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Immunological and Molecular Study of Human Cytomegalovirus contribution to Anemia in patients with Chronic Kidney Disease

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Abstract

Background: Renal failure is a condition in which the kidneys fail to remove metabolic end-products from the blood and regulate fluid, electrolytes and pH balance of extracellular fluids. The aim of current study was to investigate the role of human cytomegalovirus in renal failure and its contribution to anemia among patients with renal failure. **Methods:** This study was carried out at AL-Sadder Medical City Hospital during the period from December 2018 to February 2019. The study involved a total of 50 patients (32 males and 18 females) with kidney disease with age range between (20-70) years and 20 healthy individuals considered as controls. The human cytomegalovirus antibody were detected by cassette (rapid test) and compared the results of cytomegalovirus diagnosis (by rapid test) with molecular technique (Real time-Polymerase chain reaction) results. Erythropoietin hormone concentration was detected by ELISA technique. **Results:** Data obtained from rapid test showed that positive patients for human cytomegalovirus were 32(64%) for all cases, while patients negative for human cytomegalovirus were 18(36%) compared with those of controls. Real time-Polymerase chain reaction amplification for presence of human cytomegalovirus DNA in serum samples revealed that human cytomegalovirus genome was detected in 10(20%) of the 50 serum samples. Erythropoietin hormone showed lower concentration in patients than controls. Erythropoietin showed significant decrease (P<0.05) in all groups of patients compared to those of control group. **Conclusion:** Human cytomegalovirus seemed to have relationship with chronic and acute renal failure and can affect patient's immune status. Also, the decrease of erythropoietin hormone is the mean cause of anemia in renal failure patients.

Keywords: *renal failure, Human Cytomegalovirus virus, Erythropoietin hormone, Real time-Polymerase chain reaction technique.*

Introduction

Renal failure is a systemic disease and usually turns into a route cause for several kidney and urinary tract diseases. Renal failure induces a slow and progressive decline of kidney function enhanced by various factors including infections, diabetes, auto immune diseases, endocrine disorders, cancer and toxic chemicals [1]. The immunity of patients with hemodialysis becomes weak and this causes viral infections such as Human Cytomegalovirus (HCMV) [2]. It is commonly a result of complications arising from other serious medical conditions. Unlike acute renal failure, which happens speedily and suddenly, chronic renal failure occurs gradually (over a period of weeks, months or years) as the kidneys slowly stops working leading to an end-stage renal disease (ESRD) [3,4].

Anemia is a frequent complication during the later stages of chronic kidney disease. When present, it may cause symptoms such as fatigue and shortness of breath. The pathogenesis of anemia in chronic kidney disease is complex, but a central feature is a relative deficit of erythropoietin (EPO) [5]. The latter is a glycoprotein produced in the kidney under hypoxic conditions. It functions as the principal regulator of red blood cell production by controlling the proliferation, survival

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and differentiation of immature erythroid progenitors into mature red cells [6].

On the other hand, HCMV is one of the causes of inflammation in the kidney in the developed world; 40–60% of individuals are infected by time they reach adulthood, with seroprevalence approaching 100% in some populations [7]. Although initial HCMV infection is often asymptomatic in healthy individuals, it can cause severe and sometimes fatal disease in immune compromised individuals and neonates [8].

HCMV is one of the most common causes of birth defects resulting from an infectious agent, with 20% of congenitally infected infants exhibiting permanent neurological squeals including blindness, deafness and/or mental disability [9]. HCMV can also cause severe diseases in organ transplant recipients and AIDS patients after either primary infection or reactivation of a latent infection [10]. HCMV establishes a persistent infection, remaining silent in the host and undergoing productive reactivation cycles that contribute to its efficient transmission. HCMV infects and replicates in a wide variety of cells including epithelial cells of gland and mucosal tissues, smooth muscle cells, fibroblasts, macrophages, dendritic cells, hepatocytes and vascular endothelial cells [11,12]. The aim of current study was to investigate the role of human cytomegalovirus in renal failure and its contribution to anemia among patients with renal failure.

Patients and Method

This study was carried out at AL-Sadder Medical City Hospital during the period from December 2018 to February 2019. A total of 50 patients (32 males and 18 females) with kidney disease, with age range between (20-70) years, and 20 healthy individuals considered as controls were included. A 5-ml blood sample was collected from each participant. The blood samples were obtained by vein puncture from all patient after cleaning the skin with 70% alcohol, then blood left for about 30 minutes at room temperature, then they were centrifuged for 5 minutes at 3000rpm to separate serum and then transferred into other tubes. Serum samples were kept in capped plastic tubes and frozen at -20°C until being used. Each sample was labeled and given a serial number together with patients' name.

Immunological tests

CMV cassette rapid test: The sample and test components were prepared to room temperature if they were refrigerated or frozen. The sample was mixed thoroughly before the test was performed, after which the cassette was placed on a clean surface. The test strip was numbered with the patient's sample number. The pipette was filled with sample and the droplet retained. One drop (about 10µl) of the test sample was placed in each sample hole. The 2 drops (80µl) from the sample diluents were added directly to each specimen well. The results were read within 15 minutes.

Molecular tests: Sample extraction: (G- spin DNA extraction kit).

A 150-µl sample was added into microcentrifuge tube and a 570-µl volume of VNE Buffer was added into the sample and mixed well by vortexing and then incubated for 10 minutes at room temperature. A 570-µl volume of ethanol was added to the sample mixture and mixed well by plus-votexing. VNA Column was combined with collection tube and transferred up to 700ml of sample mixture to the VNE Column and then centrifuged at 8, 000×g for 1min then discarded the flow-through and then combined with the VNA Column with used collection tube. The rest of sample mixture was transferred to the VNA Column and centrifuged at 8,000xg for 1min. A 500-µl volume of wash Buffer1 was added to the VNA Column and centrifuged at 8, 000×g for 1min. A 750-µl volume of wash Buffer2 was added to the VNA Column and then centrifuged at 8, 000×g for 1min. A 750-µl volume of wash Buffer2 was added to the VNA Column and then centrifuged at 8, 000×g for 1min. Then centrifuge at full speed (18, 000×g) for an additional 3min to dry the VNA Column and then discarded the flow-through and the Collection tube. The VNA Column was combined with elution tube and added 50µl of preheated RNase-free water to the membrane center of the VNE Column and then the VNE Column left to stand for 2min, centrifuged for 2min to elude the nucleic acid. Nucleic acids were stored at -70°C.

Erythropoietin hormone assay procedure

A 100-µL volume of dilutions of standard, blank and samples were added into the appropriate wells. Wells were covered with the sealing plate and incubated for 1 hour at 37°C. The time was started after the last sample addition. The liquid of each well was removed without wash. A 100-µL volume of detection reagent A was added to each well and they were covered with plate sealer and incubated for 1 hour at 37°C. The

solution was aspirated and washed with 350µL of 1× wash solution to each well using a squirt bottle, and let it sit for 1~2 minutes. Then the remaining liquid was removed from all wells completely by snapping the plate onto absorbent paper. Totally washed 3 times and after the last wash, any remaining wash Buffer was removed by aspiration. The plate was inverted and blotted against absorbent paper. A 100-µL volume of detection reagent B was added to each well and the wells were covered with plate sealer and incubated for 30 minutes at 37°C. The aspiration/wash process was repeated for 5 times as conducted. A 90-µL volume of substrate solution was added to each well. Wells were covered with a new plate sealer, incubated for 10-20 minutes at 37°C and protected from light. The liquid will turn blue by the addition of substrate solution. Fifty-µL volume of stop solution was added to each well. The liquid will turn yellow by the addition of stop solution. The liquid was mixed by tapping the side of the plate. Any drop of water and fingerprint on the bottom of the plate were removed and confirmed there was no bubble on the surface of the liquid. Then, the microplate reader was run and conducted measurements at 450nm immediately.

Statistical analysis

All values were expressed as means ± SE. The data were analyzed using of SPSS (T test) version 23 and Microsoft Excel computerized programs. *P* value less than 0.05was taken as the lowest limit of significance.

Results

Fifty patients with kidney failure, who referred to Central laboratory at Al-Sadder Teaching Hospital in AL-Najaf Governorate with age range of (20 to 70) years, were distributed according to gender 32(64%) males and18(36%) females (Table 1).

Table (1) Distribution of patients with renal failure according to gender

Gender	Controls No.(%)	Patients No.(%)
Male	10(50)	32(64)
Female	10(50)	18(36)
Total	20(100)	50(100)

Age groups and percentages of the study groups

In the current study, renal failure patients' age ranged between (20-70) years. The current study showed that patients within the age group (20-30) years represented 18% of the study sample, while the age group (>50) years represented the highest percentage of 32%; (Table 2). In addition, result showed that 20% and 30% were within age groups (31-40) and (41-50) years, respectively, while the age of control group ranged between (20-60) years (Table 2).

Table (2) Distribution of patients according to age groups

Group	Age/yr		
		Controls No.(%)	Patients No.(%)
A1	20-30	6(30)	9(18)
A2	31-40	6(30)	10(20)
A3	41-50	4(20)	15(30)
A4	>50	4(20)	16(32)
Total		20(100)	50(100)

Detection of HCMV by rapid test

Out of the 50 patients, only 32(64%) were positive for HCMV, whereas 18(36%) showed negative results for HCMV by rapid test (Figure 1).

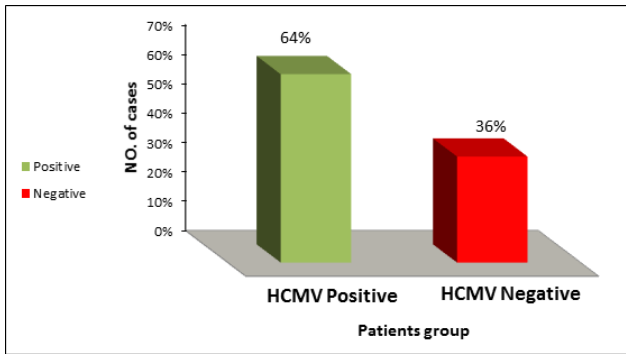

Figure 1 Serology of HCMV among renal failure patients.

Detection of HCMV nucleic acid (DNA) by RT-PCR

The results of RT-PCR amplification for presence of HCMV DNA in serum samples (Figure 2) showed that HCMV genome was detected in 10(20%) of the 50 serum samples tested from patients suffering from renal failure with viral loads ranged from (25×10^2 to 281×10^4IU/ml (Table 3).

Table (3) Percentage of HCMV in serum samples by RT-PCR

Results	Number of samples	%
Positive	10	20
Negative	40	80
Total	50	100

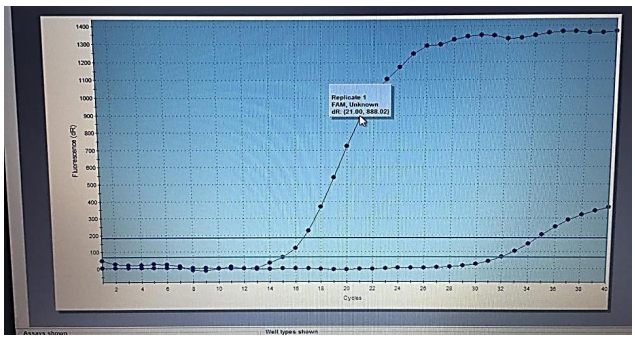

Figure (2) Real-time PCR amplification.

Erythropoietin hormone detection with participants' gender

In the present study, there was a decrease in the level of erythropoietin (EPO) hormone concentration for patients with renal failure compared with control groups. The concentration of erythropoietin hormone in males (positive and negative for HCMV) decreased to (3.8905pg/ml and 9.0727pg/ml), respectively, compared with male controls (14.71pg/ml), while the concentration of EPO in females (positive and negative for HCMV) decreased to (3.6909 pg/ml and 8.7286pg/ml), respectively, compared with female controls (14.62pg/ml) with significant difference (P<0.05; Figure 3).

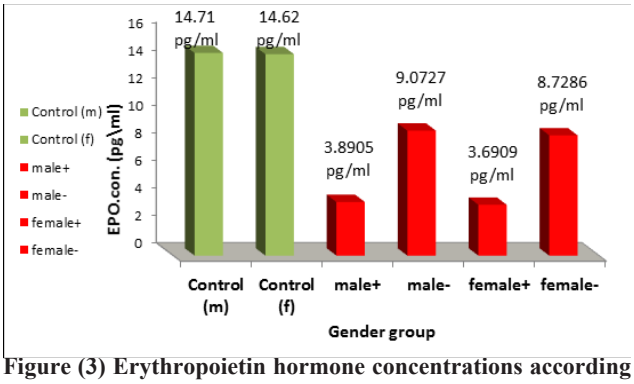

Figure (3) Erythropoietin hormone concentrations according to participants' gender.

Discussion

The present study showed that the percentage of males (64%) with kidney failure was more than females (36%). There was no reason to give a response to another category in terms of infection, but the weakness of immune system may be the main cause of the disease. This result was agreed with the results of [13, 14, 15] who shown that males with renal diseases are more susceptible to infections than females, due to daily efforts of males as compared to females in addition to the increase in muscle mass in males than females leading to high proportion of creatinine in males and kidney damage. In this study, one of the common immunological techniques, cassette technique, was used. After the examination of samples of patients with renal failure and control samples, current results showed that out of 50 patients, only 32 (64%) were positive for HCMV, whereas 18(36%) showed negative results for HCMV by rapid test. The results were consistent with the results obtained by [16] who found the presence HCMV antibody in patients with kidney failure (83%) compared to control group. The proportion of positive serological test differed for this virus in patients with hemodialysis and that the cause of this variation was linked to the number of patients screened, the geographical distribution and sensitivity of the immunological techniques used to determine the Anti-CMV antibodies [17].

The results of current study were positive for human cytomegalovirus among the group of patients with renal dysfunction for males and females using one of the sensitive molecular techniques, RT-PCR. The results in this study reported that there was about 10(20%), out of 50, samples gave positive viral DNA existence and these results were in agreement with [18] who found that about 32(32.7%) of the samples tested gave positive results for viral genome and the difference in such subject areas may be ascribable to the sample type or in some case

disease type and stage [19]. RT-PCR has been developed to detect HCMV because of its time-saving feature and high sensitivity and specificity [20].

Current study showed a significant decrease in erythropoietin hormone, for both genders (negative for HCMV), responsible for the production and development of red blood cells and these lead to cause anemia in these patients, this result was in agreement with [21]. The results showed that the level of EPO in renal failure patients (positive for HCMV) decreased more than in patients negative for HCMV, and this study was in agreement with [22]. HCMV targets renal glomerular, vascular, epithelial, interstitial and tubular cells, including fibroblast-type cells in the renal cortex that activate EPO production in the setting of local tissue hypoxia and these lead to decrease EPO production and cause anemia.

Conclusion

Human cytomegalovirus seemed to have relationship with chronic and acute renal failure and can affect patient's immune status. Also, the decrease of erythropoietin hormone is the mean cause of anemia in renal failure patients.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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