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Interleukins 7, 9 and HLA-DRB1 genes polymorphism in hemodialysis patients with cytomegalovirus in Diyala province

A Thesis
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By

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1.1 Introduction

Renal failure (RF) is a common disease throughout the world, which occurs because the kidneys can't perform excretory functions resulting in nitrogenous waste product retention from the blood (Levey *et al*., 2013). Kidney functions include volume and electrolyte regulation, nitrogenous waste excretion, exogenous molecule elimination, e.g., drugs, synthesis of different hormones, e.g., erythropoietin as well as low molecular weight protein metabolism e.g., insulin (Calderon-Margalit *et al*., 2018). Renal failure pathophysiology is a series of events that occur as acute insult during the acute renal failure as well as gradually over a period of time in chronic kidney disease conditions (Mills and He, 2016).

Human cytomegalovirus (HCMV) is the most complicated genetic virus among all pathogenic viruses of humans and the largest typical one among all herpesviruses (Karbach, 2012). HCMV is the prototypic member of human *Herpesveridae* family, subfamily Betaherpesverinae, about (20 to 300 nm) in diameter, and it is an enveloped double-stranded DNA virus (Alston, 2017). Primary infection with HCMV in immunocompromised people is asymptomatic or mild, or produces fever-like with mononucleosis-like symptoms (Cannon *et al.*, 2010).

The HCMV causes opportunistic infections to immunocompromised patients such as cancer and kidney failure patients, while it rarely causes complications to healthy people; however, in fetuses, neonates and immunocompromised patients, infection with HCMV may lead to a series of harmful clinical effects. Below is a summary on pathogenesis, incidence and clinical features of HCMV infection among different patients (Adler S, 2007).

The seroprevalence of HCMV was shown to be highest in South America, Africa and Asia, while it is the lowest in Western Europe and the United States. Approximately 70% of adults in developed countries develop asymptomatic latent CMV infections. It can be reactivated among

immunosuppressed individuals. The prevalence rate of HCMV infection is high in kidney failure patients (Schottstedt *et al*., 2010).

A combination of adaptive and innate immune responses contributes to control the primary HCMV infections. The immune responses to HCMV comes from innate interferon and natural killer (NK) cell responses, which are essential to immediately control of viral infections (Lam and Lanier *et al*., 2017). Despite macrophages function as vehicles for spread and also regulate T-cell response, protective adaptive response is shown to be T-cell mediated, with antibodies playing secondary roles in most conditions, in spite of the presence of some exceptions (Litjens *et al*., 2017).

A huge number of diseases have been shown to link with various human leukocyte antigen (HLA) genotypes, such as rheumatoid arthritis, ankylosing spondylitis, coeliac disease, insulin-dependent Diabetes Mellitus (DM), multiple sclerosis, kidney failure, tuberculosis. In the last two decades, findings regarding relationship of HCMV infection with HLA were recorded (Varga *et al*., 2008).

Human leukocyte antigen correspond to the Major Histocompatibility Complex (MHC) class I (A, B and C), all of which are the HLA class 1 group present peptide from inside cells, so, if cells are infected with the virus, the HLA system brings the viral fragments to the cell surface so as the cell is destroyed by the immune systems. The digested proteins produce peptides that are broken down in the proteasomes (Sadiq and Pakianathan, 2007).

Human leukocyte antigen correspond to MHC class II (DP, DQ, and DR), which provide antigens from outside of cells to T-lymphocytes. These antigens stimulate T-cell (or CD4+ T-cells) multiplication, which in turn stimulates B-lymphocytes to produce antibodies to that antigen (Ten Brooke *et al*., 2013).

Human leukocyte antigen polymorphism may thus play an essential role in the immune response development or antibody development to medicines

or to protein medicines which result protein modification in the body (e.g., by medicine or metabolite binding to proteins) (Crux and Elahi, 2017).

Production of cytokines may be influenced by cytokine gene polymorphism via the effect of this gene on transcriptions. The single nucleotide polymorphism (SNP) induces resistance or susceptibility to infections, and can be used for the diagnosis of some factors involved in inflammatory diseases or cancers. The SNP occurs in regulatory region of the cytokine gene (Jacob Barnes; 2017).

Interleukin-7 (IL-7), a 25 kDa cytokine produced by bone marrow and thymic stromal cells, plays a basic role in lymphocyte homeostasis. It has been proven that naive T-cell development in the thymus and naive and memory T-cell homeostasis in the periphery rely upon IL-7 functions (Toghraie *et al*., 2017). IL-7 already has a known function in B cell precursor and functions on both mature and immature T-cells, leading to regulation of homeostasis of the T-cell populations, e.g., IL-7 level increases when there is a T-cell depletion for any cause (Gonçalves *et al.*, 2019).

In humans, Interleukin-9 (IL-9) is situated on chromosome 5 (5q31–35) between IL-3 gene and early growth response-1 (Namkung *et al.,* 2011). The activated IL-9 receptor plays a basic role in immunologic processes like T-cell development. It also contributes to apoptosis prevention. It is able to induce the release of chemotactic factors from smooth muscle cells and bronchial epithelial cells (Noelle and Nowak, 2010).

1.2 Aims of study:

Study association among IL-7, IL-9 and HLA-DRB1 genotype and HCMV effect on hemodialysis patients in Diyala by these approaches.

1- Evaluate latent infection of Human Cytomegalovirus in hemodialysis patients s by ELISA method.

2- Diagnostic of Human Cytomegalovirus DNA in hemodialysis patients by molecular techniques polymerase chain reaction (Real Time PCR).

3- Demonstrate the role of single nucleotide polymorphism (SNP) of IL-7 and IL-9 in immunocompromised patients of hemodialysis with Human Cytomegalovirus infection.

4- Study the immune role of HLA-DRB1 genotype in hemodialysis patients s with Human Cytomegalovirus infection.

2. Literature review

2.1 Renal Failure

Renal failure happens when the kidneys can't do their job: waste filtration from the blood, help blood pressure regulation, and salt and water balance regulation in the body. When blood flows through kidneys, it is filtered, then wastes are removed and sent to the bladder as urine. When kidney function is impaired, acute (rapid) or chronic (gradually developing) kidney failure may take place. With acute kidney failure, kidney function can return to normal state if the underlying cause of the failure is detected and successfully treated. (Farhan, 2013) In accordance to the global burden of diseases, renal failure is now considered as a worldwide public health epidemic. (Akpan and Ekrikpo, 2015)

There is an apparent increase in morbidity and mortality with End Stage Renal Disease (ESRD) around the world. In 2010, 2.62 million people underwent dialysis in the world and the need for dialysis was planned to be doubled by 2030. (Liyanage, 2015) The total cost of treating the milder chronic renal disease types worldwide appears to be much more than the total cost of treatment of end-stage kidney diseases. (Luyckx *et al*., 2018) Renal diseases are associated with age since the functions decline with time leading to acceleration of diabetes, hypertension, obesity and primary renal disorders. (La Russa *et al*., 2019) There are two types of kidney disorders:

2.1.1 Acute Renal Failure (ARF)

Acute renal failure (ARF) is the rapid kidney function loss, occurring over hours or days and leading metabolic waste product accumulation and the extracellular volume dysregulation and electrolyte homeostasis. ARF is particularly found in the elderly people ≥65 years. It was estimated that it occurs in 2–7% of all hospital admissions, and even at higher rates in

elderly patients. (Abdel-Kader and Palevsky, 2009) Acute renal failure is the syndrome where glomerular filtration declines suddenly (hours to days) and is often reversible. Acute kidney injury (AKI) is diagnosed when there is a creatinine increase of 0.3 mg/dL in 48 hours, creatinine increases to 1.5 times baseline within the last 7 days. The rate creatinine production depends on muscle mass. Hence, the rate where creatinine concentrations increase in acute renal failure relies on both glomerular filtration rate and muscle mass. Definitions depend upon absolute alteration in creatinine level regardless of the main modifying impacts of age, sex and ethnicity on muscle mass and creatinine generation. (Luo *et al*., 2014) The term acute kidney injury (AKI) has recently replaced the term acute renal failure (ARF) since AKI denotes the complete clinical spectrum from mild serum creatinine elevation to obvious renal failure. (Awdishu *et al.*, 2016)

2.1.2 Chronic Renal Failure (CRF)

Chronic kidney disease (CKD) is defined as a constant kidney function impairment, in other words, abnormally increased serum creatinine for more than 3 months or a Glomerular filtration rate (GFR) less than 60 ml / minute / 1.73m^2. CKD usually involves a progressive kidney function loss that needs renal replacement therapy (transplantation or dialysis). When renal replacement therapy is required, the condition is called end-stage renal disease (ESRD). (Chertow, 2005) chronic kidney disease (CKD) is a syndrome of persistent changes in kidney function, structure or both. (Zoccali *et al.* 2017) chronic kidney disease is a progressive irreversible disease defined by kidney function decline. The diagnosis of CKD depends on a measurable reduction in the kidney function, and obvious damage of the kidney structure lasting for more than three months. (Johnson *et al*. 2013) A single kidney has about 1-2 million nephrons, and each nephron contains a glomerulus for blood filtration and tubules for reabsorption and

balancing of fluids and electrolytes. (Eckardt *et al*. 2013) The glomerular filtration rate (GFR) is used to measure the kidney functions and is calculated depending on the creatinine levels in the blood. (Levey and Coresh, 2012) A low GFR is seen along with renal damage markers damage e.g., proteins, albumin or blood in the urine, or abnormal imaging, indicating the presence of renal diseases. (Johnson *et al.* 2013) When these changes persist for less than three months, the disease is said to be acute kidney injury (AKI), while if they continue for three months or more the diagnosis can be reported as CKD. (Macedo *et al*. 2021)

2.2 Human Cytomegalovirus (HCMV)

2.2.1 General Characterization

Human Cytomegalovirus (HCMV) is a ubiquitous virus that is globally distributed. (Al Mana *et al*., 2019) This virus is the most important infectious agent causing congenital diseases, and it is an important opportunist in the immunocompromised people and an occasional cause of febrile diseases in addition to infectious mononucleosis in the general community. (De Melo Silva *et al*., 2020) HCMV infects a majority of the people and spreads effectively during life all over the world by direct contact with human body secretions. (Burrell *et al*., 2017) Like other herpesviruses, HCMV can’t be entirely cleared and persists latent for the host’s life. Viruses that are shed sporadically and persistently are important recurrent sources for viral transmission. (Forte *et al*., 2020) The susceptibility to HCMV infection is correlated with a compromised immune system, especially correlated with defects in cell-mediated Cluster of differentiation 4 CD4 and Cluster of differentiation 8 CD8 T-cell functions. During gestation, intrauterine transmissions to the fetus lead to sensorineural damages. (Fields, 2013)

Human Cytomegalovirus (HCMV) belongs to the human herpesvirus family which includes 8 viruses: Herpes simplex virus-1 (HSV-1), Herpes simplex virus-2 (HSV-2), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Human herpes virus-6 A and B (HHV-6A and B), Human herpes virus-7 (HHV-7) and Human herpes virus-8 (HHV-8). Herpesviruses can be further classified into subfamilies: α−herpesviruses (HSV-1, HSV-2 and VZV), β-herpesviruses (HCMV, HHV-6 A/B and HHV-7) and γ-herpesviruses (EBV and HHV-8). The enveloped Herpesviruses have icosahedral capsids that enclose double-stranded DNA genomes as shown in figure (2-1). The largest member of the human herpes virus family is HCMV, which has a genome of 236 kbp and more than 200 open reading frames (ORFs) that encode more than 80 viral proteins, such as glycoproteins B (e.g., gB), phosphoproteins 65 (e.g., pp65) as well as other transcription/replication proteins. (Balázs *et al*., 2017)

2.2.2 Classification

The initial classification of Human Cytomegaloviruses infecting animals and human-beings is comparative biology, physicochemical characteristics and virion morphology. During the past 20 years, genome sequence analysis eclipsed other approaches in viral taxonomy. HCMV belongs to Group I of the Baltimore classification, specifically to the subfamily Betaherpesverinae within the *Herpesveridae* family show figure (2-1)

Group: dsDNA

Order: Herpesvirales

Family: *Betaherpesverinea*

Gene: *Cytomegalovirus*

Species: *Human Cytomegalovirus*

(Gugliesi *et al*., 2020)

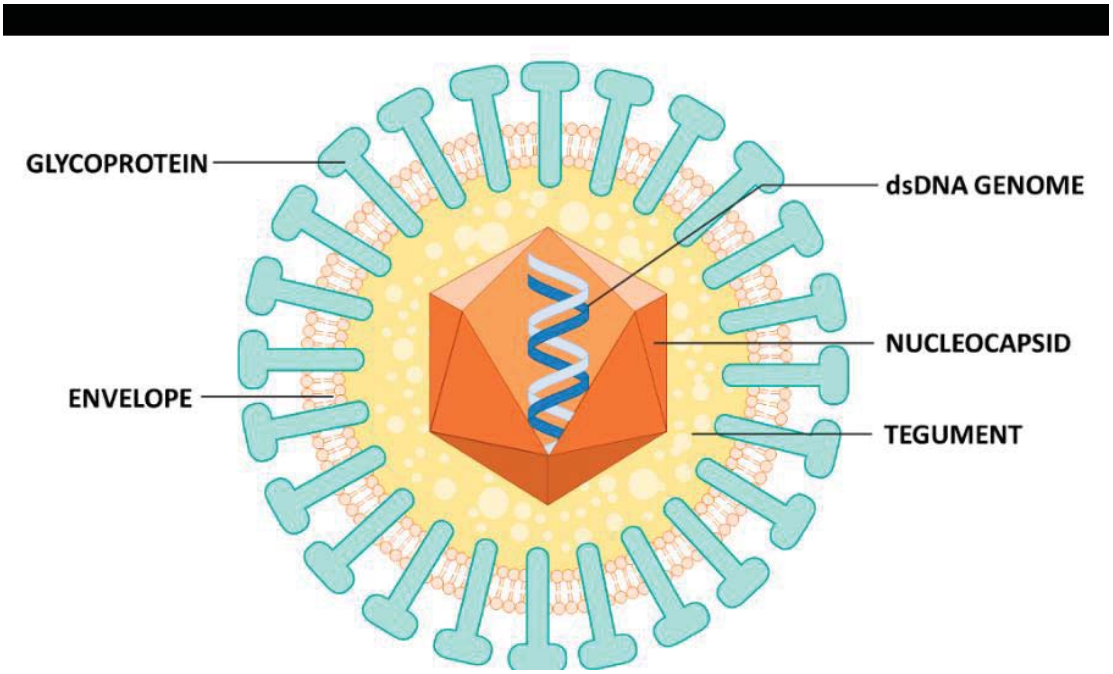

Figure (2-1): Structure of HCMV virion. Mature virions are coated by an envelope, from which viral glycoproteins protrude, and contain a double-stranded DNA genome enclosed within an icosahedral symmetry capsid, that is surrounded by tegument (Gugliesi *et al*., 2020).

2.2.3 History of Human Cytomegalovirus (HCMV):

The intranuclear inclusions of cytomegalovirus infections were first observed in 1881 by Ribbert who thought they represent protozoa. He noticed large cells in renal sections of a luetic stillborn and in a child's parotid gland. (Ribbert ,1904) In 1907, Lòwenstein detected such cells in parotid gland of 4 out of 30 infants. This appears to be the first description of typical cytomegalic cells with intranuclear inclusion. (Ho, 1982) In 1950, Wyatt et al. proposed the name, "generalized cytomegalic inclusion disease (CID)", although its viral cause was having not been known yet. (Wyatt *et al*., 1950) In 1953, Minder first noticed the virus by the electron

microscope as 199 nm particle in the clear halo surrounding the intranuclear pancreatic cell inclusions in a case of CID. (Minder *et al*., 1953) In 1970, Weller et al. named the virus as "Cytomegalovirus". (Weller, 1970)

2.2.4 Life cycle of HCMV in a human cell

HCMV gets access to human cells either by direct fusions or via the endocytic pathway. This virus attaches the cell through interaction between viral glycoprotein (e.g., gB and gH) and specific surface receptor(s) (e.g., platelet-derived growth factor), followed by the envelope fusion with the cell membrane to release nucleocapsid into the cytoplasm. This nucleocapsid is translocated into the nucleus, where viral DNA is released (Nguyen and Kamil, 2018), leading to initiation of IE-1/IE-2 gene expressions. The stimulation and parallel accumulation of viral synthesis functions is followed by viral maturation and replication. This process includes encapsulation of replicated viral DNA as capsids, which are then transferred from the nucleus to the cytoplasm. Secondary envelopments take place in the cytoplasm at the endoplasmic reticulum (ER)-Golgi intermediate compartments as observed in figure (2-2), followed by a complex two-stage final egress and envelopment resulting in release of virions by exocytosis at the plasma membranes. (Wang and Zhao, 2020)

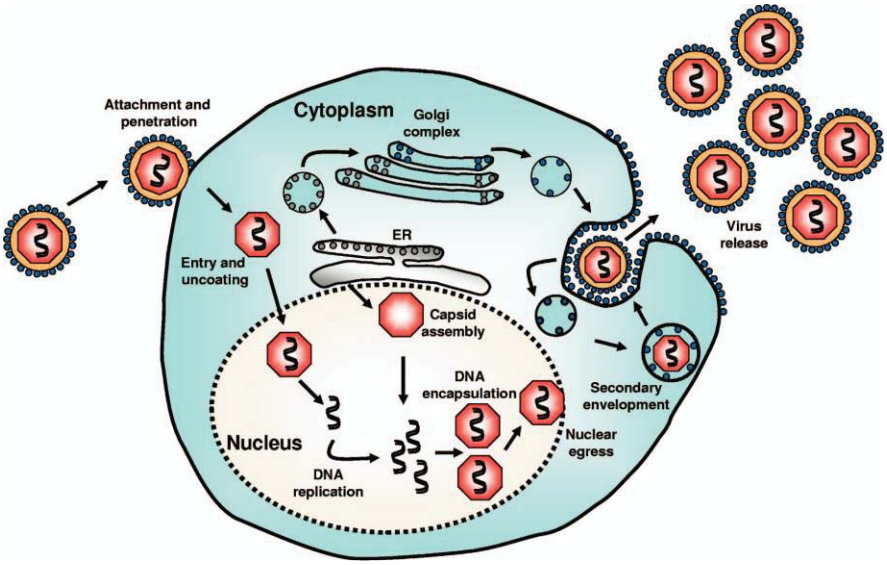

Figure (2-2): Life cycle of HCMV in a human cell. (Crough and Khanna, 2009)

2.2.5 Infection and viral latency

Human cytomegalovirus infection was first described by (Klemola and Kaariainen, 1965) as a condition similar to infectious mononucleosis caused by Epstein Barr virus; therefore, CMV infection was also called "mononucleosis-like" disease. The incubation period for HCMV ranges from 4 to12 weeks, where it can be identified. In most cases, on the progression of the infection, it becomes asymptomatic or subclinical in immunocompetent individuals.However, when symptoms exist, the infected person may show persistent fever, myalgia as well as cervical lymphadenopathy (Nangle *et al*., 2018). The most serious symptoms are typically seen in infected fetuses and patients with aggravate immunodeficiency, either as disseminated infection or in specific organs (Laurens, 2019). In patients with immunodeficiency, HCMV is opportunistic, and infection is correlated with diverse clinical symptoms and signs. In human immunodeficiency virus (HIV) seropositive individuals for example, infection with HCMV compromises different organs, resulting in important disease development like renal failure in

الخلاصة:

يعد الفيروس المضخم للخلايا البشرية (HCMV) أحد مسببات الأمراض المهمة في الأفراد الذين يعانون من نقص المناعة. لقد تم توثيقه جيدًا أن مرضى غسيل الكلى يعانون من ضعف الاستجابة المناعية ، مما قد يؤدي إلى معدلات إيجابية أعلى للعدوى الفيروسية ، بما في ذلك HCMV. قد تكون العدوى في هؤلاء المرضى ناتجة عن عدوى أولية أو ، بشكل أكثر شيوعًا ، عن طريق إعادة تنشيط الفيروس الكامن أو إعادة العدوى بفيروس خارجي المنشأ ، والذي قد يتم إدخاله عن طريق نقل الدم.

هدفت الدراسة الحالية إلى تحديد أبرز السمات المناعية الخلوية والخلطية لمرضى غسيل الكلى (IL-7، IL-9، HLA-DRB1) ، واستكشاف تعدد أشكال جينات مضيفة معينة قد تؤثر على قابلية مرضى غسيل الكلى المصابين بعدوى HCMV.

اشتملت هذه الدراسة على (100) مريض عراقي (62 ذكر و 38 أنثى) مصابين بالفشل الكلوي تتراوح أعمارهم بين (13-76) سنة من مركز ابن سينا للغسيل الكلوي وأمراض الكلى في مستشفى بعقوبة التعليمي و (50) عينة سيطرة (38) ذكور و 12 إناث) تتراوح أعمارهم بين (18-45) سنة خلال الفترة من يناير 2020 إلى يونيو 2021.

تضمنت الخطوة الأولى في الدراسة الحالية تشخيص الفيروس المضخم للخلايا البشري (HCMV) في المجموعات المدروسة بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) ، وكانت الخطوة الثانية هي اكتشاف الفيروس المضخم للخلايا البشري (HCMV) عن طريق تفاعل البلمرة المتسلسل في الوقت الحقيقي. ، في حين تضمنت الخطوة الأخيرة الكشف عن العديد من الأشكال المتعددة للنيوكليوتيدات المفردة (SNP) في Interleukin 7 (IL7) ، و Interleukin 9 (IL9) و Human Leukocyte Antigen (HLA) class II (HLA DRB1).

أظهرت النتائج وجود معدلات إيجابية عالية 100 (100٪) من موجب مصل HCMV- IgG ، بينما تم الكشف عن HCMV- IgM إيجابي في 15 (15.0٪) من مرضى غسيل الكلى باستخدام تقنية ELISA. تم العثور على أعلى معدلات إيجابية لإيجابية مصل HCMV-IgG عند الذكور ، بينما تم العثور على أعلى معدلات إيجابية لإيجابية مصل HCMV-IgM في الإناث. كما أوضحت النتائج أن أعلى معدلات إيجابية إيجابية لمصلي HCMV-IgM تم اكتشافها في المرضى ضمن الفئة العمرية (50-70) سنة. تم الكشف عن الحمض النووي HCMV (DNA)