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Techniques

Association of Human Herpes Virus 6 with IL_17 polymorphism in Patients with Acute Myeloid Leukemia

A research submitted to Hilla University College to obtain a bachelor's degree in the Department of pathological analyzes

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بِسمِ اللَّه الرحمن الرَحيم

﴿قَالَ الَّذِي عِنْدَهُ عِلْمٌ مِنَ الْكِتَابِ أَنَا آتِيكَ بِهِ قَبْلَ أَنْ يَرْتَدَّ إِلَيْكَ طَرْفُكَ فَلَمَّا رَآهُ مُسْتَقِرًّا عِنْدَهُ قَالَ هَذَا مِنْ فَضْلِ رَبِّي لِيَبْلُوَنِي أَأَشْكُرُ أَمْ أَكْفُرُ وَمَنْ شَكَرَ فَإِنَّمَا يَشْكُرُ لِنَفْسِهِ وَمَنْ كَفَرَ فَإِنَّ رَبِّي غَنِيٌّ كَرِيمٌ﴾

صدق الله العلي العظيم

سورة النمل آية (٤٠)

Dedication

I dedicated this study Report

To the purest heart to my role model, and my ideal in life; He is the one who taught me how to live with dignity and loftiness… My dear father

To the heaven of God on earth, to the bridge that ascends me to heaven, to my ideal… My mother

To the eyes and heartbeat You were and still my support, lean and fame in all stages of life. ...to … My brother & sisters

To anyone who helped me and was by my side throughout the study period, to everyone who was happy for my joy and prayed for me with all his heart,

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Acknowledgments I would like to express my deep gratitude to God Almighty for allowing me to undergo this life-changing operation. Thanks to him, I was able to undertake and complete this study. To Him I give honor and glory. My sincere gratitude and appreciation to my father, mother, and supervisor whose supervision, constructive criticism, and support contributed greatly to the writing of this project. Thank you all for the academic feed. I would also like to thank and appreciate the ongoing help and advice from my classmates. That's why I will always cherish the memories we made together. Finally, I thank you all for the unwavering moral and material support you have given me throughout my studies.

Abstract

Human herpes virus-6 (HHV-6) was first isolated from peripheral blood leucocytes of patients with lymphoproliferative disorders, including lymphoma and leukemia. Th17 cells are blamed for being accused in the pathogenesis of acute myeloid leukaemia. Th17 cells are CD4+ cell subtype. They produce IL-17A and IL-17F. Genetic polymorphisms leading to defects in the IL-17-axis may alter the ability to elicit effective immune responses.

Acute myeloid leukaemia (AML) is an aggressive haematological malignancy resulting from the clonal expansion of abnormal myeloid progenitors (1) . T helper lymphocytes produce pro-inflammatory interleukin-17 (IL-17) that plays a critical role in many types of cancers and inflammatory diseases (2) IL-17 increases in the bone marrow and blood in AML patients more than in healthy controls (3) . IL-17A (rs2275913) polymorphisms might be responsible for chronic inflammatory diseases, autoimmune diseases and malignancies as an inducer of inflammatory chemokines secretion also release cytokines stored in neutrophils and macrophages (4) . We aimed to evaluate the expression of IL-17A gene polymorphism and IL-17 serum level in adult AML patients and to clarify its prognostic significance.

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1. Introduction

Acute myeloid leukemia (AML) is the most common leukemia among the adult population and accounts for about 80% of all cases. It is characterized by clonal expansion of immature "blast cells" in the peripheral blood and bone marrow resulting in ineffective erythropoiesis and bone marrow failure. With recent advancements in the management guidelines, the cure rates have increased up to 15% in patients older than 60 years and about 40% in patients below 60 years of age. Despite advancements in therapeutic regimens, the prognosis remains very poor in the elderly population (Naymagon *et al*.,2019).

Human herpesvirus 6 (HHV-6) was the sixth human herpesvirus discovered. It belongs to the β-Herpesvirinae subfamily. Although horizontal transmission is considered to be the main route of HHV-6 infection, it can be genetically transmitted from parent to child as inherited chromosomally integrated HHV-6 (iciHHV-6) (Miura *et al*.,2018).

The complete HHV-6 genome is integrated into every nucleated cell of an individual with iciHHV-6. Extremely high copy numbers of HHV-6 DNA can be detected in clinical specimens containing nucleated cells, which can lead to a misdiagnosis of active viral infection. Theoretically, a parent with iciHHV-6 has a 50% chance of transmitting the integrated HHV-6 genome to the next generation (Gaccioli *et al*.,2020) HHV-6 can integrate into human chromosomes, resulting in genetic transmission from parent to child. Individuals of either sex with inherited chromosomally integrated human herpesvirus 6 (iciHHV-6) harbor the virus in every cell. Viral reactivation from the integrated HHV-6 genome can occur in pregnancy (Miura *et al*.,2020). IL-17 stimulates expansion of myeloid progenitors and proliferation of mature neutrophils. IL17 is an indirect antigenic factor. It enhances tumor micro-vessel e (SNPs) can

alter gene functions and protein expression or function thus influencing cell proliferation increasing cancer risk.

1.2. Aim of Study: -

Considering all these points, this research study was designed to detection of Human Herpes Virus-6 A&B **(HHV-6 A& B), IL-17 A rs2275913** and genes polymorphism from patients with Acute Myeloid Leukemia (AML) through achieving the following Objectives:

1- Determine the relation of HHV-6 A& B in blood specimens that range from apparently healthy persons to patients with AML .

2- Estimation of IL17A rs2275913 and genes polymorphism in patients With AML

3- Find the association between these of IL-17 A rs2275913 ; gene polymorphism and HHV-6 A& B among study population .

CHAPTER TWO \ Literature RevieW

1-Acute Myeloid Leukemia (AML)

Acute myeloid leukemia (**AML**) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal cells that build up in the bone marrow and blood and interfere with normal blood cell production. Symptoms may include feeling tired, shortness of breath, easy bruising and bleeding, and increased risk of infection. Occasionally, spread may occur to the brain, skin, or gums. As an acute leukemia, AML progresses rapidly, and is typically fatal within weeks or months if left untreated **(**Bain *et al*.,2019**).**

1-1Risk Factors

Most cases of AML do not have exposure to any identified risk factors. However, a number of risk factors for developing AML have been identified. These include other blood disorders, chemical exposures, ionizing radiation, and genetic risk factors. Where a defined exposure to past chemotherapy, radiotherapy, toxin or hematologic malignancy is known, this is termed secondary *AML* (Maleki *et al*.,2021).

I. Other blood disorders

Other blood disorders, particularly myelodysplastic syndrome (MDS) and less commonly myeloproliferative neoplasms (MPN), can evolve into AML; the exact risk depends on the type of MDS/MPN. The presence of asymptomatic clonal hematopoiesis also raises the risk of transformation into AML (Khoury *et al*.,2022).

II.Chemical exposure

Exposure to anticancer chemotherapy, in particular alkylating agents, can increase the risk of subsequently developing AML. Other chemotherapy agents, including fludarabine, and topoisomerase II inhibitors are also associated with the development of AML; most commonly after 4–6 years and 1–3 years respectively. (Sachiko, 2012).

1-2 Pathophysiology

Acute myeloid leukemia is characterized by mutations of the genes involved in hematopoiesis. These mutations result in a clonal expansion of undifferentiated myeloid precursors (blasts) in the peripheral blood and bone marrow resulting in ineffective erythropoiesis and bone marrow failure. Recent studies also revealed that it could arise from a series of recurrent hematopoietic stem cell genetic alterations which get accumulated with age . In most cases, AML appears as *de novo* in a previously healthy person. The exact cause of genetic mutations is unclear, but few risk factors include exposure to radiation, chemotherapeutic agents, and smoking. AML can also evolve from myeloproliferative disorders (MPD), myelodysplastic syndrome (MDS), paroxysmal nocturnal hemoglobinuria, and aplastic anemia. Familial causes of genetic mutations should also be considered (Schmid *et al*.,2019).

Acute myeloid leukemia is a highly heterogeneous disease with a variable prognosis. It can result from genetic mutations, chromosomal translocations, or changes in molecular levels. About 97% of the cases have been studied to have genetic mutations. Despite its heterogeneity, it can be categorized into favorable, intermediate, or adverse-risk groups based on cytogenetics.

1-3 Viral Infection and AML

The most common viruses to cause trouble in AML patients belong to the herpes family of viruses, mostly Human herpes virus 1 (HHV1), chicken pox virus (or varicella zoster virus (VZV), cytomegalovirus (CMV) and Human herpes virus 6 (HHV-6) (Innao *et al.,* 2020; Voigt *et al.,* 2021).

2-HUMAN HERPES VIRUS-6 (HHV-6)

2.1 Historical Preview:

Herpesvirus was established as a genus in 1971 in the first report of the International Committee on Taxonomy of Viruses (ICTV).

During 1986, Syed Kaki Salah Uddin, Dharma Ablashi, and Robert Gallo cultivated peripheral blood mononuclear cells from patients with AIDS and lymphoproliferative illnesses. Short-lived, large, refractile cells that frequently contained intranuclear and/or intracytoplasmic inclusion bodies were documented.

Electron microscopy revealed a novel virus that they named Human BLymphotrophic Virus (HBLV) (Kawabata etal.,2011).

HHV-6 was divided into subtypes. Early research (1992) described two very similar, yet unique variants: HHV-6A and HHV-6B. The distinction was warranted due to unique restriction endonuclease cleavages, monoclonal antibody reactions, and growth patter ns (Kawabata *et al.*,2011).

Shortly after its discovery, Ablashi ***et al.,(2006)*** described five cell lines that can be infected by the newly discovered HBLV. They published that HSB-2, a particular

T-cell line, is highly susceptible to infection. Ablashi's pioneering research concluded by suggesting that the virus name be changed from HBLV to HHV-6, in accord with the published provisional classification of herpes viruses.

2.2 Taxonomy and Classification of HHV6

The family Herpesviridae was divided into 3 subfamilies (alphaherpesvirinae , betaherpesvirinae and gammaherpesvirinae) and 5 unnamed genera; 21 viruses were recognized as members of the family as the following (Rizzo *et al.*,2017;Yaara *et al.*,2020).

I.α herpesviruses: Herpes simplex virus types 1 and 2, and varicella-zoster virus, which have a short replicative cycle, induce cytopathology in monolayer cell cultures, and have a broad host range.

II.β herpesviruses: Cytomegalovirus, and human herpesviruses 6 and 7, with a long replicative cycle and restricted host range.

2.3. Morphology and Structure of HHV-6

2.3.1 HHV-6 Particles:

The diameter of an HHV-6 virion is about 2000 nanometer. The size of the HHV-6 virion increases from 120 nm to approximately 300 nm after the inclusion of the tegument and envelope (Roizman *et al.*,2001).

The virion's outer portion consists of a lipid bilayer membrane that contains viral glycoproteins and is derived from that of the host. Below this membrane envelope is a tegument which surrounds an icosahedral capsid, composed of 162 capsomeres. The protective capsid of HHV-6 contains double stranded linear DNA (Kawabata et al ;2009)

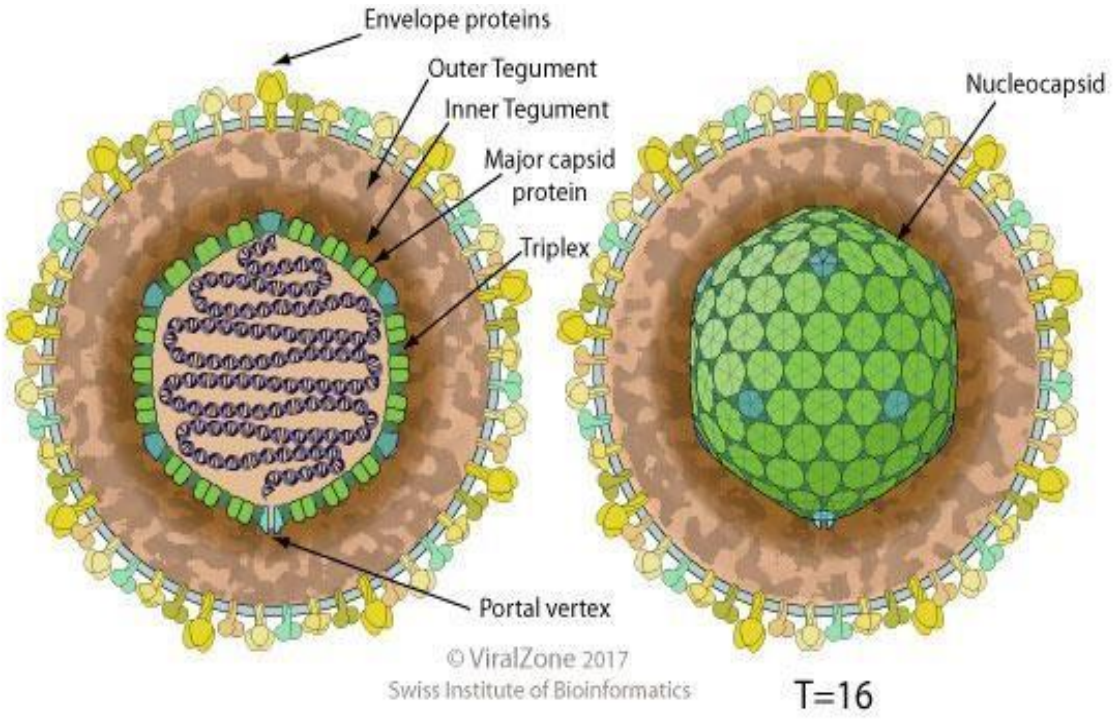


Figure (2.1): HHV-6 structure (Julia *et al*.,2014)

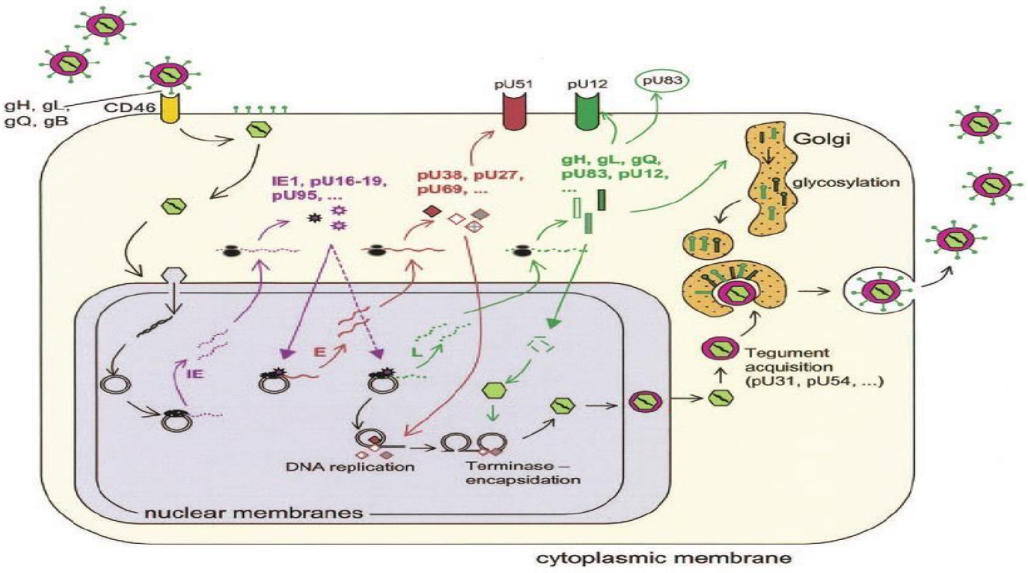
2.3.2. HHV-6 Genome Organization

The genomes of HHV-6A and HHV-6B, similar to those of other herpesviruses, consist of large linear double stranded DNA molecules, 160 kb in length, containing a unique segment flanked by direct repeats (Finkel Y. *et al*.,2020).

The genetic material of HHV-6 is composed of linear (circular during an active infection), double stranded DNA which contains an origin of replication, two 8–10 kb left and right direct repeat termini, and a unique segment that is 143–145kb (Tang *et al*.,2010).

2.3.4: Replication Cycle of HHV6

The HHV6 replication cycle takes 72 hours to complete and consists of four general steps as shown in figure (2-5)



I.HHV-6 receptor and Host Tropism

HHV-6 infects a wide range of human cells in vitro, but it preferentially replicates in activated $CD4^+$ T lymphocytes. HHV-6A and HHV-6B recognize their specific receptors by differentiating gQ1 and gQ2 (Tang *et al*.,2014).

III. HHV6 Attachment

HHV-6 attaches to its cell receptor by means of a tetrameric viral ligand complex made up of the glycoproteins H (gH), L (gL), Q1 (gQ1), and Q2 (gQ2) (Yamanishi *et al*.,2013).

IV. Entry and Un-coating

Following attachment, HHV-6 entry into cell occurs through a fusion between the viral envelope and the cell membrane by a mechanism which involves gB and gH functions but remains poorly understood. The nucleocapsid is then transported through the cytoplasm to the nucleus, likely using the pathway of the microtubule network. HHV-6 DNA is released into the nucleoplasm (Henri *et al*.,2015).

V. Impacts of HHV6 Gene Expression on Cell Functions

Viral genes are expressed in a temporally ordered manner, starting with immediate early (IE) genes from the IE-A locus, which is constituted of two genetic units, IE1 and IE2 . Those genes are transcribed in the absence of de novo protein synthesis, and this step is followed by the transcription/expression of early (E) and late (L) genes. The replication of the genome occurs after the synthesis of E proteins, which have enzymatic activities dedicated to nucleotide metabolism and DNA synthesis, i.e., phosphotransferase, ribonucleotide reductase, uracil-DNA glycosylase, origin-binding protein, DNA polymerase, polymerase processivity factor, major DNA-binding protein, and helicase-primase complex activities (Tsao *et al*.,2009) .

VI. HHV6 Releasing

The capsids exit the nucleus, acquiring an intermediate envelope by budding through the inner part of the nuclear membrane, are de enveloped by fusion with the external part of this membrane, and appear as tegumentary forms in the cytoplasm. The acquisition of the final envelope carrying viral glycoproteins

2.3.6: Latency and Reactivation

Like other human herpesviruses, HHV-6 persists indefinitely in its host and is capable of reactivation, meaning the active production of detectable mature virions in some body compartments following a phase of apparently complete clearance. These properties rely on the putative capacity of its genome to be maintained in a nuclear latent form or to drive a low-level productive infection in some cells while inducing a fully lytic infection in other cells. For other human herpesviruses, such as herpes simplex virus, the latent DNA genome has the form of a covalently closed circular episome associated with cellular nuclear proteins. The existence of such a latent nuclear form has not been demonstrated formally for HHV-6, although an episomal state was shown after experimental infection of cervical carcinoma cell lines (Tweedy *et al*.,2015).

3. Transmission of HHV6

Transmission is believed to occur most frequently through the shedding of viral particles into saliva. Both HHV-6B and HHV-7 are found in human saliva, the former being at a lower frequency. Studies report varying rates of prevalence of HHV-6 in saliva (between 3–90%), and have also described the salivary glands as an in vivo reservoir for HHV-6. The virus infects the salivary glands, establishes latency, and periodically reactivates to spread infection to other hosts (Arbuckle *et al*.,2011; Araujo *et al*.,2011).

Vertical transmission has also been described, and occurs in approximately 1% of births in the United States. This form is easily identifiable as the viral genome is contained within every cell of an infected individual(Araujo *et al*.,2011).

4. HHV-6 and Disease Association

Several diseases have been associated with HHV-6A/B reactivation in adults, although the causal correlations are still unproven. Most studies on pathogenic association do not specify the HHV-6 virus species, which, however, can be inferred by the reference strains used in the methods(Elisabetta *et al*.,2020)

I.HHV-6A/6B-associated with neurological diseases

II.HHV-6A/6B correlated to multiple sclerosis

III.HHV-6A correlated with Hashimoto's thyroiditis

III.H HV-6A and infertility

IV. HHV-6A and fulminant hepatic failure (HHV-6A/6B)

V. HHV-6A and chronic fatigue syndrome (HHV-6A)

VI. HHV-6A and neoplasia(Miyagawa *et al*.,2016).

VII. HHV-6A and myocarditis, drug reaction with eosinophilia, and systemic symptoms (HHV-6A/6B) (Miyagawa *et al*.,2016).

VIII. HHV-6A and Alzheimer's disease

IX. HHV-6B and COVID-19 patient

X.HHV6 and Spontaneous Abortion

5. ***HHV-6A & B and Leukemia***

The preponderance of data suggests no association between HHV-6 and leukemia. Contrasting findings, and differences in HHV-6 species predominating in bone marrow of leukemia patients, may stem from the use of different probes for HHV-6A and/or divergence in HHV-6A across geographical areas. one group has investigated HHV-6 antigen expression in leukemia, with intriguing results: HHV-6 early antigen p41 was detected in bone marrow cells—blasts and megakaryocytes (Eliassen *et al*.,2018).

On other study in iraq were revealed only 14% (14 out of 100) and **16**% (16 out of 100) of the AML specimens results are positive for HHV-6A and HHV-6 B genome detection, respectively(n. worood2023). The present result of HHV6 is compatible with Petr *et al*.,(2009) who found the percent of HHV6 in in patients with AML was 26.8% (107 samples from 91 patients).

Voigt *et al*.,(2021) who report a case of a clinically manifest human herpesvirus6 (HHV-6) encephalitis in a neutropenic patient with acute myeloid leukemia (AML) in a non-transplant setting while on antimicrobial prophylaxis including aciclovir.

6 Immune Response to HHV6

6.1 Innate Immune Responses To HHV6 Infection

Both HHV-6A and -6B establish a latent infection in the host following resolution of primary infection. Reactivations in the adult have been associated to the development of multiple symptomatic diseases often characterized by immune dysregulation (multiple sclerosis, Sjögren's syndrome, autoimmune thyroiditis, and others). Both viruses are considered lymphotropic, showing an elective tropism for CD4+ T-lymphocytes and being able to infect several different cell types of the immune system, including NK cells (Rizzo *et al*., 2017).

6.2. Adaptive Immune Responses To HHV6 Infection

I. Cellular Response to HHV-6

Information on HHV-6-specific T cell responses is still limited, in particular regarding CD8 T cells . It was shown early that healthy virus carriers have CD4 T cells that respond to HHV-6 lysate or infected cells (Becerra *et al*.,2014).

Target antigens and epitopes of the specific CD4 T cell response were identified first in a study on six selected structural proteins , and more recently by a proteomic approach that has identified ten viral antigens targeted by CD4 T cells (Becerra-Artiles *et al*.,2015).

II. Antibody Response to HHV-6

During primary infection, anti-IgG and anti-IgM antibodies are produced, with IgM antibodies being the first to be detected . IgG titer begins to increase about one week post infection and peaks a week later. Additionally, there is an increase in IgG avidity over the course of infection (Ward ,2013).

7. Interleukin- 17 A (IL -17A)

7.1.Defention

Interleukin-17A is a protein that in humans is encoded by the *IL17A* gene. In rodents, IL-17A used to be referred to as CTLA8, after the similarity with a viral gene (O40633) (Chen *et al*.,2017).

7.*2.* Structure

IL-17(A) is a 155-amino acid protein that is a disulfide-linked, homodimeric, secreted glycoprotein with a molecular mass of 35 kDa. Each subunit of the homodimer is approximately 15-20 KDa. The structure of IL-17 consists of a signal peptide of 23 amino acids (aa) followed by a 123-aa chain region characteristic of the IL-17 family. An N-linked glycosylation site on the protein was first identified after purification of the protein revealed two bands, one at 15 KDa and another at 20 KDa (Chen *et al.*,2017).

Comparison of different members of the IL-17 family revealed four conserved cysteines that form two disulfide bonds. IL-17 is unique in that it bears no resemblance to other known interleukins. Furthermore, IL-17 bears no resemblance to any other known proteins or structural domains (Mohammed & Al-Janabi , 2021).

7.*3.* Gene expression

The gene for human IL-17A is 1874 base pairs long and was cloned from CD4+ T cells. Each member of the IL-17 family has a distinct pattern of cellular expression. The expression of IL-17A and IL-17F appear to be restricted to a small group of activated T cells, and upregulated during inflammation (Eileen and Kepeng ,2022).

7.*4. Function*

The protein of IL-17 A encoded by this gene is a proinflammatory cytokine produced by activated T cells. This cytokine regulates the activities of NF-kappaB and mitogen-activated protein kinases. This cytokine can stimulate the expression of IL6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide *(Mandy et al.,2019).*

7.5. IL-17 A Polymorphism and AML

IL-17 is a proinflammatory cytokine family produced by Th-17 cells and has been found to be implicated in the pathophysiology of many cancers including acute myeloid leukemia (AML). Since single nucleotide polymorphism (SNP) alters the genetic functions and cancer susceptibility, we studied SNPs in two members of IL-17 family, IL-17A (rs2275913; G-197A) and IL-17F (rs763780; A7488G) which are the most common loci associated with IL-17 activity and cancer risk, and correlated the results to AML susceptibility and response to therapy (*Rania et al.,2020)*.

IL-17 increases in the bone marrow and blood in AML patients more than in healthy controls . IL-17 can activate MAPK, PI3K/Akt, NF-κB, and STAT3 downstream signaling pathways to regulate AML progression. IL-17A (rs2275913) polymorphisms might be responsible for chronic inflammatory diseases, autoimmune diseases and malignancies as an inducer of inflammatory chemokines secretion also release cytokines stored in neutrophils and macrophages (Ahmed *et al.,2020)*.

8. Laboratory Diagnostic of HHV6

8.1. Indirect (Serology)

Assays for IgG and IgM detection by

immunofluorescence assay (IFA) ; enzyme-linked immunosorbent assay (ELISA) and avidity assays (Ana Lia *et al* ., 2020).

8.2. Tissue Culture and Cell lines

I. Cell lines and HHV6

Single-cell cloning integration assay.

8.3. Antigen detection

8.4. Qualitative viral DNA PCR

8.5. Quantitative viral DNA real-time PCR

8.6. Detection of viral transcripts by RT-PCR

8.7. Droplet digital PCR

Conclusions

The Following Conclusions are obtained from the Present Study

- Age factor has a significant association with viral infection, rather than with the differences of mean ages of the HHV-6.
- HHV-6 might be one of the most recently identified viruses in Iraqi patients suffering from AML in the Iraqi population. these findings lead to the proposal that HHV-6 acts as cofactor in patients suffering from acute myeloid leukemia.
- Our study indicated that ***IL-17rs2275913*** genes polymorphisms may be considers as risky factors for AML Patients.
- Serum IL-17A levels can be considered a useful diagnostic and prognostic factor in AML patients, like IL-17A .
- The significant correlation between the gene polymorphism of *IL-17A* with HHV-6 infection could indicate highly important role of these molecular factors in patients suffering from AML.
- High interleukin is caused by a viral infection, and as a result of increased interleukin secretion, it leads to a malfunction in the immune system and thus a greater ability to develop leukemia.

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