



# DETECTION OF THE IL-1B GENE POLYMORPHISM AMONG RENAL FAILURE PATIENTS WITH AND WITHOUT CMV BY RFLP-PCR TECHNIQUE, IRAQ

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## Abstract

Renal failure results in the abrupt loss of kidney function, leading to the retention of waste products, electrolyte disturbances, and volume status changes. The estimation of glomerular filtration rate (eGFR) has been recognized as the best standard screening for kidney disease. The study aimed to estimate the level of HCMV IgM/ IgG antibody in renal failure patients and the use of the RFLP-PCR technique in detecting the polymorphism of IL-1B. The study conducted on fifty renal failure patients (34 males and 16 females) and 20 randomly healthy subjects as a control group. The study demonstrated that 22(44%) of patients' blood samples were positive for Cytomegalovirus antibody rapid test. plasma concentration IL-1 $\beta$  was estimated in all study groups, the level of IL-1 $\beta$  was significantly elevated in all renal failure patients compared with healthy persons. Digested DNA bands at 190 bp and 115 bp were produced by homozygote genotype of IL-1 $\beta$  -511(C/C), digested DNA band at 305bp, 190bp, and 115bp were produced by heterozygous genotype of IL-1 $\beta$  -511 (C/T) whereas homozygote genotype of IL-1 $\beta$ -511 (T/T) was produced one fragment 305bp and remained undigested. Conclusion. The renal failure patients are highly susceptible to infection with CMV. CMV IgM/IgG antibody test is an important test in the diagnosis of CMV. The severity of this disease may be associated with the presence of CT genotype and T allele of IL-1 $\beta$  in renal failure patients.

**Key words :** Kidney failure, Human Cytomegalovirus RFLP-PCR (Restriction fragment length polymorphism) PCR Technique.

## Introduction

Cytomegalovirus (CMV) is the largest member of the virus family *Herpesviridae* and is a ubiquitous virus that infects almost all humans at some time in their lives. The virus was first isolated by three different groups of investigators; Rowe and colleagues, Weller and colleagues, and Smith simultaneously in 1956 (Kenneson and Cannon, 2007). CMV infection may be acquired for the first time during pregnancy (primary infection) or women may experience secondary CMV infection, either by reactivation of prior CMV infection or by a new infection with a different strain of the virus. Transmission of the virus to the fetus can occur antenatal by the trans placental route, during labor and delivery through contact with cervicovaginal secretions and blood (Boppana *et al.*, 2001). Also, CMV is transmitted by close contact between individuals, through contamination from urine,

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saliva, semen, cervical secretions, and breast milk, while droplet contamination is thought to be less important (Stagno *et al.*, 2006). After primary infection with CMV, the virus becomes latent and can be reactivated to produce a secondary infection, particularly during episodes of immunosuppression. Cytomegalovirus is secreted in saliva, urine and breast milk, and intermittent shedding of the virus is common, particularly in infected infants, children, and pregnant women) Britt *et al.*, 1996). CMV has been recognized as an important cause of morbidity and mortality in immunocompromised hosts such as patients with acquired immunodeficiency syndrome (AIDS), and recipients of solid organ and stem cell transplants (Winston *et al.*, 1990). Renal failure is a medical condition in which the kidneys are functioning at less than 15% of normal (Sarah *et al.*, 2017). The term renal failure denotes the inability of the kidneys to perform excretory function leading to retention of nitrogenous waste products from the blood. Acute kidney injury is a

medical emergency characterized by a rapid (hours to days) fall in glomerular filtration rate. Most people who experience acute kidney injury have some degree of pre-existing chronic kidney disease (CKD) ( Biolatti *et al.*, 2018). In a study of over 1700 patients with acute kidney injury requiring dialysis, 74% had an estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 m<sup>2</sup> in the months before admission (Hilton, 2006). Changes in kidney function are detected by a change in biomarkers, the most common biomarker being serum creatinine (SCr). Serum creatinine is an imperfect biomarker for recognizing AKD, given that an increase in SCr often lags (48–72 hours) behind the onset of the injury. Besides, SCr is not in a steady-state condition in critically ill patients, leading to inaccurate estimates of glomerular filtration rates (eGFRs) (Mehta and Chertow,2003). Restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence. The term may refer to a polymorphism itself, as detected through the differing locations of restriction enzyme sites (Cable *et al.*,2014)

### Materials and Methods

A total of fifty patients with renal failure (34 males, 16 females), ages ranging from (20 -60) years who admitted to a renal dialysis unit at AL-Sadder medical city in AL-Najaf city. The renal failure patients were involved in two groups, with (22) and without (28) Cytomegalovirus infection. 5 ml of venous blood were collected from each patient and control person. 2 ml of

blood was placed in (EDTA) tubes for DNA extraction. Serum was separated by place 3ml of blood in the gel tube to the detection of Cytokine (IL 1 $\beta$ ) by using an ELISA kit (Elabscience Company, USA) designed to measure human IL-1  $\beta$  in plasma. All samples were tested for HCMV antibodies using IgG/IgM antibody rapid test that was applied according to procedure in leaflet kit (Biotech Company, USA). Creatinine and blood urea were assessed in 20 healthy persons.

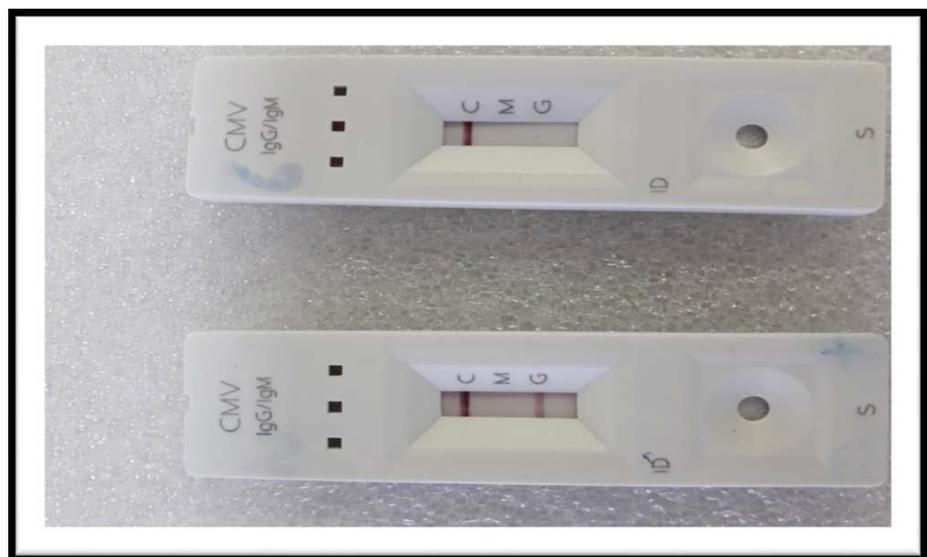
Genomic DNA was extracted from peripheral blood samples. PCR-based genotyping

of the IL-1 $\beta$  polymorphism was used according to the leaflet of the kit (Sacace Company, Italy). The primers were used in our study: IL-1  $\beta$  Forward 5'-TGGCATTGATCTGGTTCATC-3' and Revers 5'-GTTTAGGAATCTTCCCA, CTT-3'. *Ava*I restriction enzyme was used to digest the PCR product of IL-1  $\beta$  at 37°C, electrophoresis on 2% agarose gels, and pictured with ethidium bromide underneath UV light illuminator. The Chi-square test was applied to determine the statistical significance of the data. P value of B 0.05 was considered significant.

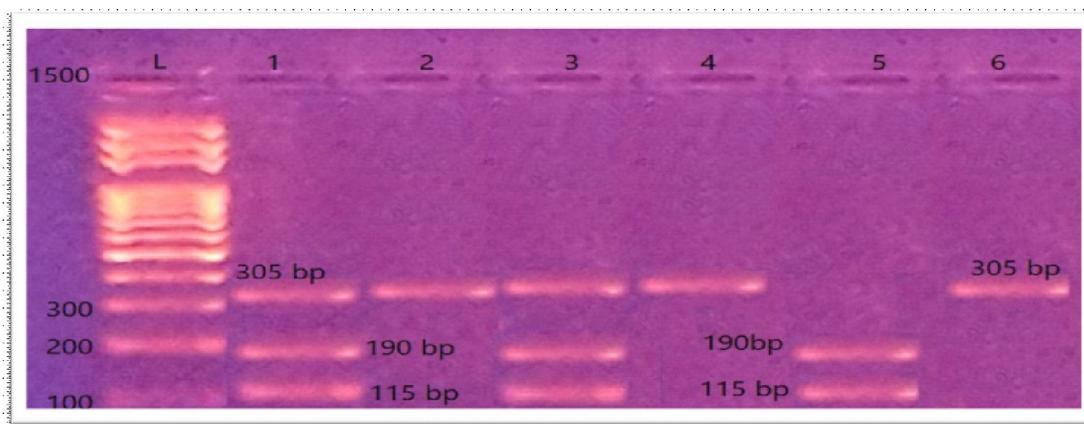
### Results and Discussion

Fifty renal failure patients and twenty healthy persons were enrolled in this study. Most renal failure patients were males (68%) than female (32%) Our results were not consistent with Brown et al ( Brown *et al.*,2003). who stated that renal failure is a high prevalence in both men and women, but women had a tendency to develop renal failure than men ( Chadban *et al.*, 2003). reported significant sex dissimilarity in women than men in renal failure patients. Generally, a higher occurrence of renal failure disease in women or men may be various in ethnic groups.

This study revealed that patients age in more than fifty years (38%) were at high risk of developed renal failure disease compare with other study groups. Our findings were in agreement with the results of other studies( Sepehrv *et al.*, 2010; Zhang and Rothenbacher,2008; Hsu *et al.* 2008). They showed that the incidence of renal failure disease increases with age.



**Fig. 1:** Antibody rapid test: In case of infection with CMV or healthy persons, the red color will appear on the letter G, non-infection with CMV, color appears only on the letter C.



**Fig. 2:** Agarose gel electrophoresis picture of the RFLP-PCR test to the determinant of the IL-1 $\beta$  gene as follow:  
 Sample: blood samples  
 Restriction enzyme: *Ava*I enzyme, Lan 1: CT heterozygote at 305bp, 190bp, 115bp, Lan 2: TT homozygote at undigested 305bp  
 Lan 3: CT heterozygote at 305bp, 190bp, 115bp, Lan 4: TT homozygote at undigested 305bp  
 Lan 5: CC homozygote at 190 bp, 115bp, Lan 6: TT homozygote at undigested 305bp

**Table 1:** Genotype and allele frequency of the IL-1  $\beta$  -511 C/T in renal failure patients infected with CMV and renal failure patients non-infected with CMV.

Genotype and allele frequency		Patients infected with CMVNO =22 (%)	Patients non-infected with CMVNO= 28 (%)	Control group NO=20 (%)
Genotype	CT	13 (59)	6 (21.4)	9 (45)
	TT	7 (31)	2 (7.1)	5 (25)
	CC	2 (9)	20 (71.4)	6 (30)
Allele frequency	C	17 (38.6)	46 (82.1)	21 (52.5)
	T	27 (61.4)	10 (17.9)	19 (47.5)

Increase the development of renal failure disease among patients in age more than fifty years maybe because most peoples in this age are suffering from chronic diseases such as heart disease, diabetes, hypertension, in addition to a decrease in several glomerular filtration rates (GFR) (Druml *et al.*,2010; Meylan and Manuel, 2007).

Antibody against Cytomegalovirus in serum can be detected by specific rapid test named CMV IgG/IgM antibody rapid test. When renal failure patients are infected with CMV, red color appear on letter G, also in healthy persons, but in case, renal failure patients are not- infected with CMV color appears only on the letter C as in Fig. 1.

In the present investigation, antibody rapid test was positive in 22 (44%) and negative in 28 (56%) of renal failure patients, but all healthy persons were negative for this test. The current study demonstrated that higher proportion from renal failure patients infected with cytomegaloviruses were females (75 %) than males (29.4%) (Staras *et al.*,2006). Who reported that 50% to 80% of women showing serological evidence of previous infection with CMV. Many females can acquire a CMV infection when contact with

young children within 1 year and approximately 80% of young children will develop CMV within two years in daycare centers, they will continue to shed virus for years after initial gaining (Bhide and Papageorghiou, 2008; Lazzarotto *et al.*,2011).

**RFLP – PCR for IL-1 $\beta$  gene**

The present study showed that digested DNA bands at 190 bp and 115 bp were produced by homozygote genotype of IL-1 $\beta$  -511(C/C), digested DNA band at 305bp, 190bp, and 115bp were produced by heterozygous genotype of IL-1 $\beta$  -511 (C/T) whereas homozygote genotype of IL-1 $\beta$ -511 (T/T) was produced one fragment 305bp and remained undigested as in Fig. 2.

**Table 2:** Show IL-1 $\beta$  level of genotype in renal failure patients infected or non- infected with CMV.

Genotype IL-1 $\beta$	CMV-infection	Non-CMV infection	Control
CC	13.8995 pg/ml $\pm$ .09300	10.9366 pg/ml $\pm$ .30236	5.4248 g/ml $\pm$ .17269
CT	19.2933 pg/ml $\pm$ .26589	7.8721 pg/ml $\pm$ .21906	4.4056 pg/ml $\pm$ .10210
TT	17.3609 pg/ml $\pm$ .38346	4.8408 pg/ml $\pm$ .51605	3.1536 pg/ml $\pm$ .09750

The cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ) is a key mediator of the inflammatory response. Essential for the host-response and resistance to pathogens, it also exacerbates damage during chronic disease and acute tissue injury (Lopez-Castejon and Brough, 2011). Single nucleotide polymorphisms in the interleukin-1 locus, their functional consequences, and their association with susceptibility to and severity of various chronic inflammatory diseases (Cannon *et al.*, 2010). Development of renal disease is related to polymorphism of IL 1 $\beta$  and the polymorphism gene of interleukin IL-1 $\beta$  C/T has a role in the existence and progress of human Cytomegalovirus (HCMV) infection (Puius and Snyderman, 2007; Meyer and Hostetter, 2007).

The current study demonstrate that percentage of the genotype frequencies of polymorphism in renal failure patients infected with CMV were (59%, 31%, 9%) for genotype CT, TT, CC, respectively, whereas in renal failure patients non-infected with CMV were (21.4%, 7.1%, 71.4%) for genotype CT, TT, CC, respectively, and in control group were (45%, 25%, 30%) for genotype CT, TT, CC, respectively as in (Table 1).

The present study revealed a significant difference in the genotype and allele frequency of IL-1 $\beta$  -511 C/T polymorphism among renal failure patients infected and non-infected with CMV compare with the control group. The CT (59%), genotype and T (61.4%), alleles were highly frequent in renal failure patients infected with CMV. The CC (71.4%) genotype and C (82.1%), alleles were highly frequent in renal failure patients non-infected with CMV. The CT (45 %) genotype and C (52.5 %), alleles were highly frequent in the control group as in (Table 1). Also, this results demonstrate a significant difference in the genotype concentration among renal failure patients infected and non-infected with CMV of IL-1 $\beta$  compare with control group  $P < 0.05$ , as showed in table (2), in renal failure patients infected with CMV, CT (19.2933), TT (17.3609 ) genotype, are produce high amount of IL-1 $\beta$ , in renal failure patients not-infected with CMV, CC (10.9366 pg/ml), CT (7.8721 pg/ml ) genotype are produce high amount of IL-1 $\beta$ .

Our results were consistent with Johnson *et al.*, (Levey and Coresh, 2012) and Wang *et al.* (Johnson, 2013) who showed that the persons carrying the (CT) genotypes and (T) allele are at higher risk to develop renal failure disease when infected with CMV compare with non-infected with CMV renal failure patients that carrying the (CC) genotype and (C) allele as a high frequently genotype and allele while healthy persons carrying the (CT) genotype and (C) allele as a high frequently genotype and allele.

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