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CYTOMEGALOVIRUS INFECTION;

PREVALENCE IN HEMODIALYSIS PATIENTS IN DIALYSIS UNIT OF LAHORE GENERAL HOSPITAL

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ABSTRACT… Cytomegalovirus can cause lifelong latency and reactivates when the patient become immunocompromised and can cause severe complications. Patients on hemodialysis are on risk of CMV infection due to multiple blood transfusions and impaired immunity. Serology of the patient does not detect the latent infection. **Objectives:** To check the frequency of cytomegalovirus (CMV) infection in patients on hemodialysis. **Design:** Observational study. **Setting:** Gulab Devi Chest Hospital & Lahore General Hospital Lahore. **Period:** six month. **Material and Methods:** 31 patients that were on hemodialysis were enrolled in this study. CMV DNA detection was done from the peripheral blood with consent from the patients. The 222bp band corresponding to the size marker and positive control was considered as positive. The data was analyzed using SPSS version 20.0. **Results:** Age, gender and socioeconomic status had no association with CMV infection. No patient was found positive for CMV DNA. Serum creatinine levels were significantly associated with the duration on dialysis. Hypertension and diabetes were diagnosed as major co-morbidities. In this study none of the sample tested positive for CMV DNA. **Conclusions:** There is a need to conduct large population based study to establish the sero-prevalance of CMV infection in Pakistani population as no description is available yet. Furthermore, the blood should be screened for CMV viremia or antibodies prior to transfusion to rule out risks associated with reactivation of latent infection in critically ill patients.

Key words: Cytomegalovirus, Hemodialysis, Polymerase Chain Reaction.

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INTRODUCTION

Human Cytomegalovirus (CMV) is a DNA enveloped virus which establishes lifelong latency after the primary infection. The presence of actively replicating virus either from primary infection or reactivation of latent infection during pregnancy results in transmission to the fetus causing congenital complications.[1] CMV causes wide spread infections and 50 to 85% of adults are infected by CMV by the age of 40. However, as compared to the congenital infections or in infants, CMV does not pose a serious threat in adults until the person become immune-compromised.[2,3] CMV can cause a serious problem in HIV-infected individuals, organ transplant recipients on immune suppressive drugs and critically ill patients with severe trauma and injury. Clinical syndromes observed in this setting include encephalitis, pneumonitis, colitis, hepatitis, uveitis, retinitis and graft rejection.[4,6]

CMV is endemic in most areas of the world and sero-prevalence varies from 60 to 100% in different geographic areas and in different socio-economic groups.[2,6] The patients with end organ kidney disease and those on hemodialysis are susceptible to the reactivation of CMV due to multiple blood transfusions and dysregulated immune system.[2]

Early diagnosis and detection of the virus is important for the proper prognosis of the disease in these patients. While most common method for the detection of CMV is the presence of anti-CMV antibodies in the blood[7,8], it cannot detect the latent virus, which upon transmission into immune-compromised host can reactivate and cause serious complications. Also the false sero-positive results causes difficulties in obtaining sufficient sero-negative blood donations.[9]

Therefore, the screening of blood with more sensitive and reliable methods is necessary before transfusions to the immune-compromised host. Polymerase chain reaction (PCR) is highly sensitive methods which detects the presence of CMV DNA in the patient's blood either latent or reactivated. Moreover, PCR viremia can rule out the reactivation of CMV despite the sero-positive status of the patient.[10]

The aim of the present study was to detect the presence of CMV DNA in patients on hemodialysis and with renal disease using biological specimen.

MATERIAL AND METHODS

The nonprobability purposive sampling was done. 31 patients that were on hemodialysis were selected for this study. The blood samples were taken from the dialysis unit of Lahore General Hospital with patient's consent. Five ml of blood was drawn and divided into two halves and was sent to the pathology laboratory for CBC and RFT (Renal function test) and 2 ml were used for the extraction of DNA from whole blood. The study was done at Post Graduate Medical Institute Gulab Devi Chest Hospital after approval from the ethics committee.

DNA was extracted with DNA Extraction kit purchased from Thermo Scientific USA (Catalog no. k0512). The manufacturer's protocol was followed with slight modifications and briefly descried here. The DNA was precipitated with isopropanol from the aqueous phase after lysis of the whole blood. DNA was stored at -20 °C in DNAse free water until further use.

Polymerase Chain Reaction

Beta actin was amplified from the extracted DNA as the internal control and to verify the extraction procedure and DNA quality. All the reagents for PCR were purchased from Thermo Scientific (USA) unless otherwise specified and were put on ice prior to reaction. A total 50 μl of reaction volume was made containing 0.2 μmoles of each primer, 1X PCR buffer, MgCl2 (1.5 mM), dNTP mix (0.2 mM) and TAQ DNA Polymerase 1U. Forward and reverse primer with the sequence GCAACCTTGGGAACAATACG and CCACGTTGTCCATGAAGAGG respectively was used. The PCR cycling parameters was as follow, initial denaturation was performed at 95 °C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 45sec then annealing at 54°C for 45sec then extension at 72°C for 45sec. Final extension was done at 72°C for 5 min. 10 µl of PCR product was analyzed on 2% agarose gel and stained with ethidium bromide (0.5 μg/ml) and visualized under UV light. The band equivalent to the 110bp size marker was considered as positive.

CMV PCR Amplification

PCR amplification for CMV DNA was done with the CMV PCR detection kit (CinnaGen Inc. Catalog no. PR7836C). The manufacturer's protocol was followed as briefly described here. Prior to the reaction all the tubes were spin and were put on ice. The final volume of each reaction was 25 µl. New 0.2 ml sterile tubes were labeled for amplification reaction for test, positive and negative control. The following reagents were added into each tube on ice, 1X PCR MIX 20 µl, TAQ DNA polymerase 0.3 µl. 5µl of DNA was then added. All the tubes were closed and mixtures were spin for 3-5 sec on micro centrifuge and tubes were placed in preheated thermal cycler. The cycling parameters for CMV DNA amplifications was initial denaturation at 95°C for three minutes, annealing at 62 °C for 40 seconds, and extension at 72°C for 40 seconds, then 35 cycles of denaturation at 93°C for 40 seconds, annealing at 61°C for 40 seconds and extension at 72°C for 40 seconds. 10 μl of amplified product was analyzed on 2% agarose gel directly without adding loading buffer. The bands corresponding to the 222bp size marker and positive control were considered as positive. The bands were visualized with ethidium bromide under UV light.

Data Analysis

Data analyses were done with SPSS version 20. All the data were presented as mean ± S.E of mean. Chi square was used to find out the statistical significance. P value of ≤ 0.05 was considered as positive.

RESULTS

Minimum age of the patient in this study was 21 years and maximum age was 80 years. The mean age was 44.42 ± 17.24 years. Out of 31 patients 21 (67.7%) were male and 10 (32.3%) were female as shown in Table-I. According to socioeconomic status 3 (9.68%) patient were from upper class, 20 (64.52%) were from middle class and 8 (25.81%) were from lower class. Relating to the renal failure 6 (19.35%) patients were with acute renal failure and 25 (80.65%) patients were with chronic renal failure. The mean blood urea level was 118.9 mg/dl + 41.8 mg/dl. The mean level of serum creatinine was 6.38 mg/dl + 3.86 (Table-II). The mean hemoglobin was 9.40 g/dl + 1.42 g/dl. The mean level of total leukocyte count (TLC) was 7.13 × 103/μl + 2.42 × 103/μl. Mean level of Lymphocyte was 30.9% + 9.1%. Mean level of Neutrophils was 68.5 % + 9.5 %. The mean level of Platelets was 192 × 103/μl + 65.04 × 103/μl as shown in Table-II.

Blood transfusion was done to 26 (83.87%) patients and 5 (16.13%) patients had no history of blood transfusion. In this study 5 (16.13%) patients were presented with Hypertension and Diabetes and 26 (83.87%) patients were presented with only Hypertension. According to the duration of the dialysis 2 patients (6.45%) were on dialysis from 3 months, 1 patient (3.23%) was on dialysis from 4 months, 2 patients (6.45%) were on dialysis from 5 months, 2 patients (6.45%) were on dialysis from 6 months, 5 patients (16.13%) were on dialysis from 1 year, 8 patients (25.81%) were on dialysis from 2 year, 9 patient (29.03%) were on dialysis from 3 years, 2 patient (6.45%) were on dialysis from 4 years.

The results showed significant association between duration on dialysis and serum creatinine levels (P value 0.017) as shown in Table-III. Whereas, for hemoglobin, reduced hemoglobin levels were observed in patients that were on dialysis for more than two years as well as for one year, although we did not find statistical significance in this case as shown in Table-IV. Those patients that were on dialysis for more than two years were also presented with hypertension and diabetes and had higher creatinine levels from all other patients (11 out of 31). Of these, 7 had only hypertension while 4 were presented with both hypertension and diabetes as shown in Table-V. In this study no patient was found positive for CMV DNA.

	Total no	Mean	SD	Minimum	Maximum
Age (Years)	31	44.42	17.24	21	80
Blood urea (mg/dl)	31	118.9	41.8	25.6	199.0
Serum Creatinine (mg/dl)	31	6.38	3.86	0.60	12.70

Table-I. Renal function test

Descriptive statistics	Total number	Minimum	Maximum	Mean	S.D
Hb	31	6.50	13.70	9.40	1.42
TLC	31	3.40	14.30	7.13	2.42
Lymphocytes	31	6.0	45	30.9	9.1
Neutrophils	31	54	94	68.5	9.5
Platlets	31	62	328	192	65.04

Table-II. Descriptive statistics of complete blood count

Count						
		Serum creatinine				Total
		Less or equal to 1.4	1.5-3.0	3.1-6.0	More than 6	
On dialysis from	upto 6 months	5	0	0	2	7
	1 year	0	0	2	3	5
	2 year	1	0	3	4	8
	> 2 year	0	2	4	5	11*
Total		6	2	9	14	31

Table-III. Serum creatinine levels according to duration on dialysis.
*P value= 0.017

Count					
		Hemoglobin			Total
		6.0 to 8.0	8.1 to 10.0	10.1 to 14.0	
On dialysis from	upto 6 months	0	7	0	7
	1 years	1	3	1	5
	2 years	3	2	3	8
	> 2 years	0	8	3	11
Total		4	20	7	31

Table-IV. Hemoglobin levels according to duration on dialysis.

On dialysis from	Co-Morbidity	Serum creatinine				Total
		Less or equal to 1.4	1.5-3.0	3.1-6.0	More than 6	
upto 6 months	HTN	5			2	7
1 year	HTN/Diabetes			0	1	1
	HTN			2	2	4
2 year	HTN	1		3	4	8
> 2 year	HTN/Diabetes		0	3	1	4
	HTN		2	1	4	7
Total		6	2	9	14	31

Table-V. Serum creatinine levels with co-morbidities

DISCUSSION

CMV disease can occur as an opportunistic infection in patients with severe immunosuppression such as cancer, HIV and renal failure.[11] Patients on HD have incompetent immune system generally due to multiple transfusions and low lymphocyte count. Various defects in the T cell function of HD patients had been described that might be due to intrinsic T cell abnormality rather than due to HD.[12]

The rate of CMV infection and reactivation increases with age as elder age patients are more susceptible to opportunistic infections due to dysregulated immunity and low number of naïve T cells in their peripheral blood.[13] In this study maximum age of the patient was 80 years while the mean age was 44 years. No significant association was found between age, gender and socioeconomic status and the CMV infection in patients on HD.

Hemoglobin levels have significant importance in dialysis patients as low levels of hemoglobin correlates with the rate of mortality and the length of the hospital stay.[14] Furthermore, quality of life improves with the normalization of the hemoglobin levels in the hemodialysis patients.[15] In this study only 3.2% patients had the hemoglobin level above the lowest normal level. All other patients had the hemoglobin below the normal range. Total leukocyte count was raised in only 3.23% patients and all other patients had normal TLC levels. These raised levels of TLC in some patients might be due to some other infection because no patient was diagnosed with the CMV associated pathologies such as esophagitis, colitis and pneumonitis.

Hypertension and diabetes generally appears as co-morbidity which indirectly increase the severity of the disease. In this study 16.13% patients were presented with both hypertension and diabetes while 83.87% were presented with the hypertension alone. Five of the patients that were on dialysis for more than two years was hypertensive and had elevated serum creatinine levels. There was a significant association between serum creatinine levels and duration on dialysis, whereas, greatest association was found in patients that were on dialysis for more than two years (P-value 0.017, $P \leq 0.05$). These findings suggest that those patients had chronic kidney disease. Furthermore, those patients that

were on dialysis for more than six months and had serum creatinine levels in the normal range might have acute renal failure. This study showed that the serum creatinine levels increased with the increase in the duration on dialysis.

In this study no patient was detected with CMV DNA despite of multiple blood transfusions. There was no history of any anti-viral therapy given to any patient. This might be due to the fact that these patients were transfused with the packed cell volume (PCV) instead of the whole blood. Pliquett et al, reported that PCR can rule out the CMV viremia in the seropositive patients. Those findings were in concordance with this study.[10] Furthermore, the data on the sero-prevalance of CMV is significantly lacking in the Pakistani population. Previously we have found CMV DNA in leukemia patient that was immune-compromised due to prolonged chemotherapy.[16] So, there is a need to conduct a large population based study to estimate the sero-prevalance of CMV infection in Pakistan to access the risk of transmission of infection to immune-compromised and critically ill patients through blood transfusion.

CONCLUSIONS

There is a need to conduct large population based study to establish the sero-prevalance of CMV infection in Pakistani population as no description is available yet. Furthermore, the blood should be screened for CMV viremia or antibodies prior to transfusion to rule out risks associated with reactivation of latent infection in critically ill patients.

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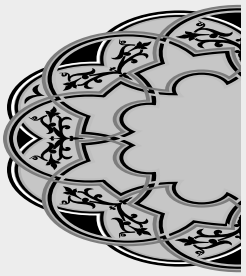

"Train your mind to see good in every situation."

Unknown

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Khalid Mahmood	Experimental work	
2	Dr. Abid Ali Hashmi	Preparation of manuscript	
3	Muhammad Umair Hanif	Proof Reading	