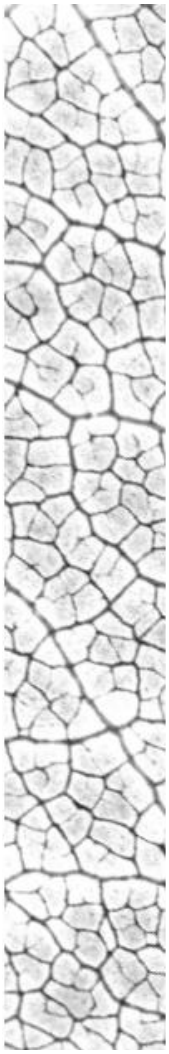

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Interleukin 1β and cytotoxic T lymphocyte-associated antigen: Role in susceptibility to cytomegalovirus infections

Shahad Abd Alrahman [1], Zainab A. Tolaifeh [1*], Israa Adnan Ibraheam [1]

[1] Biology department, College of Science for Women, University of Babylon, IRAQ
*Corresponding author: zainab.tolaifeh@uobabylon.edu.iq

Abstract

Objective: Interleukin 1β is a powerful cytokine with a variety of inflammatory functions. This cytokine plays a role in many cellular activities including cell expansion, differentiation, and apoptosis. CTLA4 is an antigen expressed in T lymphocytes has a critical role as inhibitory receptor acting as a major negative regulator of T-cell responses. **Material and Methods:** Fifty samples of the blood were obtained from CMV patients and a safe control group, then DNA was extracted and analyzed for genotypes IL-1β and CTLA-4 and Alleles with (PCR) and Gel electrophoresis using 2,25%, 2% the concentration of agarose was (respectively) investigated. Results: IL-1β mutations were detected in 58 percent of CMV patients, Although only 44% were observed in the control group, we noticed a substantial correlation between the genotype and the allele frequency and the P<0.05 group of CMV infections. CTLA-4, the G allele correlates with a substantially reduced risk of CMV infections. **Conclusion:** IL-1β and CTLA-4 are associated with CMV infections.

Keywords: Interleukin 1β, T lymphocyte, CTLA-4

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INTRODUCTION

Human Cytomegalovirus is double stranded DNA virus causes most widespread intrauterine infections, transmitted through blood, urine, genitourinary tract secretions, tears, saliva, breastfeeding and transplanted organs (AL.Dulaimi T.H.et al, 2019). Infections of HCMV can be obtained congenitally via vertical transmission of virus from infected mother to her fetus trough hematogenic expansion via placenta or at delivery time (Wujcicka et al 2017). Interleukin 1 are cytokines that affecting many cell types and organs. Inteleukin 1 cytokines bind with strong potential to three receptor-like replicates of extracellular immunoglobulin (Ig), allowing moderately little concentrations of this cytokine to cause a functional responses (Khazim et al., 2018). Interleukin 1β is a powerful cytokine with different functions in inflammation involving activation of the cellular components of acquired immunity, promotion of chronic inflammation and stimulation of acute phase response. This cytokine is produced in big quantities by monocytes through stimulation of its gene *ILB* after exposure for different endogenous and exogenous stimuli mainly related with injury or infections (Listman et al., 2008). IL-1 β acts a role in the microbial pathogenesis and development of many diseases. Overexpression of IL 1 had been identified in autoimmune diseases, inflammatory diseases, kidney and heart disease, malignancies and many infections (Khazim et al., 2018). CTLA-4, also named CD125 is an antigen that is expressed in T cells in response to stimulation of the T-cell receptor and acts as a significant negative regulator of the cellular immunity. A reduced function of CTLA-4 may be enrolled in autoimmune processes, it has a significant lymphoproliferative and lethal condition. Hence, molecular variations in CD125 receptor lowered its capability to inhibit and regulate the expansion of T lymphocytes (Danilovic et al., 2012; Abushama, et al, 2014). The gene of CTLA4 is located on chromosome 2 q33, with nucleotide size about 6.2 kb, composed of 4 exons and 3 introns. Frst exon codes sequence of leader peptide, exon two encodes for an immunoglobulin domain, exon three encodes for the hydrophobic transmembrane domain, and fourth exon encodes for the cytoplasmic domain (Misra et al., 2015).

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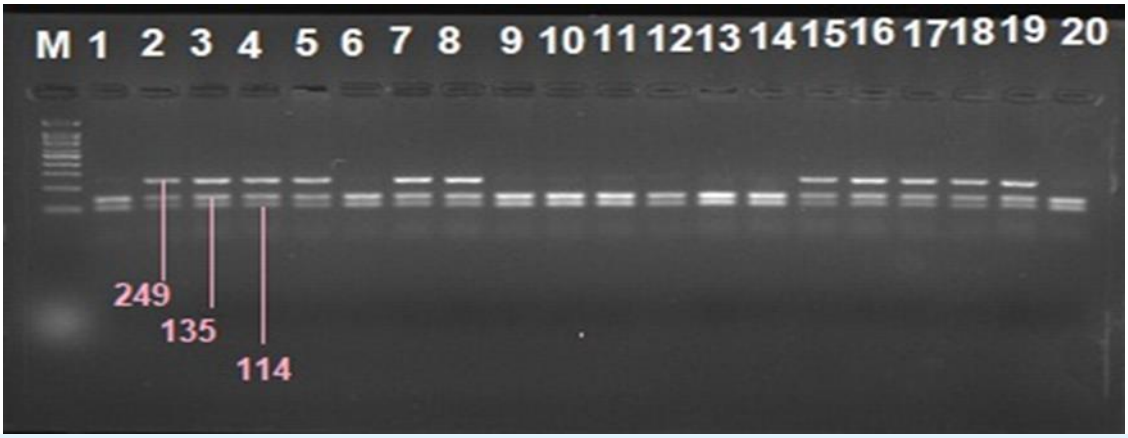

Fig. 1. Gel electrophoresis for PCR product of IL-1β gene polymorphism for Cytomegalovirus infected: M: (100-bp) DNA ladder, Lane(2-3-4-5-7-8-15-16-17-18-19) heterozygote CT (249, 135, 114 bp).Lane (1-6-9-10-11-12-13-14-20) Homozygotes CC (135,114), Gel con. (2.5%)

MATERIALS AND METHODS

Subjects

The present study is a case-control analysis that involves 100 females, divided into two classes, the 50 females with Human Cytomegalovirus and 50 females that are apparently stable. Four ml of venous blood were drawn from all participants using disposable syringe, four ml of blood was obtained for genetic study and slowly pushed into the EDTA tube and given the patient's name at -20 ˚C.

Extraction DNA

In addition to the human DNA extraction kit of Intron Biotechnology, and following the directions of the manufacturer, the DNA was extracted from each patent and control group blood using 200ml of whole blood.

Genotyping DNA

The genotyping of IL-1β gene polymorphism was observed by the use of RFLP technique PCR (Polymerase Chain Reaction) and the use of Green master mix (promega), the genotyping of IL-1β gene polymorphism using the primary pair as previously used in the study (Balding et al., 2004). The total volume of reaction was 25 µl, containing 12.5 µl of master mixture combined with 7 µl of DNA and 100 P mole (1 µl) of the specific C primer with 100 P mole(1 µl) of the specific primary T and 1 µl of the primary antisense and 3.5 µl of the purified water. Using this condition PCR method, the PCR mixer uses amplified: front denaturation 94 ° C for 4 min, and 37 loop 94 ° C for twenty seconds, 59 ° C for 55 seconds, 72°C for 25 seconds, then last stage 72°C for 2 min. Restriction fragment length gene polymorphism was performed using Taq1 restriction enzyme by reaction containing 10 µl pcr drug, 0.5 µl R.E. Serum bovine albumin 0.2, buffer E 2 µl, and D.W 7.3. CTLA-4 gene polymorphism was genotyped using the PCR (Polymerase Chain Reaction) technique and then using the Green Master Mix (Promega) process. Genetic manipulation using the priming pair as used in the study of CTLA-4 gene polymorphism (Dursun et al., 2018). The volume of reaction was twenty µl composed of ten µl master mix, 0.6 µl of both forward and reverse, 6 µl of the DNA sample, and 2.8 µl of the distilled water. With this condition PCR device the PCR mixer uses amplified. Front denaturation 94 ° C for 3 min, and 39 period for 94 °c for twenty seconds, 54.6 °c for 25 seconds,72 °c for 25 seconds and finally 72 °c for 2 minutes. RFLP was performed using restriction enzyme Ncol by 10 µl pcr drug, 0.5 µl R.E. 0.2 bovine serum albumin, 2 µl d and 7.3 D.W.

Analyzing

Gel electrophoresis was performed on 2.5% agarose gel for IL-1β gene polymorphism and 2% agerose gel for CTLA-4 gene polymorphism, containing 4,5 µl red safe. The gel was analyzed and genotypes determined using transilluminator.

Statistical Analyses

Potential associations of IL-1β and CTLA-4 with the risk of cytomegalovirus infection were analyzed by comparing IL-1β and CTLA-4, in control group patients use chi-square ($P < 0.05$ find significant) and odd ratio (OD) check CI 95% to measure the effect of this mutation on the infected group relative to the control group.

RESULTS

The genotype of whole 50 patients of Cytomegalovirus was analyzed for detection the presence of normal or mutant genotype of IL-1β, The PCR results showed the polymorphism of IL-1β displayed C and T alleles and three genotypes (CC, CT and TT) **Fig. 1**. The T allele results in an undigested polymerase chain reaction product of 249 bp (homo),and C allele contributed to a digested PCR product of two 135 and 114 bp fragments, while the AC genotype resulted in three 249, 135, 114 bp (hetero)

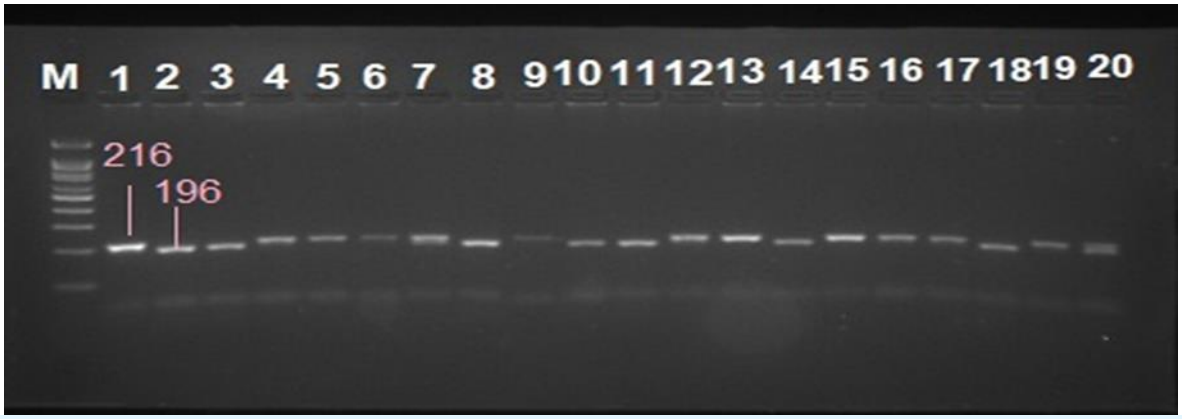

Fig. 2. Gel electrophoresis for PCR product of *CTLA-4* gene polymorphism for Cytomegalovirus infected: M: (100- bp) DNA ladder , Lane (7-20) heterozygote AG(216-196-20 bp).Lane (1-4-5-6-9-12-13-15-16-17-19) Homozygotes GG(216 bp), (2-3-8-10-11-14-18) Homozygotes AA(196,20 bp), Gel con. (2.25)

Table 1. Allele and Genotype frequencies of IL-1β gene polymorphism between positive and negative Cytomegalovirus patients (uninfected controls)

Genotype Il-1b	Patients	Control	P- Value	OR=(95%CI)
TT[a]	2(4%)	8(16%)		
TC	29(58%)	22(44%)	0.035*	0.19(0.03-0.98)
CC	19(38%)	20(40%)	0.09	0.26 (0.04- 1.40)
Total	50	50		
Allele				
T	33	38	0.27	0. 8(0.45-1.43)
C	67	62		

P ≤ 0.05; OR=(95%CI); [a] reference

Table 2. CTLA-4 gene polymorphism allele and genotype frequencies among Cytomegalovirus positive and healthy patients (uninfected controls)

Genotype Ctl-4	Patients	Control	P- Value	OR=(95%CI)
AA[a]	11(25.58%)	6(13.95%)		
AG	6(13.95%)	2 (4.65%)	0.48	0.61(0.09-4.02)
GG	26(60.46%)	35(81.39%)	0.09	2.46 (0.80- 7.53)
Total	43	43		
Allele				
A	28	14	0.01*	2.48(1.19-5.14)
G	58	72		

P ≤ 0.05; OR=(95%CI); [a] reference

fragments. Revealed that 19(38%) patients infected with Cytomegalovirus has CC Allele, and 2(4%) patients infected with Cytomegalovirus has TT, while 29(58%)patients has TC allele which consider as mutant, compared with the control group, 20(40%) containing the CC allele and 8(16%) containing the TT allele,and 22(44%)contain the TC allele (**Table 1**). The CTLA-4, in **Table 2** no significant difference in AG, GG genotypes frequency between patient and control groups (P-value =0.61,2.46 respectively) where AG genotype was found in 13.95% and GG genotype was found in 60.46% of patient group, while AG genotype was found in 4.65% and GG genotype was found in 81.93% of control group. The G allele frequency (i.e., the AG, GG genotypes) has increased substantially in the control group relative to the patient group, where 72 (83.7%) of control compared to 58 (67.4%) of patient. (**Fig. 2**) control compared to 58 (67.4%) of patient (**Fig. 2**).

DISCUSSION

A variety of genetic polymorphisms were related to high risk of CMV infections. IL1 β +3954, increasing in the TC genotype frequency in CMV patients when compared to uninfected control group, lead to suggest an association between TC genotype and increase infection with CMV. The analysis of allele frequency among patients with cytomegalovirus and the healthy persons (control group) also showed that no obvious association between allele frequency and CMV infection, these result was matched to the previous study done by Ali Abdul Hussain et al 2018 in Baghdad, Iraq, and Wujcicka et al 2017. IL1 β +3954 C>T polymorphism was describe to be correlated with other disease such as tuberculosis (Meenakshi et al., 2013; Hall et al.,2014) and the lipodystrophic syndrome (LD) in Caucasian persons infected with HIV(Asensi et al.,2008).CTLA4 is an immunomodulatory enzyme, since it plays a

significant role in stimulation and negative control of T cells expansion. However, the precise role of CTLA4 in cellular immunity following transplantation in combination with symptomatic CMV infections is not well understood. (Misra et al., 2015) According to result of current study the rs3087243 SNPs of CTL-4 gene had no association with CMV infection and may be protect effect against HCMV infection in female.

In contrast with other research, Misra et al 2014 findings in India indicate that variants of CTLA4 could be implicated in the clinical aspects of HCMV infections, where this study reported that A allele has protective effect in HCMV asymptomatic groups, and G allele had risk association with HCMV. Other study done by Thio, Chloe L., et al. 2004, It was observed that those with +6230A in tree untranslated regions were 1.3 times more vulnerable to hepatitis B infection (Thio et al., 2004) In addition, +6230 was tested for organ transplantation, cancer, diabetes type 1, lupus erythematous and different diseases (Guo et al., 2013). This difference in result between two studies might because difference in sample size, or misinterpretation of PCR result or because difference in race, lifestyle of patient.

CONCLUSION

The IL-1β polymorphism, the significant increasing in the TC genotype frequency in CMV patients when compared to uninfected control group. According to CTLA-4 gene polymorphism, G allele significantly correlates with lowered susceptibility for CMV infections.

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