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ASSOCIATION BETWEEN POLYMORPHISMS IN CYTOKINE IL7 GENE AND CMV IN RENAL FAILURE PATIENTS IN DIYALA PROVINCE

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Abstract

Background: The human cytomegalovirus (HCMV) is an important human pathogen primarily affecting immunocompromised patients, like Renal failure patients, transplant recipients or HIV-infected individuals. Renal failure diesease is a wide dissemination among kidney patients in Ba'quba City.

Aim: the study was carried out to detect the HCMV and detection of several single nucleotide polymorphisms (SNP) in IL7 among100 patient suffering from acute or chronic renal failure whom admitted to Ibn Sina Center for kidney Dialysis in Baquba Teaching Hospital.

Patients and Methods: This study was conducted for the period from 1/12/2019 to 15/6/2020 in Baquba city in Iraq, with age ranged between (13-76) years. The samples were diagnosed by serological and molecular tests.

Results: The obtained results showed that HCMV antibody was detected in renal failure patients by ELISA IgG (100%) while IgM were (15.0%) . Also, Distribution of genotypes for the (IL7) rs7007634 gene samples of renal failure patients 15 out of 20, Chi-Square (9.375) investigated samples (P value 0.002) . The alignment results of the 323 bp samples revealed the presence of one genetic variation variably distributed in some of the analyzed samples in comparison with the referring IL7 genetic sequences. Distribution of genotypes of the rs7007634(IL7)gene in renal failure patient samples according to CMV infection 12 sample infection in CMV and 3 sample without CMV.

Keywords: CMV, Renal Failure, IL7, Genotype.

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1. Introduction

Renal failure occurs when the kidneys are unable to do their job: to filter wastes from the blood, help regulate blood pressure, and regulate salt and water balances in the body. As blood flows through the kidneys, it is filtered, and wastes are removed and sent to the bladder as urine. If kidney function becomes impaired, acute (rapid) or chronic (gradually developing) renal failure may occur. With acute renal failure, kidney function can return to normal if the underlying cause of the failure is discovered and successfully treated. (Farhan,2013) There are two type of kidney infection: Acute Renal Failure (ARF) and Chronic Renal Failure (CRF).(1)

Human cytomegalovirus (HCMV) is a ubiquitous virus infection with worldwide distribution. The virus is the most significant infectious cause of congenital disease, an important opportunist in the immunocompromised host like renal failure .Cytomegalovirus (CMV) is a member of the human herpesvirus family Herpesviruses are enveloped viruses with an icosahedral capsid that encloses a double-stranded DNA genome, CMV is the largest member of the human herpesvirus family, with a genome of 236 kbp and more than 200 open reading frames (ORFs) encoding more than 80 viral proteins, including glycoproteins (e.g., gB), phosphoproteins (e.g., pp65), and other transcription/replication proteins.(2) CMV is one of the most successful of human pathogens, since it can be transmitted both vertically and horizontally, usually with little effect on the host. CMV is shed in body fluids (e.g., saliva, urine, semen, breast milk).Transmission usually occurs from person to person, including through the intrauterine route, but it can also be spread during transfusion or organ transplantation. Risk factors for primary CMV infection include blood transfusion (treatment for clotting factors, and etcetera), infected transplants, hemodialysis, and the frequency of dialysis in a week. CMV seroprevalence has been shown to be highest in South America, Africa, and Asia, while it is lowest in Western European countries and the United States.(3) In developed countries, about 70% of adults have asymptomatic latent CMV infection. It can be reactivated if individual becomes immunosuppressed. CMV is the most common viral infection after kidney transplantation.(4) Depending on the socioeconomic status, seropositivity for adult population over forty years ranges from 60 to 100% possibly as a result of transmission through intrauterine (or at parturition), breastfeeding, blood transfusion, sexual contact and spread from children. It tends to be lower in the developed countries than the developing countries.(5) Globally, between 60 and 90% of the general population is infected with CMV (6) with generally higher rates in developing countries. The high seroprevalence rate in Sweden (70-80%) is believed to be related to the high rate of breastfeeding and the widespread use of daycare facilities for children. (7) CMV is usually acquired during early childhood, with 40% of all individuals being seropositive at 4 years of age (8); the prevalence of infection increases with age after infancy and ongoing seroconversion is seen through out life (9) diagnosis of CMV disease has been made superseded by serological by EliSA and Molecular assays for determining the level of CMV replication such as Polymerase Chain Reaction (PCR). (10)

CMV infection triggers a forceful immune reaction in the human body, including both antibody- and T-cell-mediated responses. Because of its effective immune evasion strategies, (11) B-cell immune responses Primary CMV infection elicits a transient IgM response within 1-3 weeks that is followed by the development of persistent IgG antibodies. These antibodies play a minor role in clearing the infection but are believed to play an important role in reducing the severity of CMV disease in adults and protecting the fetus from congenital infection. The antibodies are directed against at least 15 different proteins; the most immunogenic is pp150, against which nearly 100% of the CMV-seropositive individuals have antibodies. (12) Another important immunogenic protein is pp65; the antibody response against this protein is very high during the acute phase of the infection. The best characterized protein, however, is glycoprotein B (gB), and up to 50-70% of the host's neutralizing antibody response is accounted for by the response to his protein . (13)

Interleukin 7 (IL-7), a 25 kDa cytokine secreted by bone marrow and thymic stromal cells, plays acrucial role in lymphocyte homeostasis . (14) It has been confirmed that naive T cell development in the thymus and naive and memory T cell homeostasis in the periphery depend on IL-7 function. (15) Human IL7 is located on chromosome 8q12–13 and contains 6 exons distributed over more than 33-kbp. (16) IL-7 already has a recognized function in B cell precursors and acts on both mature and immature T cells , regulating homeostasis of the T cell population , for example, IL-7 levels increase when T cell depletion is present for any reason . (17) IL-7 is a member of the common γ chain (γc-CD132) family of cytokines that include interleukin-2 (IL-2), IL-4, IL-7, IL-9,IL-15, and IL-21. (18) Like other members, IL-7 plays multiple important roles during B cell lymphopoiesis. Prior to very early B cell development, IL-7 promotes the commitment of CLP cells to the B-lineage. (19) Cytokine Gene polymorphisms was difference in DNA sequence among individuals that may underlie differences in health. Genetic variations occurring in more than 1% of a population would be considered useful polymorphisms for genetic linkage analysis. Polymorphisms can be in coding regions or more commonly, in non-coding regions, and often vary by ethnicity. The most common

type of polymorphism is a change in one nucleotide (base pair) in a DNA sequence, referred to as an SNP. Other polymorphisms are insertion and deletion of multiple sequential nucleotides ('indels'); variable numbers of repeats, such as doublets or triplets; or large-scale duplications or deletions. Although some genetic variants are known to alter protein abundance or function, the functional consequences of most polymorphisms are unknown.(20) Single-nucleotide polymorphism (SNP)Single nucleotide polymorphisms (SNPs) (pronounced: snip) are an alteration in a lone DNA order structure building block unit: (A, T, C, or G) which termed a nucleotide. (21) It is the simplest formula of genetic difference among persons. SNPs are the most frequent occurrence from all genetic variants, which happen usually in a person's DNA. It is a ratio of occurrence near 90% of human genomic variants. (21) They may be occurring one time in each 300 nucleotides on usual, that is, average is about 10 million SNPs in the individual's genome. Greatest frequently, those SNPs are set between genes or within genes. They may perform as living signs and/or hereditary indicators, aiding experts find sequence, which are linked with disease. As soon as SNPs happen inside a gene or in an adjusting area nearby agene, they might show an additional strong impact in disease via stirring the gene's role. However, the SNPs generally have no influence on the general state of health. Moreover, investigators have instituted that SNPs might assist and guess a person's reaction to definite medications. Additionally, they are utilized for a pathway of genetic factors of malady inside relatives. (22)

Materials and Methods:

Samples collection: A search was performed through The sample of study was 100 Iraqi patients with renal failure at age range (13-76 years) from Ibn Sina Center for kidney Dialysis in Baquba Teaching Hospital and 50 healthy controls from Blood donors at the main blood bank in Baqubah at age range (18–45) years during the period from January to March 2020.The patients in this study included 62 males, 38 females , aswellas healthy controls 38 males, and 12 females. First step includes Human Cytomegalovirus (HCMV) diagnosis in studied groups by ELISA and, detection of several single nucleotide polymorphisms (SNP) in IL7.

Collection and Preparation of Samples:

Seven ml of vein blood samples were collected included 2 ml from whole blood with EDTA tube and storage in -20°C until use to detecting single nucleotide polymorphisms SNP . While residual volume 5 ml put in gel tube and left 15 min to allow clotting at room temperature about 20 -25 °C , after that the serum was separated by 3000 rpm for 10 minutes in centrifugation. The serum was collected and distributed in small tubes and stored in -20°C until use to diagnosis HCMV in study groups.

Serological detection of Human Cytomegalovirus:

Serological investigation included detection of CMV-IgG antibodies and CMV-IgM antibodies by using enzyme-linked immunosorbent assay (ELISA) (MyBioSource , USA). The procedure was carried out according to the manufacturer's instructions.

Cytokine Polymorphism Parameters:

1.Genomic DNA Extraction

Genomic DNA was isolated from blood sample according to the protocol ReliaPrep™ Blood gDNA Miniprep System, Promega.

2.Primer preparation

These primers were supplied by Macrogen Company in a lyophilized form.

Table 1 : Components of IL7 (rs7007634)Primer

Primer Name	Vol. of nuclease free water (µl)	Concentration (pmol/µl)						
rs7007634-F	300	100						
rs7007634-R	300	100						
IL7 (rs7007634)	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity
Forward primer	GAGCTGCAGGAAAGGCAATGTA	Plus	22	490	511	61.20	50.00	6.00
Forward primer	GAGCTGCAGGAGAGGCAATGTA	Plus	22	490	511	61.20	50.00	6.00
Reverse	TACTTGATGTTGTTA	Minus	25	62	60	59.5	40.00	3.00

primer	GAATGTGCCC			7	3	9		
Product length	138							

3.Primer optimization

To examine the optimum annealing temperature of primer, the DNA template was amplified with the same primer pair, (Forward) (Reverse), at annealing temperatures of 55, 58, 60, 63 and 65°C. PCR amplifications were performed with 20µl volumes containing 10µl GoTaq Green Master Mix (2X); 1µl for each primer (10pmol); 6µl nuclease free water and 2µl of template DNA. PCR cycling was performed with PCR Express (Thermal Cycler, BioRad, USA) with the following temperature program: denatured at 94oC for 4 min followed by 30 cycles of denaturation at 94°C for 30 sec; annealing at 55, 58, 60, 63 or 65°C for 30 sec; and extension at 72°C for 30 sec. A final extension incubation of 7 min at 72oC was included, followed by a 10 min incubation at 4oC to stop the reactions.

Results and Discussion:

The sample of study was 100 Iraqi patients with renal failure during the period from January to March 2020 This study was conducted to detect the prevalence of cytomegalovirus infection (CMV) among patients undergoing hemodialysis by using CMV/IgG, CMV/IgM. And association of IL7 genes polymorphism with the kidney failure associated with cytomegalovirus infection.

The result of infection current research revealed that CMV-IgG was found in 100 out of 100 (100.0%), while CMV- IgM was detected in 15 out of 100 (15.0%) , Chi-Square(8.333) of hemodialysis patients. The high prevalence of IgG seropositive was probably due to cumulative effect of previous infection ; reactivation or new infection lead to high percentage of seropositivity, because renal failure patients conceder immunosuppressed individuals. A positive test for CMV IgG indicates that a person was infected with CMV at some time during their life when a person was infected The seroprevalence of CMV varies in different studies. (23) revealed that CMV-IgG was found in 102 out of 116 (87.9%), while CMV-IgM was detected in 10 out of 116 (8.6%) of hemodialysis patients in Tikrit city. (24) found that the rate of CMV infection among hemodialysis patients (HD) was 98% using CMV IgG and 11% using CMV/IgM. Astudy was carried out in Antakya, Turkey to determine the rate of CMV infections revealed that anti-CMV IgG and IgM was found in 99.6% and 0.4% respectively of the HD patients (25). The rate of anti-CMV IgG (39%) obtained by the current study was similar to that obtained by (26) (34%),but differ with (27) (69%) . HCMV IgG antibody levels increased by increasing frequency of exposure and transmission viacrowded and poor living conditions, (28). These variations in the results may be attributed to several factors including endemicity of infection, study population, the techniques used for diagnosis and immune status of the patients

Table 2: Anti CMV IgM Ab & Anti CMV IgG Ab frequency and percentage in patients' group by ELISA technique

Groups	Anti CMV IgM Ab	
	Patient	Control
	No. (%)	No. (%)
Positive	15 (15.0)	0
Negative	85 (85.0)	50
Total	100 (100.0)	50
Chi-Square	8.333	
Df	1	
P value	0.004	
Groups	Anti CMV IgG Ab	
	Patient	control
	No. (%)	No. (%)
Positive	100	0 (0.0)

Negative	0	50 (100.0)
Total	100 (100.0)	50
Chi-Square	150.000	
Df	1	
P value	0.000	

Moreover of this study results was found that the CMV-IgG was found in 62% in males. while females ,was detected in 38%.The laboratory investigation concerning CMV- IgM among hemodialysis patients revealed that 33.3% of males and 66.7% of females have CMV- IgM antibodies.

Table 3 : anti-CMV IgG and IgM frequency distribution according to the sex of the patient's group

Groups	Patients group		Control groups	
Anti-CMV IgG status	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)
Males	62 (62)	0 (0.0)	0	40
Females	38 (38)	0 (0.0)	0	10
Total	100 (100.0)	0 (0.0)	0	50
Anti-CMV IgM status				
Males	5 (33.3)	57 (67.1)	0	40
Females	10 (66.7)	28 (32.9)	0	10
Total	15 (100.0)	85 (100.0)	0	50

revealed 69 male, CMV-IgG was found in 59 (85.5%) of them. Regarding females, CMV-IgG was detected in 43 out of 47 (91.5%). However, that CMV- IgM among hemodialysis patients revealed that 7 out of 69 (10.1%) of males and 3 out of 47 (6.4 %) of females have CMV- IgM antibodies. In Turkey, gender did not contribute independently to the seroepidemiology of CMV($p > 0.01$). (29) Also, (30) reported that there was no difference in CMV prevalence between males (87.9%) and females (96.3%). (31) showed non- significant relation concerning sex status of the CMV-cases. and (32) in U.S. reported that females had higher seroprevalence than males. It is possible that the gender difference in CMV seroprevalence reflects females' exposure to young children. The relationship of child care to CMV infection has been presumed to be attributable to the presence of CMV at high titers in urine and/or saliva. Nevertheless, a previous study suggested that, similar to females, adolescent males are at an increased risk of CMV infection when exposed to young children in the household.

Varying methodologies perhaps may have contributed to the disparities observed. Although immunocompromised patients are at risk for morbidity due to wide variety of pathogens, few, if any of these are capable of producing such widspread disease as CMV. CMV-related morbidity follows a progressive, relentless course in the absence of effective therapeutic intervention. Thus, rapid diagnosis of active CMV infection is of great importance to avoid over treatment with immunosuppressive drugs and to guide antiviral therapy. In recent years, treatment of CMV infection in high-risk patients prior to the onset of clinical disease is preferred. (33)

Seroprevalence of CMV in the study groups according to age An increase in the seroprevalence rates was observed with age . Also, a significant association between increase of the age and increment of the seroprevalence confirmed that according to age, a progressive increase in seropositivity was observed in hemodialysis patients.

CMV- IgM was detected at a highest rate in patients within the age group was found among age group 50-70 years. Many investigators observed that 0lder patients were at higher risk of CMV infection (Seed et al, 2009). From the previous studies and our study there was an agreement. This may be due to that patients with highest ages will have the low immune response.

This study was conducted to investigate the possible association of IL7 genes polymorphism with the kidney failure associated with cytomegalovirus infection. The genetic locus was amplified in this study, 232 bp.To elucidate the positions of the targeted rs7007634 SNP with regard to its deposited database of the

sequenced 323 bp fragment, the corresponding position of the IL7 gene was retrieved from the dbSNP server (https://www.ncbi.nlm.nih.gov/projects/SNP/). To find out the nature of this SNP, a graphical representation was performed concerning the IL7 dbSNP database within chromosome 8 (GenBank Acc. no. NC_000008.11) (Fig. 1). However, this SNP was found to be positioned in the intronic portions of IL7 gene sequences. Interestingly, the targeted SNP locus did not exhibit the transition of Adenine to Guanine as it was deposited in the dbSNP. Instead, it was entitled that the currently observed transition from the Adenine to Thymine is a new polymorphic pattern that is not registered in the dbSNPs yet.

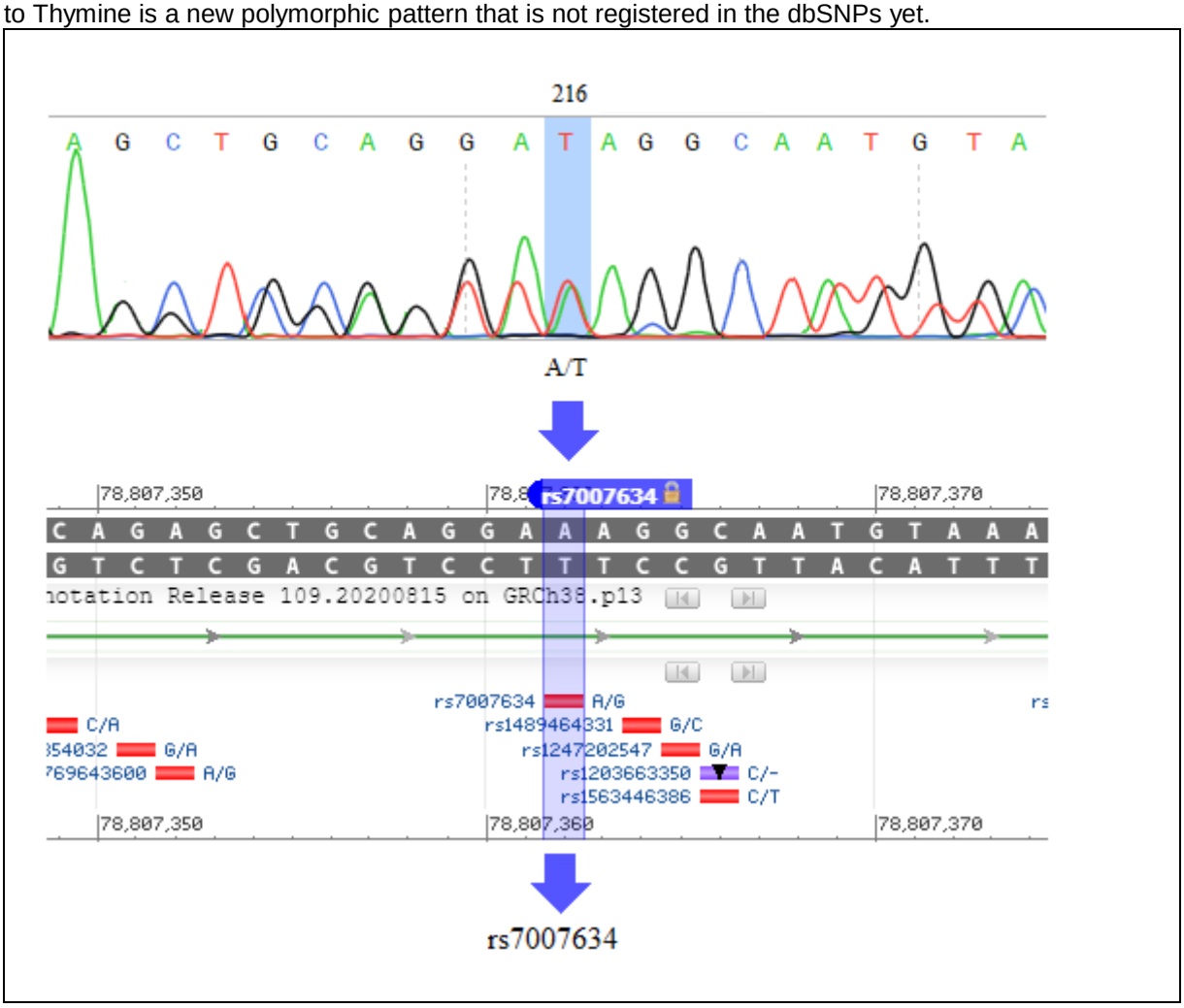

Fig. 1. The details of the targeted rs7007634 SNP's within the IL7 sequences using dbSNP server. The identified SNP is marked with a blue color. The targeted rs7007634 SNP was placed in the forward strand.

Table 4 : Distribution of genotypes for the (IL7) rs7007634gene samples of renal failure patients and healthy controls

Genotypes	renal failure patients	Control	OR (95%CI)	P value Fisher – test	preventive or etiological fraction
and alleles	No. 20 %	No. 15 %			
AA homo	5 (25%)	5 (100%)	0.2500 (0.0515 to 1.2140)	0.0855	20%
AT hetro	15 (75%)	0 (0%)	8.3171 (0.4270 to 162.0069)	0.162	50%
Total	20 (100%)	5 (100%)			

Chi-Square	9.375				
Relative Risk (95% CI)	0.5000 (0.2366 to 0.7634)				
Df	1				
P value	0.0047 **				
	Allele frequencies				
A	25 (63%)	10 (100%)	0.6250 (0.2279 to 1.7142)	0.3612	20%
T	15 (37%)	0 (0%)	8.0370 (0.4436 to 145.6076)	0.1585	28.57%
Relative Risk (CI 95%)	0.7143 (0.5495 to 0.9192)				

The current study is considered the first study in Iraq. It shows the effect of genetic variability in some pro-inflammatory interleukin genes producing from IL-7 on renal failure patients and controls.This current study was to investigate the association between gene polymorphisms of IL-7 with renal failure patients associated with HCMV infection patients.

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