

# Evaluation the Occurrence of Human Cytomegalovirus (HCMV) and its Relation to Some Biochemical Markers in Hemodialysis Patients from Kirkuk City/Iraq

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**Annotation: Background and Objectives:** Across all socioeconomic categories, Cytomegalovirus (CMV) is extremely common worldwide and affects 50.0% to 85.0% of persons over 40. Most of its infections are asymptomatic in children and are typically prevalent in the community. The seroprevalence of CMV varies between 40 and 60 percent in Africa and 80 and 100 percent in Latin America, respectively, and between 65 and 85 percent in Brazil. Cytomegalovirus (CMV) infection is one of the main causes of death for individuals receiving hemodialysis (HD). **Aim:** To evaluate prevalence of anti-HCMV antibodies among Hemodialysis patients and their correlation with some biochemical parameters. **Materials and Methods:** The present study (case-control study), was achieved at the Hemodialysis department of Kirkuk General Hospital, Kirkuk city, Iraq, during the period of first of July 2024 to 15 of June 2025. The participants included 128 patients (70 male and 58 female) who have had Hemodialysis and aged (40-80) years old and 93 individuals who used as control group (60 male and 33 female) who their ages were close to age groups of the patients. Some immunological and biochemical parameters had measured for the participants. **Results:** It was documented that the highest rate, 22 (31.42%), was found in male patients aged

(56–65) years, while the lowest proportion, 1 (1.72%), was found in female patients aged 25–35 years. Likewise the present study showed significant lowering of the mean concentration levels of vitamin D3 ( $15.32 \pm 6.41$  ng/mL) among the hemodialysis patients in comparison to ( $27.40 \pm 8.80$  ng/mL) recorded for the control group. Concerning the Serum Creatinine and B. Urea mean levels, they recorded extremely significant variances ( $P < 0.0001$ ), between the patients and controls. For those patients who have both (IgM +, and IgG +) displayed 51 (39.84%), while the control group showed 8 (8.60%). **Conclusion:** Old men who are on Hemodialysis are more susceptible to get reactivated HCMV. Low Vitamin D3, and S. Albumin could increase the severity of the infection. Patients on Hemodialysis could get reactivation with HCMV easily.

**Keywords:** Anti-HCMV IgM, IgG, Hemodialysis, Creatinine, Urea, Albumin.

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## 1. Introduction:

Chronic renal failure (CRF) is a chronic condition that typically develops over years and is defined as an irreversible decline in renal function brought on by a decline in glomerular filtration rate (GFR). It initially shows up as aberrant biochemical results because to the kidneys' incapacity to maintain normal low levels of the products of protein metabolism (like urea and creatinine), normal blood electrolyte levels (like sodium and potassium), and acid-base balance (1). Among maintenance hemodialysis (MHD) patients, paroxysmal atrial fibrillation attacks are among the most prevalent tachyarrhythmias. In addition to impairing the normal operation of dialysis, paroxysmal atrial fibrillation attacks can raise the risk of death for patients receiving (MHD) (2).

A common human-specific DNA virus that is a member of the *Herpesviridae* family is the human *Cytomegalovirus* (HCMV). The HCMV has a significant global prevalence that could be ranged from 34% to 80% in wealthy nations to 100% in some African countries, and there is a significant chance that hemodialysis patients will come into direct contact with human *Cytomegalovirus* (3, 4). The United States and Western European nations have the lowest CMV seroprevalence, whereas South America, and Asia have the highest (5). In renal transplant recipients, HCMV infection may be primary or recurrent. While the former refers to HCMV detection in a person who was previously seronegative (infected by a different strain), recurrent infection includes both reinfection (with the same HCMV strain) and reactivation (4, 6, 7).

Reviewing earlier research from various nations across the world, it was discovered that the seroprevalence of CMV-IgM and CMV-IgG antibodies in hemodialysis patients ranges from 0–10% and 90–100%, respectively (8, 9). According to other research, 20–60% of recipients of kidney transplants also had CMV infection (9).

Hypoalbuminemia may be brought on by chronic systemic inflammation that leads to diabetic

end-stage renal disease (ESRD) because of decreased albumin production or increased breakdown (10). Moreover, hunger and insufficient dialysis may also be factors in hypoalbuminemia (10). Numerous studies have shown that poor one-year graft outcomes, including CMV infections in kidney and pancreas transplant recipients, were linked to post-transplant hypoalbuminemia (10).

## **2. Rational:**

The frequency of HCMV in hemodialysis patients and its correlation with some biochemical indicators were not well documented in Iraq, especially in Kirkuk City. By examining these associations, laboratory patterns associated with viral activity can be found, routine surveillance can be guided, and therapeutic and preventive measures to lessen HCMV-related problems in this high-risk population can be informed.

## **3. Aim:**

To determine whether Human Cytomegalovirus (HCMV) infection is present in Kirkuk City, Iraqi hemodialysis patients as evidenced by anti-HCMV-IgM and anti-HCMV-IgG antibodies, and to determine how these antibodies relate to specific biochemical markers including: (Vitamin D3, C-reactive protein, creatinine, urea, and albumin).

## **4. Objectives:**

- 1- To ascertain the frequency of anti-HCMV-IgM and anti-HCMV-IgG antibodies in Kirkuk City hemodialysis patients.
- 2- To assess the research population's serum levels of albumin, creatinine, urea, C-reactive protein (CRP), and vitamin D3.
- 3- To examine any potential relationships between biochemical markers and HCMV serostatus (IgM and IgG).
- 4- To supply baseline information that could aid in the creation of hemodialysis patients' HCMV infection screening and treatment plans.

## **5. Materials and Methods:**

### **5.1. Design and context of the study**

The present study (case-control study), was achieved at the Hemodialysis department of Kirkuk General Hospital, Kirkuk, Iraq, during the period of first of July 2024 to 15 of June 2025. The participants included 128 patients (70 male and 58 female) who have had Hemodialysis and aged (40-80) years old and 93 individuals who used as control group (60 male and 33 female) who their ages were close to age groups of the patients. Basic data such as age, sex, etc were gathered from the participants through utilizing of designated questionnaire form.

### **5.2. Immunological tests:**

#### **5.2.1. Testing for anti-CMV- IgM and IgG antibodies**

Serum samples were taken from both the patients and control group to test for the presence or absence of anti-CMV-IgM and IgG antibodies through using of a qualitative method (TORCH Panel Rapid Test, from CTK Biotech, Inc, Ref. No. R0253C, USA). This technique was Immunochromatography technique and the results were read within 10 mints.

#### **5.2.2. C-Reactive Protein Latex test:**

Agglutination between the patient's serum CRP and latex particles coated with anti-human CRP antibodies is the basis for the test. A noticeable agglutination will occur within a short period of time (generally two minutes) if the serum contains CRP at a concentration equal to or higher than the detection limit, which is typically approximately 6 mg/L. The latex suspension stays consistently milky and smooth without clumping if there is no CRP or if its concentration is

below the detection threshold (From SPINREACT, Ref No. 1200302, Made in Spain).

### **5.3. Biochemical analysis:**

#### **5.3.1. Evaluation of Vitamin D3 concentration:**

The test is a fluorescence immunoassay (FIA) that uses the sandwich technique using biotin and streptavidin. Extraction buffer frees the sample's 25-hydroxyvitamin D (25-OH D) from binding proteins.

After that, it forms immune complexes by binding to biotin-conjugated antibodies and detector antibodies that have been fluorescence-labeled.

Through the interaction between biotin and streptavidin, these complexes move along a nitrocellulose membrane before being caught at the test line by immobilized streptavidin. The ichroma™ analyzer measures the ensuing fluorescence signal, and the strength of the signal is directly proportional to the amount of total 25-OH Vitamin D present in the sample (From Boditech Med INC, Ref: CFPC-133, Made in Korea).

#### **5.3.2. Measurement of serum Albumin:**

The dye-binding technique known as Bromocresol Green (BCG) is used in the Cromatest albumin assay. In this technique, the anionic dye Bromocresol green is particularly bound by albumin in the serum at an acidic pH. The dye-albumin complex's absorption (wavelength) maximum changes when albumin binds to BCG as opposed to when the dye is unbound. As a result, the hue changes. The complex's color intensity is directly correlated with the amount of albumin present in the sample. The concentration of albumin can be computed by measuring the colored complex's absorbance, or optical density (From Cromatest, LiNEAR Chemicals, S. L. Ref: 1101000, Made in Spain).

#### **5.3.3. Estimation of Serum Creatinine:**

The foundation of the Biolabo kinetic Creatinine assay is the Jaffé reaction, a colorimetric reaction that occurs between Creatinine and picric acid in an alkaline environment. The initial rate approach measures the change in absorbance over a predetermined period of time rather than waiting for the reaction to reach an endpoint because it is kinetic. The development of a yellow-orange complex (Creatinine-picrate) whose concentration rises with time is the cause of the absorbance shift. The amount of Creatinine present in the serum or plasma sample determines the rate of growth, or slope. The working reagent is used to perform the measurement spectrophotometrically at approximately 490 nm (or 490-510 nm) (From BIOLABO, Ref: 80107, Made in France).

#### **5.3.4. Measurement of Blood Urea:**

Based on the urease–Berthelot enzymatic colorimetric method, the test is performed:

Ammonia and carbon dioxide were produced when Urea was hydrolyzed by urease. The resulting Ammonia forms a blue-green indophenol complex by reacting with hypochlorite and phenolic chemicals. The urea concentration in the sample was directly correlated with the color's intensity (From BioResearch, Lot No. L24020088. Jordan).

### **5.4. Exclusion criteria:**

Exclusion of patients who were less than 18 years old. Patients who were on current chemotherapy, immunosuppressive treatment, or an active cancer were also excluded from the study. Pregnant women were also excluded.

### **5.5. Statistical Investigation:**

For computerized statistical analysis, SPSS (Statistical Package for Scientific Services) version 26 was utilized. The unpaired t-test was also used to compare the numerical variables. P values

were classified as statistically significant (S) if they were less than 0.05, as significant if they were less than 0.05, and as highly significant (HS) if they were less than 0.01. A value of  $P > 0.05$  was considered non-significant. Pearson correlation test was also utilized.

## 6. Results:

Regarding the distribution of patients' and controls' ages and genders, it was found that the highest rate, 22 (31.42%), was found in male patients aged 56–65, while the

lowest proportion, 1 (1.72%), was found in female patients aged 25–35. Table 1.

In the control group, the largest proportion (12, or 36.36%) of the controls were females between the ages of 46 and 55, while the lowest proportion (1, or 3.03%) of the controls were females aged 75 and older. Table 1.

**Table (1): The patients' and controls' age and gender distributions**

Age groups In years	Patients		Control	
	Male No. (%)	Female No. (%)	Male No. (%)	Female No. (%)
25-35	2 (2.8)	1 (1.72)	10 (16.66)	4 (12.12)
36-45	15 (21.42)	6 (10.34)	12 (20)	10 (30.30)
46-55	19 (27.14)	13 (22.41)	15 (25)	12 (36.36)
56-65	22 (31.42)	17 (29.31)	17 (28.33)	4 (12.12)
66-75	8 (11.42)	12 (20.68)	4 (6.66)	2 (6.06)
75 and older	4 (5.71)	9 (15.51)	2 (3.33)	1 (3.03)
Total	70 (100%)	58 (100%)	60 (100%)	33 (100%)

The present study showed significant lowering of the mean concentration levels of vitamin D3 ( $15.32 \pm 6.41$  ng/mL) among the hemodialysis patients in comparison to ( $27.40 \pm 8.80$  ng/mL) recorded in the control group. The differences were highly significant ( $P < 0.0001$ ). Table 2.

Concerning the Serum Creatinine and B. Urea mean levels, they recorded highly significant variances ( $P < 0.0001$ ), between the patients and controls as shown in table 2.

Furthermore, the current study revealed that the lowest mean levels of S. Albumin ( $2.26 \pm 0.54$  g/dL) was found among hemodialysis patients while the greatest mean level ( $4.02 \pm 0.49$  g/dL) was detected among control group. The variations were highly significant as displayed in table 2.

**Table (2): Certain biochemical measures' values in patients and controls**

Biochemical Parameters	Patients	Controls	P. Value
Mean $\pm$ SD			
Vit. D3	$15.32 \pm 6.41^a$	$27.40 \pm 8.80^b$	$< 0.0001$
S. Creatinine	$17.19 \pm 10.13^a$	$0.81 \pm 0.45^b$	$< 0.0001$
B. Urea	$77.96 \pm 25.66^a$	$22.84 \pm 7.54^b$	$< 0.0001$
S. Albumin	$2.26 \pm 0.54^a$	$4.02 \pm 0.49^b$	0.007

### By Unpaired t-test

About the Immunoglobulin distribution among the patients and control group, our study exhibited that patients with (IgM +, IgG-) revealed 22 (17.18%), whereas controls within the same criteria showed only 10 (10.75%) Table 3.

For those patients with have both (IgM +, and IgG +) displayed 51 (39.84%), while the control group showed 8(8.60%). Table 3.

Moreover, patients with (IgM -, IgG +), had 10 (7.81%), but the controls exhibited 5(5.37%).

Table 3.

Furthermore, patients within (IgM -, IgG-) revealed 45 (35.15%), while the control group showed an upgrade up to 70 (75.26%). Table 3

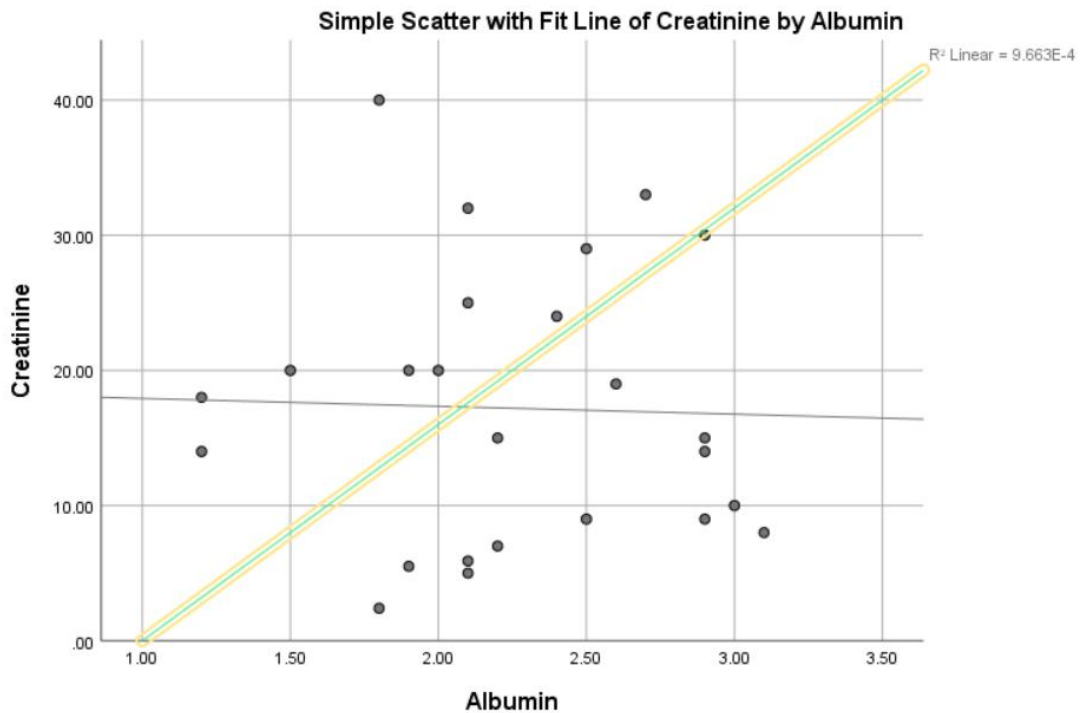
**Table (3): Anti-HCM-IgM and IgG antibody seroprevalence in hemodialysis patients and the control group**

Seroprevalence of CMV Antibodies	Seroprevalence of CMV Antibodies	
	Patients No. (%)	Control Group No. (%)
<b>IgM (+) IgG (-)</b>	22 (17.18)	10 (10.75)
<b>IgM (+) IgG (+)</b>	51 (39.84)	8(8.60)
<b>IgM (-) IgG (+)</b>	10 (7.81)	5(5.37)
<b>IgM (-) IgG (-)</b>	45 (35.15)	70 (75.26)
<b>Total</b>	128 (100%)	93 (100%)

Figure (1), exhibit Pearson correlation: Albumin= 1, Creatinine=-.031

P. Value=0.883

Non-significant decreasing of Albumin concentration noticed when S. Creatinine levels increased. Non-significant negative correlation.



**By Pearson correlation test**

**Figure (1): Correlation between S. Creatinine levels and S. Albumin levels among patients group**

**Discussion:**

Across all socioeconomic categories, Cytomegalovirus (CMV) is extremely common worldwide

and affects 50.0% to 85.0% of persons over 40. Most of its infections are asymptomatic in children and are typically prevalent in the community. The seroprevalence of CMV varies between 40 and 60 percent in Africa and 80 and 100 percent in Latin America, respectively, and between 65 and 85 percent in Brazil(11). Cytomegalovirus (CMV) infection is one of the main causes of death for individuals receiving hemodialysis (HD)(11).

In the current study we found that the highest rate of the patients were aged between 56 and 65 years old, These were near to a study conducted by Astor BC *et al.*, (12), who found the majority of the patients were male with mean age  $59.87 \pm 14.38$  years (12). Likewise our study was in line with Abidi MZ *et al.*, (13), who found that the majority of the patients were male with mean age  $44.72 \pm 9.97$  years (13).

Hemodialysis patients' mean vitamin D3 concentration levels ( $15.32 \pm 6.41$  ng/mL) significantly decreased, according to the current study, which was in agreement with Mehdiabadi FM *et al.*, (14), who revealed that in the case group, the mean blood 25-hydroxy vitamin D level was 14.42 ng/mL (14). Moreover, the present investigation showed an agreement with Astor BC *et al.*, (12), who denoted that there was an association between vitamin D3 deficiency and CMV-infection among patients with renal injury.

We also found that S. Creatinine and B. Urea levels increased significantly among patients group. These were in contrast to a study conducted in Pakistan (15), where they found that S. Creatinine and B. Urea levels elevated significantly among patients with CMV and on Hemodialysis (15). Furthermore, our study was in close to Al Atbee MY and Tuama HS (16), who detected significant correlation between S. Creatinine and B. Urea levels and CMV prevalence among Hemodialysis patients (16).

It was also noted that S. Albumin levels decreased among patients infected CMV- and on Hemodialysis. These were agreed with Donadello Tessarolo L *et al.*, (17), who noticed a relationship between low serum Albumin levels and existence of CMV among patients with renal infection (17).

The current research revealed that the greatest rate of CMV-infected patients, had 51 (39.84%) of anti-CMV-IgM and IgG together. These were in line with Vilibic-Cavlek T. *et al.*, (18), who showed high percentage of anti-CMV-IgM, IgG (reactivation) among hemodialysis patients (18).

Furthermore, non-significant decreasing of Albumin concentration noticed when S. Creatinine levels increased. Non-significant negative correlation, these were close to what was reported earlier by Wang J. *et al.*, who detected a correlation between elevation of S. Creatinine levels and decreasing of S. Albumin levels(19).

### **Conclusion:**

- 1-Old men who are on Hemodialysis are more susceptible to get reactivated HCMV.
- 2-Low Vitamin D3, and S. Albumin could increase the severity of the infection.
- 3-Patients on Hemodialysis could get reactivation with HCMV easily.

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