsias, M., Tschoepe, C. and Schultheiss, H.P., 2002. Highly variable expression of virus receptors in the human cardiovascular system. *Zeitschrift für Kardiologie*, *91*(12), pp.978-991.

Prichard, M.N. and Whitley, R.J., 2014. The development of new therapies for human herpesvirus 6. *Current opinion in virology*, *9*, pp.148-153.

Revello, M.G. and Gerna, G., 2010. Human cytomegalovirus tropism for endothelial/epithelial cells: scientific background and clinical implications. *Reviews in medical virology*, *20*(3), pp.136-155.

Rizzo, R., Soffritti, I., D'Accolti, M., Bortolotti, D., Di Luca, D. and Caselli, E., 2017. HHV-6A/6B infection of NK cells modulates the expression of miRNAs and transcription factors potentially associated to impaired NK activity. *Frontiers in microbiology*, *8*, p.2143.

Rotola, A., Di Luca, D., Cassai, E., Ricotta, D., Giulio, A., Turano, A., Caruso, A. and Muneretto, C., 2000. Human herpesvirus 6 infects and replicates in aortic endothelium. *Journal of Clinical Microbiology*, *38*(8), pp.3135-3136.

Salahuddin, S.Z., Ablashi, D.V., Markham, P.D., Josephs, S.F., Sturzenegger, S., Kaplan, M., Halligan, G., Biberfeld, P., Wong-Staal, F., Kramarsky, B. and Gallo, R.C., 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science*, *234*(4776), pp.596-601.

Sampaio, A.M., Thomasini, R.L., Guardia, A.C., Stucchi, R.S.B., Rossi, C.L., Costa, S.C.B. and Boin, I.F.S.F., 2011, May. Cytomegalovirus, human herpesvirus-6, and human herpesvirus-7 in adult liver transplant

recipients: diagnosis based on antigenemia. In *Transplantation proceedings* (Vol. 43, No. 4, pp. 1357-1359). Elsevier.

Santoro, F., Kennedy, P.E., Locatelli, G., Malnati, M.S., Berger, E.A. and Lusso, P., 1999. CD46 is a cellular receptor for human herpesvirus 6. *Cell*, *99*(7), pp.817-827.

Sedlak, R.H., Cook, L., Huang, M.L., Magaret, A., Zerr, D.M., Boeckh, M. and Jerome, K.R., 2014. Identification of chromosomally integrated human herpesvirus 6 by droplet digital PCR. *Clinical chemistry*, *60*(5), pp.765-772.

Serasanambati, M. and Chilakapati, S.R., 2016. Function of nuclear factor kappa B (NF-kB) in human diseases-a review. *South Indian Journal of Biological Sciences*, *2*(4), pp.368-87.

Shaw, J., Zhang, T., Rzeszutek, M., Yurkova, N., Baetz, D., Davie, J.R. and Kirshenbaum, L.A., 2006. Transcriptional silencing of the death gene BNIP3 by cooperative action of NF-κB and histone deacetylase 1 in ventricular myocytes. *Circulation research*, *99*(12), pp.1347-1354.

Shimazu, Y., Kondo, T., Ishikawa, T., Yamashita, K. and Takaori-Kondo, A., 2013. Human herpesvirus-6 encephalitis during hematopoietic stem cell transplantation leads to poor prognosis. *Transplant Infectious Disease*, *15*(2), pp.195-201.

Siddiqi, K., Shah, S., Abbas, S.M., Vidyasagaran, A., Jawad, M., Dogar, O. and Sheikh, A., 2015. Global burden of disease due to smokeless tobacco consumption in adults: analysis of data from 113 countries. *BMC medicine*, 13(1), pp.1-22.

Siednienko, J., Jankowska, E.A., Banasiak, W., Gorczyca, W.A. and Ponikowski, P., 2007. Nuclear factor-kappaB activity in peripheral blood

mononuclear cells in cachectic and non-cachectic patients with chronic heart failure. *International journal of cardiology*, *122*(2), pp.111-116.

Simpson, K.E., Storch, G.A., Lee, C.K., Ward, K.E., Danon, S., Simon, C.M., Delaney, J.W., Tong, A. and Canter, C.E., 2016. High frequency of detection by PCR of viral nucleic acid in the blood of infants presenting with clinical myocarditis. *Pediatric cardiology*, 37(2), pp.399-404.

Smith, A., Santoro, F., Di Lullo, G., Dagna, L., Verani, A. and Lusso, P., 2003. Selective suppression of IL-12 production by human herpesvirus 6. *Blood*, *102*(8), pp.2877-2884.

Smith, A.P., Paolucci, C., Di Lullo, G., Burastero, S.E., Santoro, F. and Lusso, P., 2005. Viral replication-independent blockade of dendritic cell maturation and interleukin-12 production by human herpesvirus 6. *Journal of virology*, *79*(5), pp.2807-2813.

Soldan, S.S., Leist, T.P., Juhng, K.N., McFarland, H.F. and Jacobson, S., 2000. Increased lymphoproliferative response to human herpesvirus type 6A variant in multiple sclerosis patients. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, *47*(3), pp.306-313.

Sorriento, D., Santulli, G., Franco, A., Cipolletta, E., Napolitano, L., Gambardella, J., Gomez-Monterrey, I., Campiglia, P., Trimarco, B., Iaccarino, G. and Ciccarelli, M., 2015. Integrating GRK2 and NFkappaB in the pathophysiology of cardiac hypertrophy. *Journal of cardiovascular translational research*, *8*(8), pp.493-502.

Strenger, V., Aberle, S.W., Nacheva, E.P. and Urban, C., 2013. Chromosomal integration of the HHV-6 genome in a patient with nodular

sclerosis Hodgkin lymphoma. *British journal of haematology*, *161*(4), pp.594-595.

Sun, S.C., 2011. Non-canonical NF-κB signaling pathway. *Cell research*, *21*(1), pp.71-85.

Sun, S.C., Chang, J.H. and Jin, J., 2013. Regulation of nuclear factor-κB in autoimmunity. *Trends in immunology*, *34*(6), pp.282-289.

Takemoto, M., Shimamoto, T., Isegawa, Y. and Yamanishi, K., 2001. The R3 region, one of three major repetitive regions of human herpesvirus 6, is a strong enhancer of immediate-early gene U95. *Journal of Virology*, *75*(21), pp.10149-10160.

Tanaka-Taya, K., Sashihara, J., Kurahashi, H., Amo, K., Miyagawa, H., Kondo, K., Okada, S. and Yamanishi, K., 2004. Human herpesvirus 6 (HHV-6) is transmitted from parent to child in an integrated form and characterization of cases with chromosomally integrated HHV-6 DNA. *Journal of medical virology*, 73(3), pp.465-473.

Tang, H., Hayashi, M., Maeki, T., Yamanishi, K. and Mori, Y., 2011. Human herpesvirus 6 glycoprotein complex formation is required for folding and trafficking of the gH/gL/gQ1/gQ2 complex and its cellular receptor binding. *Journal of virology*, *85*(21), pp.11121-11130.

Tang, H., Serada, S., Kawabata, A., Ota, M., Hayashi, E., Naka, T., Yamanishi, K. and Mori, Y., 2013. CD134 is a cellular receptor specific for human herpesvirus-6B entry. *Proceedings of the National Academy of Sciences*, *110*(22), pp.9096-9099.

Taniguchi, T., Shimamoto, T., Isegawa, Y., Kondo, K. and Yamanishi, K., 2000. Structure of transcripts and proteins encoded by U79-80 of

human herpesvirus 6 and its subcellular localization in infected cells. *Virology*, *271*(2), pp.307-320.

Tas, S.W., Vervoordeldonk, M.J. and Tak, P.P., 2009. Gene therapy targeting nuclear factor-κB: towards clinical application in inflammatory diseases and cancer. *Current gene therapy*, 9(3), pp.160-170.

Tátrai, E., Hartyánszky, I., Lászik, A., Acsády, G., Sótonyi, P. and Hubay, M., 2011. The role of viral infections in the development of dilated cardiomyopathy. *Pathology & Oncology Research*, *17*(2), pp.229-235.

Toivonen, R., Mäyränpää, M.I., Kovanen, P.T. and Savontaus, M., 2010. Dilated cardiomyopathy alters the expression patterns of CAR and other adenoviral receptors in human heart. *Histochemistry and cell biology*, *133*(3), pp.349-357.

Tomita, T., Hashimoto, H. and Yoshikawa, H., 2003. Gene therapy for arthritis. *Current drug targets*, *4*(8), pp.609-612.

Tousoulis, D., Oikonomou, E., Economou, E.K., Crea, F. and Kaski, J.C., 2016. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. *European heart journal*, 37(22), pp.1723-1732.

Toyabe, S., Harada, W., Suzuki, H., Hirokawa, T. and Uchiyama, M., 2002. Large vessel arteritis associated with human herpesvirus 6 infections. *Clinical rheumatology*, 21(6), pp.528-532.

Tsao, E.H., Kellam, P., Sin, C.S., Rasaiyaah, J., Griffiths, P.D. and Clark, D.A., 2009. Microarray-based determination of the lytic cascade of human herpesvirus 6B. *Journal of general virology*, *90*(11), pp.2581-2591.

Valen, G., 2004. Signal transduction through nuclear factor kappa B in ischemia-reperfusion and heart failure. *Basic research in cardiology*, *99*(1), pp.1-7.

Valen, G., Hansson, G.K., Dumitrescu, A. and Vaage, J., 2000. Unstable angina activates myocardial heat shock protein 72, endothelial nitric oxide synthase, and transcription factors NFκB and AP-1. *Cardiovascular research*, 47(1), pp.49-56.

Vicentini, F.T., He, T., Shao, Y., Fonseca, M.J., Verri Jr, W.A., Fisher, G.J. and Xu, Y., 2011. Quercetin inhibits UV irradiation-induced inflammatory cytokine production in primary human keratinocytes by suppressing NF-κB pathway. *Journal of dermatological science*, 61(3), pp.162-168.

Villa, A., Rizzi, N., Vegeto, E., Ciana, P. and Maggi, A., 2015. Estrogen accelerates the resolution of inflammation in macrophagic cells. *Scientific reports*, 5(1), pp.1-14.

Wallaschek, N., Sanyal, A., Pirzer, F., Gravel, A., Mori, Y., Flamand, L. and Kaufer, B.B., 2016. The telomeric repeats of human herpesvirus 6A (HHV-6A) are required for efficient virus integration. *PLoS pathogens*, *12*(5), p.e1005666.

Ward, K.N., Leong, H.N., Nacheva, E.P., Howard, J., Atkinson, C.E., Davies, N.W., Griffiths, P.D. and Clark, D.A., 2006. Human herpesvirus 6 chromosomal integration in immunocompetent patients results in high levels of viral DNA in blood, sera, and hair follicles. *Journal of clinical microbiology*, *44*(4), pp.1571-1574.

Ward, K.N., Thiruchelvam, A.D. and Couto-Parada, X., 2005. Unexpected occasional persistence of high levels of HHV-6 DNA in sera:

detection of variants A and B. *Journal of medical virology*, *76*(4), pp.563-570.

Ward, K.N., Turner, D.J., Parada, X.C. and Thiruchelvam, A.D., 2001. Use of immunoglobulin G antibody avidity for differentiation of primary human herpesvirus 6 and 7 infections. *Journal of clinical microbiology*, *39*(3), pp.959-963.

Wood, M.L. and Royle, N.J., 2017. Chromosomally integrated human herpesvirus 6: models of viral genome release from the telomere and impacts on human health. *Viruses*, *9*(7), p.184.

World Health Organization, 2019. Health topics: cardiovascular disease. Fact Sheet. Available online: http://www. who. int/cardiovascular_diseases/en/(accessed on 11 December 2020).

Wu, C.Y., Hu, H.Y., Chou, Y.J., Huang, N., Chou, Y.C. and Li, C.P., 2015. High blood pressure and all-cause and cardiovascular disease mortalities in community-dwelling older adults. *Medicine*, *94*(47).

Yamanishi, K., Mori, Y. and Pellett, P.E., 2013. Human herpesviruses 6 and 7, p 2058–2079. *Fields virology*,, *2*.

Yan, S., Zhang, X., Zheng, H., Hu, D., Zhang, Y., Guan, Q., Liu, L., Ding, Q. and Li, Y., 2015. Clematichinenoside inhibits VCAM-1 and ICAM-1 expression in TNF-α-treated endothelial cells via NADPH oxidase-dependent IκB kinase/NF-κB pathway. *Free Radical Biology and Medicine*, 78, pp.190-201.

Yazdanyar, A. and Newman, A.B., 2009. The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs. *Clinics in geriatric medicine*, *25*(4), p.563.

Yoshikawa, T., Ihira, M., Suzuki, K., Suga, S., Kito, H., Iwasaki, T., Kurata, T., Tanaka, T., Saito, Y. and Asano, Y., 2001. Fatal acute myocarditis in an infant with human herpesvirus 6 infection. *Journal of clinical pathology*, *54*(10), pp.792-795.

Youn, G.S., Kwon, D.J., Ju, S.M., Rhim, H., Bae, Y.S., Choi, S.Y. and Park, J., 2014. Celastrol ameliorates HIV-1 Tat-induced inflammatory responses via NF-kappaB and AP-1 inhibition and heme oxygenase-1 induction in astrocytes. *Toxicology and applied pharmacology*, 280(1), pp.42-52.

Zandi, E., Rothwarf, D.M., Delhase, M., Hayakawa, M. and Karin, M., 1997. The IkB kinase complex (IKK) contains two kinase subunits, IKKa and IKKb, necessary for IkB phosphorylation and NF-kB activation. *Cell*, 91(2), pp.243-252.

Zerr, D.M., Meier, A.S., Selke, S.S., Frenkel, L.M., Huang, M.L., Wald, A., Rhoads, M.P., Nguy, L., Bornemann, R., Morrow, R.A. and Corey, L., 2005. A population-based study of primary human herpesvirus 6 infection. *New England Journal of Medicine*, *352*(8), pp.768-776.

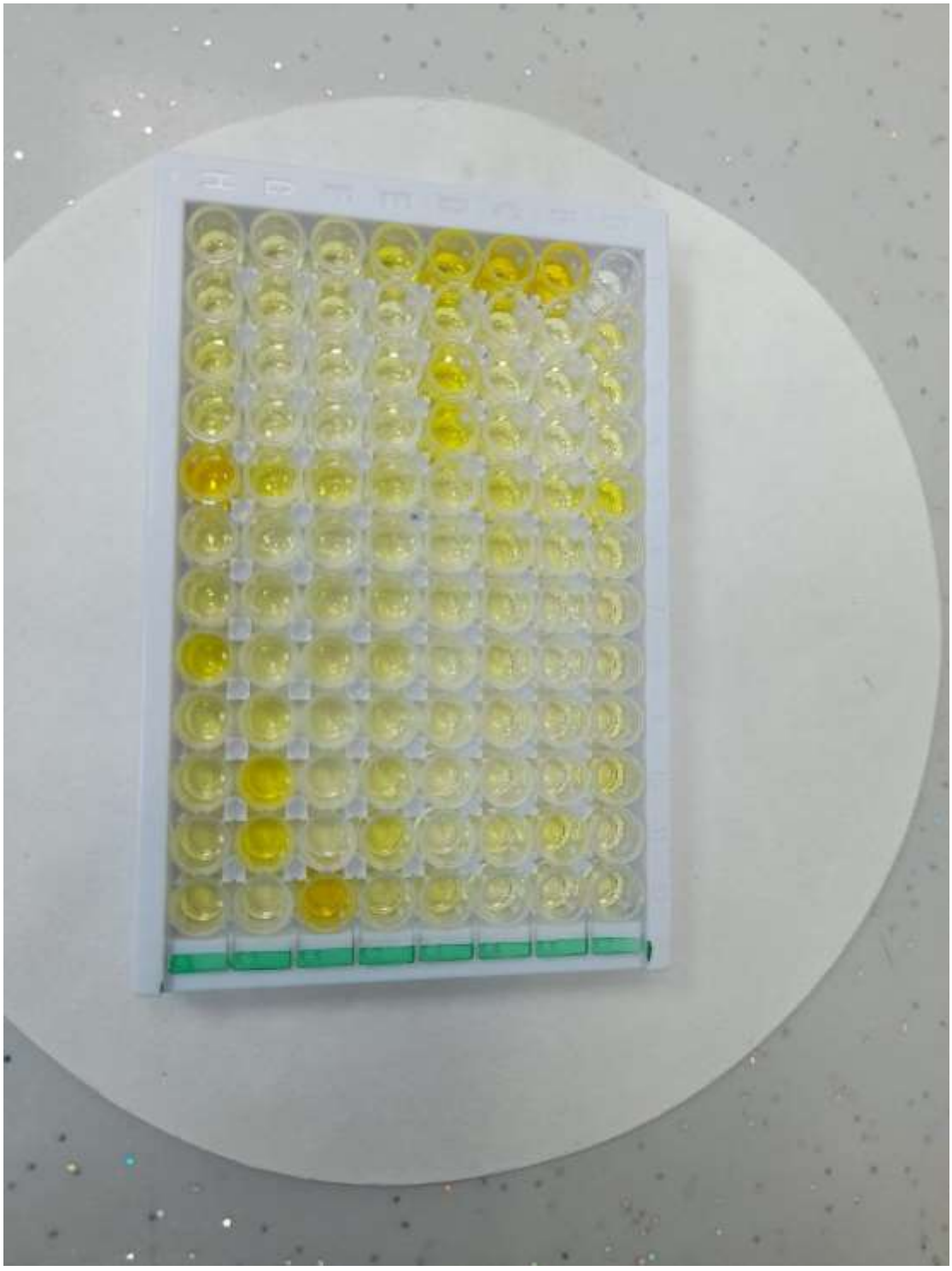
Zhang, E., Cotton, V.E., Hidalgo-Bravo, A., Huang, Y., Bell, A.J., Jarrett, R.F., Wilkie, G.S., Davison, A.J., Nacheva, E.P., Siebert, R. and Majid, A., 2016. HHV-8-unrelated primary effusion-like lymphoma associated with clonal loss of inherited chromosomally-integrated human herpesvirus-6A from the telomere of chromosome 19q. *Scientific reports*, *6*(1), pp.1-9.

Zhou, Z.H., Chen, D.H., Jakana, J., Rixon, F.J. and Chiu, W., 1999. Visualization of tegument-capsid interactions and DNA in intact herpes simplex virus type 1 virions. *Journal of virology*, *73*(4), pp.3210-3218.

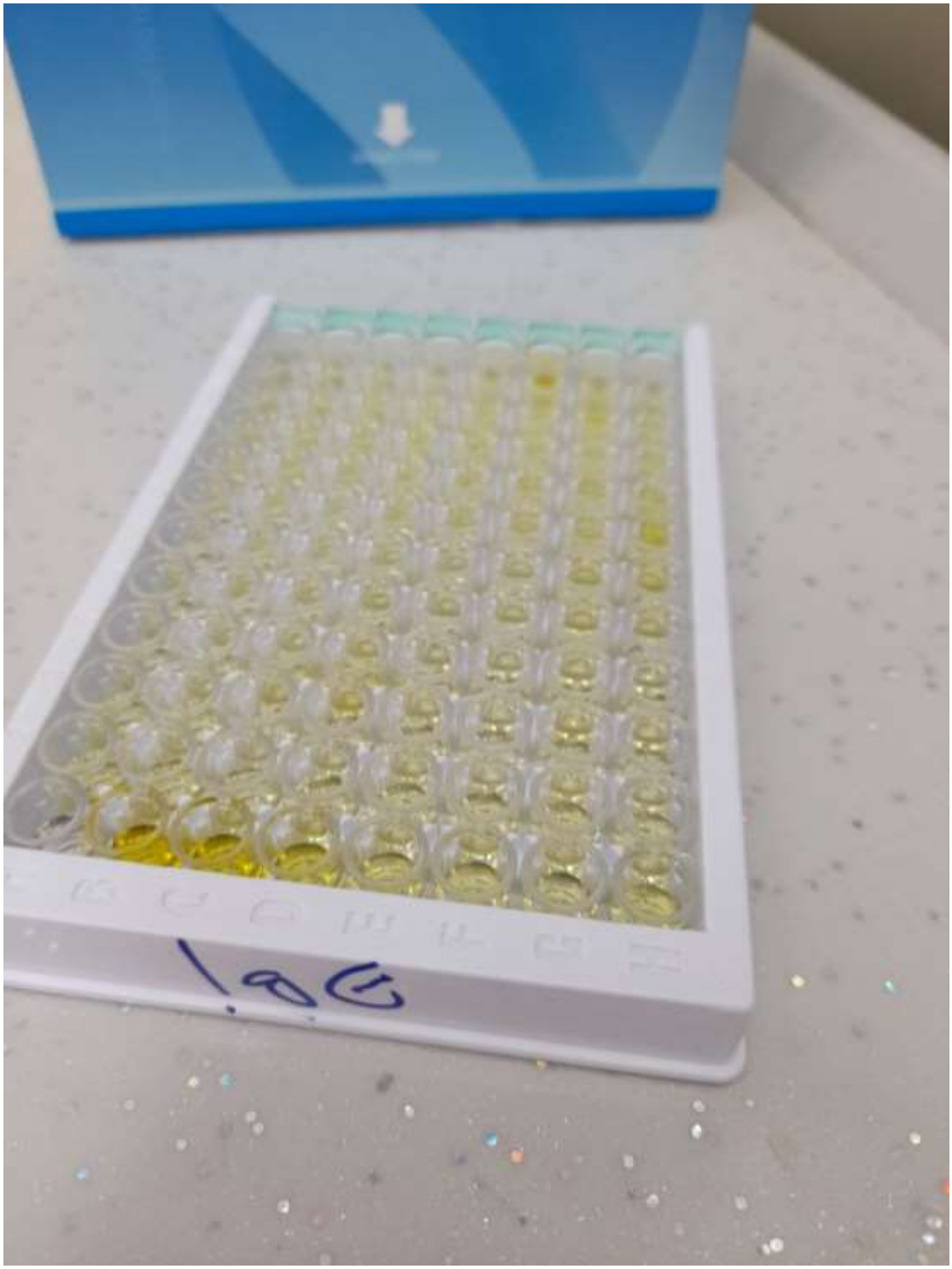
Zou, P., Isegawa, Y., Nakano, K., Haque, M., Horiguchi, Y. and Yamanishi, K., 1999. Human herpesvirus 6 open reading frame U83 encodes a functional chemokine. *Journal of Virology*, *73*(7), pp.5926-5933.

Appendiex

ELISA (BioTek)



Result of NF-kB in ELISA technique



Result of anti HHV-6 IgG in ELISA technique

questionnaires:

Name

Gender

Age

smoking history (yes/no)

type of heart disease

High blood pressure (hypertension): Yes or No

Hhstory of diabetes (diabetes mellitus): Yes or No

Address

Mobile phone number.

HHV-6 ELISA Kit

1.	**ELISA**
Name of the pro duct	**Human herpesvirus 6(HHV-6) ELISA Kit**
The list's number	SL3291 Hu
Description	ELISA human herpes virus-6 uses Sandwish-Elisa as a method for a measure of hhv-6 levels in human serum. Sensitivity:1 ng/L.
Content	• Instructions for use. • The closing plate's membrane. • Sealing bags • Stripplate microelisa . • The standard is 180 ng/L. • Standard diluent. • A conjugation reagent for HRP. • The sample's diluent. • Solution A for Chromogens . • Solution B for Chromogens • A stop Solution. • The Wash Solution
Origin	**SUNLONG(CHINA)**

Human herpesvirus 6 antibody IgM ELISA Kit

	ELISA Kit
Name of the pro duct	**Human herpesvirus 6 antibody IgM ELISA Kit**
The number of the list	SL3293Hu
Description	The Sandwish-Elisa is used to assess the HHV6 IgM level in human serum using a human he rpes virus 6 antibody IgM (HHV6 IgM) ELISA kit. Sensitivity:1 ng/L.
Content	• Instructions for use. • The closing plate's membrane. • Sealing bags. • Stripplate microelisa • The standard is 90 ng/L. • Standard diluent. • HRP conjugation reagent. • The sample's diluent • Chromogens (Solution A) • Solution B for Chromogens • A stop Solution. • The Wash Solution
Origin	**SUNLONG** (China)

Human herpesvirus 6 antibody IgG ELISA Kit

3.	**ELISA**
Name of the product	**Human herpesvirus 6 antibody IgG ELISA Kit**
The number of the list	SL 3292 Hu
Description	IgG antibody of Human herpes virus 6 (HHV6 IgG)ELISA kit is to assay HHV6 IgG level in human serum, Sandwish-Elisa is used. Sensitivity:1 ng/L.
Content	• Instructions for use. • The closing plate's membrane. • The sealed bags. • Microelisa stripplates • The industry standard is 225ng/L. • The standard diluent. • The HRPs conjugation reagent. • Solution A for Chromogens • Solution B for Chromogens • A stop Solution. • The Wash Solution
Origin	**SUNLONG(CHINA)**

ELISA Kit for Human Nuclear Factor Kappa B (NFkb)

	ELISA
Name of the pro duct	**ELISA Kit for Human Nuclear Factor Kappa B (NFkb)**
The number of the list	SL1288Hu
Description	NFKb ELISA kit for human nuclear factor kappa B to assay NFKb level in human serum ,sandwich ELISA is used ,sensitivity 1 ng/L ,
Contents	• Instructions for use. • The closing plate's membrane. • sealing bags. • Microelisa stripplated. • The standard 5.4 ng/L. • standard for diluents. • A conjugation reagent for HRP. • A diluent to dilute the sample. • The Solution A for Chromogens. • The Solution B for Chromogens. • A stop Solution. • The Wash Solution.
Origin	**SUNLONG(CHINA)**

الخلاصه:

يتسبب فيروس الهربس البشري السادس سلاله أ وفيروس الهربس البشري السادس سلاله ب- في حدوث عدوى مستمرة في المضيف تدوم مدى الحياة ، والتي يشار إليها باسم الاختباء.

العامل النووي كابا محسن السلسلة الخفيفة للخلايا البائية المنشطه هو مركب بروتيني ينظم نسخ الحمض النووي وإنتاج السيتوكينات وبقاء الخلية. العامل النووي مطلوب للتنظيم السليم وللاستجابة المناعية للعدوى. الارتباط او التنظيم غير المناسب لـ العامل النووي كابا محسن السلسلة الخفيفة للخلايا البائية المنشطه يرتبط بالسرطان والأمراض الالتهابية وأمراض المناعة الذاتية والصدمة الإنتانية والعدوى الفيروسية والتطور المناعي غير الطبيعي.

ارتبط التهاب عضلة القلب مع عدوى فايروس الهربس البشري السادس واعتلال عضلة القلب التوسعي مجهول السبب "ضعف البطين الأيسر في كل من الأشخاص الذين يعانون من نقص المناعة والأشخاص ذوي الكفاءة المناعية.

هدفت هذه الدراسة إلى الكشف عن العلاقة بين العامل النووي كابا محسن السلسلة الخفيفة للخلايا البائية المنشطه في مرضى القلب والأوعية الدموية المصابين بفيروس الهربس البشري السادس من خلال مقايسة المواد الماصه المناعيه المرتبطه بالإنزيم (ELISA) من خلال الكشف عن مستضد الهربس البشري السادس ومضاد الهربس البشري السادس نوع(HHV6 IgM) ومضاد فيروس الهربس البشري السادس نوع(HHV6 IgG) في مصل الأفراد الذين يعانون من اضطرابات القلب والأوعية الدموية.

الدراسة عبارة عن دراسة مقطعية أجريت في مدينة الصدر التعليمية في محافظة النجف من الفترة ما بين نوفمبر ٢٠٢٠ ومايو٢٠٢١. تم تضمين٢٠٠ مريض يعانون من أمراض القلب والأوعية الدموية في الدراسة ، كان هناك ٥٠ أنثى و١٥٠ ذكر وكان متوسط عمر المرضى (٣٠_١٠٤) سنة. تم إجراء الاختبارات المعملية باستخدام التقنيات المصلية ، وخاصة الساندويتش اليزا لعينات مصل المريض. تم إجراء التحليل الوصفي والإحصاء على برنامج الاس بي اس اس الإصدار٢٤.

في هذه الدراسة ،وجد ان ٦٠ (٣٠٪) من مجموع ٢٠٠ مريض يعانون من أمراض القلب والأوعية الدموية إيجابيين لـ مستضد فيروس الهربس البشري السادس . كانت هناك نتيجة مهمة للغاية لقيم المستضد نوع IgM لفيروس الهربس البشري السادس بين المرضى الذين

يعانون من وجود ايجابيه مستضد فيروس الهربس البشري السادس بقيمة احتماليه اقل من ٠,٠٠٠٠١ ، مع مستويات عالية الأهمية لمتوسط مضاد نوع IgM. كانت القيم الإيجابية لـ لمضاد فيروس الهربس البشري السادس نوع IgG بين المرضى الذين لديهم مستضد موجب لفيروس الهربس البشري السادس إيجابية بقيمة احتماليه اقل من٠,٠٠٠٠١ ، مع مستويات عالية الأهمية من متوسط مضاد الفيروس الهربس البشري السادس نوع IgG. تم العثور على نتائج مهمة للغاية لمستويات العامل النووي كابا الإيجابية في المرضى الذين يعانون من مستضد فايروس الهربس البشري السادس الإيجابي مقارنة مع أولئك الذين لديهم سلبية مستضد الهربس البشري السادس مع قيمة احتماليه اقل من٠,٠٠٠٠١ .

وجد ان هنالك ارتباط كبير بين النتائج الإيجابية لكل من مضادات فايروس الهربس البشري السادس نوع IgM و IgG مع العامل النووي .

لم يكن هناك ارتباط معنوي بين أنواع مختلفة من أمراض القلب والأوعية الدموية في مجتمع الدراسة وإيجابية علامات الدراسة مع قيمة الاحتماليه اكبر من ٠,٠٥ .

الاستنتاج : هناك نسبة ملحوظة من المرضى الذين يعانون من أمراض القلب والأوعية الدموية لديهم عدوى بفيروس الهربس البشري السادس وقد تكون الزيادة في مستوى العامل النووي بسبب عدوى فيروس الهربس البشري السادس والتهاب أنسجة القلب

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء / كلية الطب
فرع الاحياء المجهرية

تقييم العامل النووي كابا في مرضى القلب والأوعية الدموية المصابين بعدوى فايروس الهربس البشري السادس

رسالة مقدمة الى
مجلس كلية الطب جامعة كربلاء
كجزء من متطلبات نيل شهادة الماجستير في الاحياء المجهرية الطبية
من قبل

حوراء هيثم محمد الزبيدي
بكالوريوس تقنيات تحليلات مرضية/ كلية التقنيات الصحية والطبية /كوفة (2015)

بأشراف
أ.م.د. سوسن محمد جبار الحسناوي
أ.د. سيف جباریاسر المياح

2021م 1443هـ