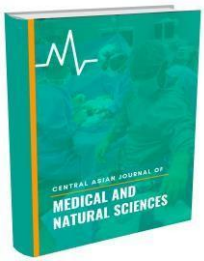

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Article

Molecular Detection of Cytomegalovirus and Bacterial Species Causing Urinary Tract Infections in Hemodialysis Patients

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Abstract: This study included 100 kidney failure patients. Five ml of blood was collected from each patient, the plasma was separated, and then the plasma samples were stored until DNA extraction was performed. Molecular detection of Cytomegalovirus (CMV) was done by polymerase chain reaction (PCR). Urine samples were also obtained for bacterial species. The results of the PCR technique revealed that 8 patients were infected with the CMV at a rate of 8 %, with 5 infections in males and 3 infections in females, with a rate of 8.9% and 6.8% respectively. Regarding the age group, the highest incidence of the virus was in 21-30 age group in males and the 41-50 age group in females. The results of bacterial detection show that *E. coli* cause the highest rate of urinary tract infections in hemodialysis patients infected with the CMV at 100%, followed by *Klebsiella pneumonia* at 60%. We conclude that patients receiving end-stage renal dialysis may be more susceptible to CMV replication while they get chronic hemodialysis.

Keywords: Cytomegalovirus, Kidney failure, Real-time PCR, UTI

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 [1]. 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 [2]. Failure of the kidneys Pathophysiology is a sequence of events that can happen both acutely during acute renal failure and gradually over time in chronic kidney disease [3].

Human cytomegalovirus (HCMV) is the most complicated genetic virus among all pathogenic viruses of humans and the largest typical one among allherpesviruses. HCMV is the prototypic member of human *Herpesveridae* family, subfamily Betaherpesverinae, about (20 to 300 nm) indiameter, and it is an enveloped double-stranded DNA virus [4]. Primary infection with HCMV in immunocompromised people is asymptomatic or mild, or produces fever-like with mononucleosis-like symptoms [5].

The most frequent cause of upper and lower urinary tract infections is thought to be bacteria. Regardless of sex, 50% of patients are infected with *Escherichia coli*. The most contagious bacteria in patients with UTIs is *Escherichia coli*.7. Inpatients are more likely to have *Klebsiella*, *Enterobacter*, *Staphylococci*, *Pseudomonas*, *Proteus*, and *Enterococci* isolated from them. It is known that *Corynebacterium urealyticum* is a significant nosocomial

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pathogen. Seldom are anaerobic microbes urinary tract pathogens. Negative coagulase Urinary tract infections are frequently caused by Staphylococci. Young women of sexually active age are susceptible to infection from Staphylococci saprophyticus.

2. Materials and Methods

Study group

The present study included 100 dialysis patients, 56 males and 44 females, of various ages between 18-60 years, who visited the dialysis center in Kirkuk city.

Sample collection

Five ml of venous blood was taken from study patients, the blood was placed in EDTA tubes and subsequently, plasma samples were obtained using the process of centrifugation at a speed of 3000 revolutions per minute for a duration of 5 minutes. Plasma samples were distributed in microcentrifuge tubes and stored at a temperature of (-20 degrees Celsius) until they were used to diagnose the virus. Urine samples were also collected from patients for the purpose of detecting bacterial species that cause urinary tract infection.

Molecular diagnosis

DNA extraction and gel electrophoresis

*I*n order to confirm that nucleic acid was present in the product and that the extraction procedures were performed correctly and precisely, the extraction products were transferred using gel electrophoresis after nucleic acid was extracted from the plasma using a specialized extraction kit. Because real-time PCR findings sometimes produce false negatives, this can ultimately result in a misdiagnosis. Figure 1 depicts this process, emphasizing the possibility of erroneous diagnostic results.

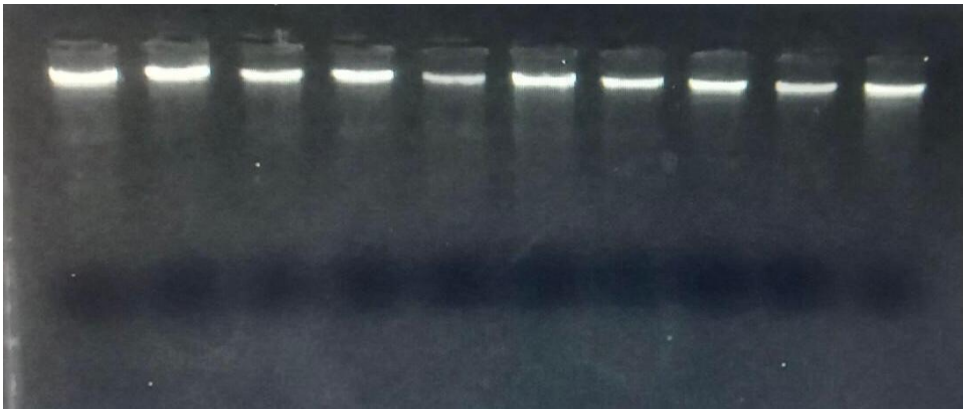

Figure 1. showed DNA extraction products

Detection of Cytomegalovirus by Real-time PCR

Primers were designed using the Primer3plus program and genes extracted from the National Center for Biotechnology Information (NCBI). Macrogen Laboratories then supplied primers in a lyophilized state table (1). To create a stock solution, the lyophilized primers were reconstituted in nuclease-free water until they reached a final concentration of 100 picomoles per microliter (100 pmol/µl). 90µl of enzyme-free water was mixed with 10µl of primer stock solution, which had been previously kept at -20 °C, to produce an efficient solution of these primers.

Table 1. Designed Primers

Primer name	Sequences	Position
CMV-UL123-F	5` AACTTGCCTCAGTGCTCTCC -3`	946-965
CMV-UL123-R	5` TACAGGGGACTCTGGAGGTG -3`	1160-1179
CMV-UL123-P	5` TCTGTCGGGTGCTGTGCTGC -3`	1442-1461

Assay procedure

The HotStarTaq DNA Polymerase is first activated by a denaturation phase in the thermal protocol. This is followed by a two-step amplification cycle and a terminal hold. Aaccording to table 2, Real-time data collection occurs during the second phase of the amplification cycle.

Table 2. Programming

Step	Degree	Duration	Cycle number
Initial denaturation	95°C	15:00 min.	1
Denaturation	95°C	00:30 min.	50
Annealing andelongation (Data collection)	54°C	01:30 min.	

Data analysis

At the end of the thermal protocol, the baseline cycles and the threshold are automatically determined by the Fluorine Detection System software. The data derived from the set standards is used to produce the standard curve, which shows the relationship between the Threshold Cycle and the Log Starting Quantity.

Detection of bacterial species

Due to their inability to urinate, hemodialysis patients do not have urine samples accessible for the detection of microorganisms. According to current worldwide norms, urine screening is not done on a regular basis in HD centers. Therefore, only five of the eight patients who tested positive for CMV had urine samples taken for this investigation.

After culturing the samples on the MacConkey and blood agar, they were incubated for 24 hours at 37°C. The phenotypic traits of the colonies on the culture media, such as their size, edge, height, and color, were used to make the initial diagnosis of the isolates after incubation. The cells were then stained with Gram stain, and their phenotypic features—such as their size, shape, grouping technique, and the outcome of the Cram stain interaction—were examined under a combined optical microscope. The Api20E system then verified the diagnosis.

3. Results

Sample Distribution

According Sex

This study included 100 Samples, 56 (56 %) were male patients and 44 (44 %) were female patients, as shown in Table (3).

Table 3. Study groups

Total No. of the study group	The study group according to the sex			
	Male		Female	
	No.	%	No.	%
100	56	56	44	44

The current study is consistent with the following studies: Ricardo *et al*., 2019, Wirya *et al* ., 2019 and Fouad *et al*., 2015 , who concluded that the rate of males among kidney dialysis patients was higher than females, the reason is believed to be due in that to biologic and psychosocial factors [7][8][9].

According Age

The ages of the patients ranged from 18 to 60 years for hemodialysis patients. The highest rate of patients were within the age groups 51—60 years (33 %),while the lowest rate of patients were within the age group **< 20** years (5 %) , as shown in Table (4).

Table 4. Distribution of the study group according to age

Age group (year)	The study group	
	No.	%
< 20	5	5%
20-30	18	18%
31-40	21	21%
41-50	23	23%
51-60	33	33%
Total	100	100

The current study showed that the most people who were subjected to dialysis were within the age groups 51-60, at rate (33 %). Little is known about the conditions in which the elderly begin dialysis. However, we believe that most people who start dialysis are those who stay in the hospital for a long time and receive different types of treatments and this is consistent with Wong *et al*., 2014 and Wirya *et al* ., 2019 [8][10].

Molecular Detection of CMV DNA in Plasma Samples (CMV viremia) using qPCR

The results of molecular detection (real-timePCR) indicated that 8 out 100 patients were infected with the CMV, 3 out of the infected individuals were female, and 5 out of the infected individuals were male. This indicates that 6.8% of the female population and 8.9% of the male population were contaminated. As shown in table (5).

Table 5. Positive rate among study groups by real-time PCR

Sex group	Positive		Negative		Total	
	No	%	No	%	No	%
Males	5	8.9%	51	92.1%	56	100%
Females	3	6.8%	41	93.2%	44	100%
Total	8	8%	92	92%	100	100%

According to the current study's findings, patients receiving end-stage renal dialysis may be more susceptible to BK virus replication if they receive chronic hemodialysis.

The hormones androgens in males and estrogens in females are probably the cause of the gender disparities in CMV [11]. It's well knowledge that men and women react to infections differently, with the female immune system frequently generating more antibodies and causing less inflammation. Mohsin et al. [12] and Mahmood et al. demonstrate that the PCR prevalence of HCMV in Diyala/Iraq was 6.6% and 6.6%, respectively. According to an Iranian investigation, CMV-DNA was present in 2.68% of samples [13].

This study supports the findings of Tofiq et al. and Firouzjahi et al., who demonstrated that males had higher rates of CMV than females [8][14], as well as Mohsin et al., who found that CMV was found in only 6% of hemodialysis patients [12].

The data in Table (6) shows the 21 to 30 age group had the highest rate of infection for males. For females, the group with the highest incidence was 41 to 50 years old.

Table 6. Positive rate among studygroups by real-timePCR technique according to patients' age and sex group.

Age groups	Male positive		Male negative		Female positive		Female negative		Total	
	No	%	No	%	No	%	No	%	No	%
≤ 20	0	0%	2	3.9%	0	0%	3	7.3%	5	5%
21 – 30	3	60%	8	15.7%	0	0%	7	17.1%	18	18%
31 – 40	1	20%	12	23.5%	0	0%	8	19.5%	21	21%
41 – 50	0	0%	17	33.4%	2	66.6%	4	9.8%	23	23%
51 – 60	1	20%	12	23.5%	1	33.4%	19	46.3%	33	33%
Total	5	100%	51	100%	3	100%	41	100%	100	100%

Detection of bacterial species

The current study showed that *E. coli* was isolated from all urine samples of patients who infected with CMV at a rate of 100%, and *Klebsiella pneumonia* were isolated from three samples at a rate of 60%. Also, *Pseudomonas aeruginosa* were isolated from two samples at a rate of 40%, while *Staphylococcus aureus* and *Staphylococcus epidermidis* were isolated from only one sample at a rate of 20%. As shown in Table (7).

Table 7. Shows Bacterial isolates

Isolated bacteria	Sample No (5)					
	Positive		Negative		Total	
	No	%	No	%	No	%
E. coli	5	100%	0	0%	5	100%
Klebsiella pneumonia	3	60%	2	40%	5	100%
Pseudomonas aeruginosa	2	40%	3	60%	5	100%
Staphylococcus aureus	1	20%	4	80%	5	100%
Staphylococcus epidermidis	1	20%	4	80%	5	100%

The current study shows that *E. coli* cause the highest rate of urinary tract infections in hemodialysis patients infected with the megaloblastic virus at 100%, followed by *Klebsiella pneumonia* at 60%, This study is consistent with the studies of Haider *et al*., 2016 and Yamashita *et al*., 2022, which showed that E. coli bacteria cause the highest incidence of urinary tract infections in patients undergoing hemodialysis, followed by Klebsiella pneumoniae [15][16].

4. Discussion

In line with the findings of this study, Beraldo-Massoli et al. reported that enteric Gram-negative bacteria, particularly E. coli, were the most frequently detected pathogens in UTIs. *Klebsiella*, *Proteus*, and *Pseudomonas* were next in line [17]. *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Citrobacter* were the most common bacteria in a study conducted by D'Addazio and Moraes [18].

5. Conclusion

This study concluded that 8% of dialysis patients were infected with the Cytomegalovirus, and the incidence was higher in women than in men. It also showed that *E. coli* cause the highest rate of urinary tract infections in hemodialysis patients infected with the CMV , followed by *Klebsiella pneumonia*.

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