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IMMUNOGENETIC DETECTION OF EBV AND TLR7 IN CKD IN BABYLON PROVINCE

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ABSTRACT

The study aims to investigate Epstein–Barr virus (EBV) and Toll-like receptor-7 (TLR-7) in chronic kidney disease infections in Babylon province. To achieve this goal, collect 480 (blood and urine) samples to each patients and control from many hospitals and outside laboratory in Babylon province (340 patients and 140 from normal person as control group). Samples were collected from the period from 1 of December 2023 to 1 November 2024. And there age between ≥20 years and ≤50 years. ((Conventional Primer-Polymerase Chain Reaction (PCR) used to detect EBV and the Single Specific Primer-Polymerase Chain Reaction (SSP-PCR), which can detect a known SNP in TLR-7.)) The result of the current study showed the distribution of samples according to the age of CKD patients and control with viral infections, the highest frequency was in age group A4 (26%) and the lowest frequency was in age group A2 (14%). While the distribution of control showed that the highest frequency was in age group A4 (22%) and the lowest frequency was in age group A4(24%). All age group were selected in order to ensure comparable frequency distribution. Chi-square = 1.894,4 and P value = 0.772 among study groups according to age distribution. The EBV DNA has been detected in PCR among CKD patients and control were classified by gender. the positive EBV DNA was detected in (89%) male and (86%) female patients respectively. And the positive EBV DNA was detected in (3%) male and (11%) female control. Chi-square = 278.3,3 ; P value = 0,0001 among male and female according to detection and distribution. The TLR-7 genotype frequency distribution patients and control groups is shown as follow: GG (28.13%), GA (42.5%) and AA (29.38%). While in control subjects was: GG (27.5%), GA (40%) and AA(32.5%).Chi-square =0.165; P value = 0.925 among patients and control group according to TLR-7 genotype detection and distribution.

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INTRODUCTION

There are numerous terms for chronic kidney disease (CKD), including Uremia, chronic renal insufficiency, or chronic renal failure (CRF) These disorders are characterized by an abnormal

glomerular filtration rate (GFR) or a gradually progressing loss of renal function over several months or a year (1). End-stage renal disease (ESRD) is the term for chronic kidney disease (CKD) that results in serious infections and requires dialysis or another type of renal replacement treatment (2). Since dialysis is a form of renal replacement therapy used in medicine to offer an artificial replacement for the kidney that lost its functions due to increasing renal failure, it is considered a life support treatment and cannot be used to cure any kidney ailments (3). Chronic kidney disease symptoms can include leg edema, fatigue, appetite loss, vomiting, or disorientation. If complications arise, they will include anemia, heart problems, and high blood pressure (4). Globally, the incidence of chronic kidney disease is rising, possibly as a result of population aging. This rising rate is also linked to an increase in other conditions including diabetes and hypertension (5).

Epstein Barr Virus is one of eight human herpesviruses that have been identified. Its genome is about 170 kb long and is made up of linear, double-stranded DNA. The genome is a circular plasmid that is located in the nucleus of latently infected cells. Both ends of the linear form of the genome contain terminal repeat (TR) sequences, which mediate the circularization in the infected cell. The EBV genome contains a surprisingly large tandemly repeated DNA sequence called the main internal repeat (IR1) (6). The EBV genome is separated into long and short unique sequences (UL and US) by the IR1 site. There are several tightly spaced genes in these sequences (7). This viral cytokine has the ability to prevent monocytes and macrophages from activating T-cells, which are required for the EBV-dependent B-cell transformation (8).

The Epstein Barr Virus enters the body through the lining of the nasopharynx and is spread by salivary contact. B-cell infection starts when the cellular complement receptor type 2 (CR2), also known as CD21, attaches itself to the main EBV outer envelope glycoprotein gp 350/220 (9). The major histocompatibility complex (MHC) class II molecule is a cofactor for B-cell infection. Cells become activated and immortalized as a result of infection. EBV's DNA genome encodes over 100 viral proteins, all of which are expressed during viral replication (10).

As a member of the innate immune receptor family, the toll-like receptor (TLR-7) can recognize pathogen-associated molecular patterns (PAMPs), control cytokine production, trigger the innate immune response, and trigger an indirectly adaptive immune response, among other functions. Members of the TLR family, including TLR-7, are able to identify single-stranded RNA in endosomes. TLR-7 is capable of identifying GU-rich single-stranded RNA. It helps prevent chickens from getting infected with viruses. An essential aspect of antiviral immunity, especially in ducks, is TRL-7 (11). Variations in cytokine production among patients with chronic kidney disease may be explained by genetic variation. TLR7 gene expression may be linked to single nucleotide polymorphisms (SNPs) in the gene. Given the part TLR7 plays in CKD, it is necessary to look into the genetic screening of TLR7 gene variants in CKD patients.

In the present investigation, we looked for variations in the TLR7 gene's coding area in both CKD patients and controls. Our goal was to find any connection between TLR7 polymorphisms and how they might affect CKD patients' susceptibility to EBV. Sensitive molecular methods, such as PCR, have been used to detect EBV infections in CKD patients and control samples.

METHODOLOGY

Collect 480 (blood and urine) samples to each patients from many hospitals and outside laboratory in Babylon Governorate (340 patients and 140 from normal person as control group). Samples were collected from 1 of December 2023 to 1 November 2024. The sample collected from patients with chronic kidney diseases and the steps of method comprise the following:

1. Collection of blood sample:

Vein punctures were used to obtain blood samples from both patients and controls. Four milliliters of blood were placed in an EDTA tube and kept at -20°C to extract human DNA and examine TLR-7 gene polymorphism.

2. Collection of Urine sample:

Fill the cup halfway with a urine sample that has been collected. A tiny amount of urine (1.7 mL) can be used to extract viral DNA. Compared to room temperature, the DNA yield was greater after 100 days of storage at 4 °C or -20 °C. DNA deterioration may have resulted from storage at room temperature.

3. Molecular detections by Using Polymerease chain reaction (PCR):

A modified methodology that works with the Blood Genomic DNA extraction kit (Solarbio Cat No.: D1800) has been used to extract DNA from urine for viruses and from human blood for humans. The procedure is as effective when applied to new samples. Please be aware that the size and density of the source material will affect the lysis time.

((Two complementary reactions make up the Single Specific Primer-Polymerase Chain response (SSP-PCR). One response has a primer specific for the normal DNA sequence and is unable to amplify mutant DNA at a particular locus, while the second reaction contains a primer unique to the mutant and is unable to amplify normal DNA, can detect a known SNP in TLR-7. Conventional PCR is used to detect EBV.))

DNA primers with amplicon widths of 265 bp were used to create primer pairs for the EBV genome (Table 1).

The amplification system contained:

1- 10 µL of Premix-Taq, 0.5 µL of forward primer (10 µM),
2- 0.5 µL of reverse primer (10 µM),
3- ddH2O, which was added to supplement the 20 µL system.

The PCR reaction conditions included:

1- An initial denaturation temperature of 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 15 s.
2- Annealing at 56 °C for 15 s,
3- Extension at 72 °C for 60 s.
4- A final extension at 72 °C for 5 min.

➧ The resulting PCR products were subjected to 2% agarose gel electrophoresis.

The Primers which are used in this study illustrate in table (1).

Table (1): Primers.

Gene	Primer	PCR product length
TLR-7G>A (rs864058)	F: CCT TGA GGC CAA CAA CAT CT R: GTA GGG ACG GCT GTG ACA TT	201bp
EBV	F: CCT GGT CAT CCT TTG CCA R: TGC TTC GTT ATA GCC GTA GT	265bp

Ethical Committe:

AlFurat Alawast Technical University oversaw and offered recommendations for this project. Every sample used in this study was obtained in accordance with the research procedures for each kind, without the use of additional materials or modifications, and was authorized by the Medical Ethics Committee of the Iraqi Ministry of Health.

Statistical Analysis:

A digital database was updated with the data. Microsoft Excel 2010 and the computer program Graph Pad Prism (version 6) were used to conduct statistical analyses. Allele and genotype frequencies of the TLR-7 genes were estimated using patient-directed counts and expressed as a percentage. The data was also compressed using the Chi-square test.The test was conducted using a significance level of 0.05.

RESULTS AND DISCUSSION

Classification of study groups

Classification The present study show the distribution of samples according to age (Figure-1). The age of CKD patients and control with viral infections showed that the highest frequency was in age group A4 (26%) and the lowest frequency was in age group A2 (14%). While the distribution of control showed that the highest frequency was in age group A4 (22%) and the lowest frequency was in age group A4(24%). All age group were selected in order to ensure comparable frequency distribution. Chi-square = 1.894,4 and *P* value = 0.772 among study groups according to age distribution.

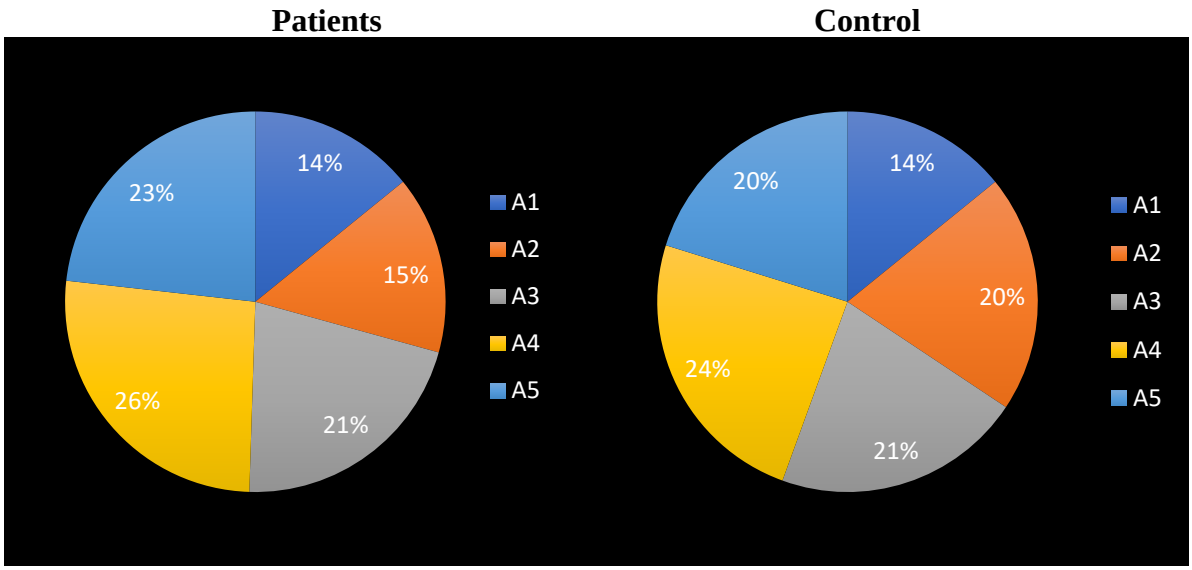

Figure-1: Distribution of patients and control , according to age

- **A1: The group of patients with age(≥20) years.**
- **A2: The group of patients with age(20-30) years.**
- **A3: The group of patients with age(30-40) years.**
- **A4: The group of patients with age(40-50) years.**
- **A5: The group of patients with age(≤50) years.**

Immunosenescence, which describes alterations in the immune system such as a weakened immunological response and persistent inflammation, typically occurs in people over 50 (12). According to statistics from multiple extremely large datasets, almost 44% of older persons over 65 had chronic kidney disease (CKD)(13). Ten to Twelve percent of people have chronic kidney disease (CKD), a progressive illness that can be exacerbated by normal aging-related physiological decline. Elderly people have a much higher prevalence of CKD. In reality, about half of people 65 to 74 years old have five or more chronic illnesses, and this percentage may increase to 70% for people over 85 (14). Sensitive molecular techniques, such as conventional PCR, have been used to detect EBV infections in samples. As far as we are aware, numerous investigations on EBV have been published using standard, everyday polymerase chain reaction (PCR).

The EBV DNA has been detected in Table -2 and Figure -2 are the detection of the EBV DNA by PCR among CKD patients and control were classified by gender. This Table shows the positive EBV DNA was detected in (89 %) male and (86 %) female patients respectively . And the positive EBV DNA was detected in (3%) male and (11%) female control. There is significant difference (Chi-square = 278.3,3 ; P value = 0,0001) among male and female according to detection and distribution.

Table-2: Illustreated the EBV infections in patients and control.

Samples	Patients		Control		ChiSquare *P*.Value
	Male	Female	Male	Female	
EBV (+)	153 (89%	144 (86%)	2 (3%)	8 (11%)	Chi-square = 278.3,3 P = 0,0001 S
EBV(-)	19 (11%)	24(14%)	68 (97%)	62 (89%)	
Total	172 (51%)	168(49%)	70 (50%)	70 (50%)	
	340 (71%)		140 (29%)		
	480 (100%)				

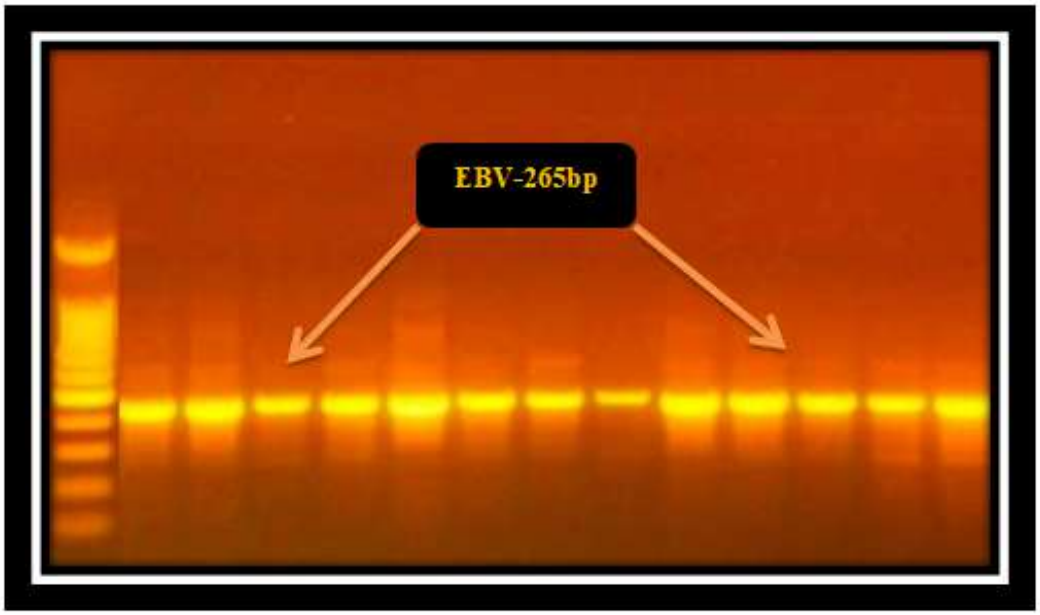

Figure-2:Agarose gel electrophoresis image that show the PCR product analysis of HBV gene in samples. PCR product was analysis by 1% agarose gel . Where M: marker (50bp – 1000bp), All lane picture showed positive bands to EBV (265 bp) in samples.

Associations with previously documented cases are evoked by the connection between the EBV infection from a few years ago and the re-emerging catastrophic renal failure. Eight occurrences of acute renal failure in juvenile EBV patients within six years were documented by a researcher from a Taipei, China hospital; two of these individuals ultimately passed away (15). Few patients with renal impairment brought on by an EBV infection get a renal biopsy. It is thought to exhibit various pathogenic forms, including minimum change disease (MCD), membranoproliferative glomerulonephritis, and interstitial nephritis (16).

According to Okada et al., a patient with a chronic active EBV infection experienced minimal change illness in addition to acute tubulointerstitial nephritis. A renal biopsy revealed lymphocytic interstitial infiltrates and papillary infoldings of atypical tubular epithelium. Some infiltrating lymphocytes had EBV DNA found by PCR, while tubular epithelial cells did not. EBV-infected T-cells (17). The TLR-7 gene's genetic polymorphism has been identified at one site, the TLR-7 G→A, which is found in patients and controls with three genotypes (GG, GA, and AA). The SSP-PCR approach has been utilized to genotype TLR-7 G-A in both control and chronic renal disease. Agarose gel electrophoresis has effectively resolved and sized the PCR results, making it simple to identify various genotypings. The gel profile has clearly identified heterozygotes and homozygotes.

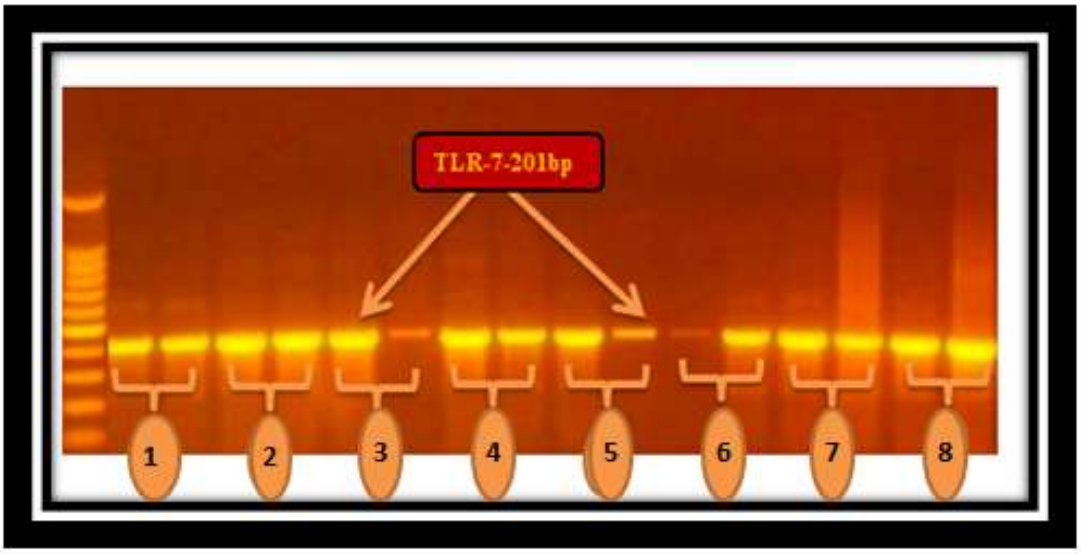

Figure-3: Agarose gel electrophoresis image that show the SSP-PCR product analysis of TLR-7 gene (G/A) in samples. SSP-PCR product was analysis by 1% agarose gel . Where M: marker (50bp – 1000bp), lane GG wild type homozygote was shown at the bands sample (1,2,4,7 and 8), lane GA mutant type heterozygote was shown at the following bands samples (3and 5) and lane AA homozygote was shown in bands samples (6).

The TLR-7 genotype frequency distribution patients and control groups is shown as follow: GG (28.13%), GA (42.5%) and AA (29.38%).While in control subjects was: GG (27.5%), GA (40%) and AA(32.5%). There was no significant difference (Chi-square = 0.165 ; *P value* = 0.925) among patients and control group according to TLR-7 genotype detection and distribution as shown in Figure (4).

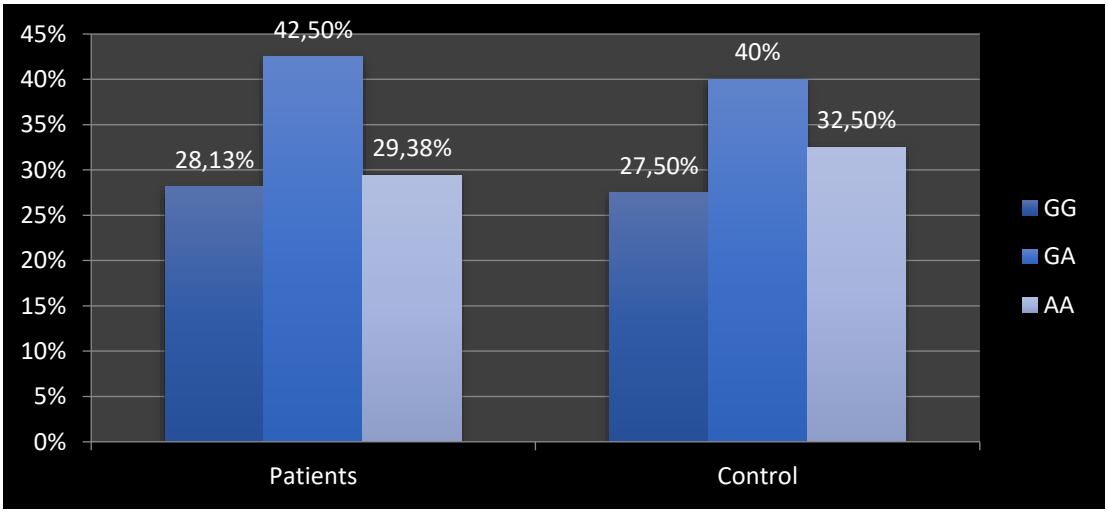

Figure-4: Distribution of patients and control gruops , according to TLR-7 Genotyping

Other researchers studying respiratory infections found a substantial association between an elevated risk of COVID-19 pneumonia and the "T/T" genotype and the "T" allele of TLR7 rs179008 as well. Likewise, the G allele and GG genotype, as well as the TLR7 genetic site rs3853839, were substantially associated with COVID-19 cases, whereas the CC genotype and C allele were associated with healthy volunteers (18). ((People in the Han Chinese community who have the C and A alleles at SNPs T-1237C and G1635A in the TLR-9 gene appear to be more likely to develop chronic kidney disease, according to additional genetic studies (19).**))**

CONCLUSION

This investigation could lead to the following conclusions: This study describes the prevalence of EBV in people with chronic kidney disease (CKD).The recent investigation found that EBV is more common in patients with chronic kidney disease. Genetic studies of TLR-7 alleles do not consider a risk factor for effection in people with and without EBV infections**.**

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