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HEMATOLOGICAL MALIGNANCIES;
FREQUENCY OF CYTOMEGALOVIRUS INFECTION IN PATIENTS

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ABSTRACT… Infection rate of CMV in adults is approximately 60% in the developed countries and almost 100% in the developing countries. **Objectives:** To evaluate the frequency of cytomegalovirus (CMV) infection in patients with different hematological malignancies. **Design:** Observational study. **Setting:** Gulab Devi Chest Hospital & INMOL Hospital Lahore. **Period:** Six months. **Materials and methods:** The blood samples were drawn from the selected patients after taking their written informed consent. The DNA was extracted from the whole blood and the polymerase chain reaction was performed for CMV DNA using CMV PCR kit (Cinnagen Inc. Catalog # PR7836C). The 222bp fragment corresponding to the size marker and positive control was considered as positive. The data was analyzed by SPSS version 16. **Results:** The mean age of patients was 36 years. Out of 16, 3 were presented with interstitial pneumonitis, 14 with retinitis, 3 with esophagitis and 5 were presented with colitis respectively. In this study one sample was tested positive for CMV DNA. **Conclusions:** CMV infection may be a serious threat for the patients with compromised immune system such as those receiving chemotherapy. The screening for CMV should be done before the blood transfusion to such patients.

Key words: Cytomegalovirus, Leukemia, PCR, ALL, AML, CML,

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INTRODUCTION

Cytomegalovirus is a member the family Herpesviridae or herpes viruses. The species that infect humans is commonly known as human herpesvirus-5 (HHV-5) or human cytomegalovirus (HCMV).[1] Infection rate of CMV in adults is approximately 60% in the developed countries and almost 100% in the developing countries. CMV is a lytic virus that causes a cytopathic effect both in-vitro and in-vivo. Enlarged cell with viral inclusion bodies is hallmark of CMV infection[2]. The sero-prevalence of CMV varies from 30 to 100% in different geographical areas[3, 11]. The transmission of virus occurs via blood, saliva, through birth canal and breast milk[4,11]. Adults with compromised immune system are at a risk of severe infection either via reactivation or via primary acquisition of the virus. Clinical symptoms that may be observed in this setting include encephalitis, pneumonitis, hepatitis, uveitis, retinitis, colitis, sinusitis, bone marrow suppression and graft rejection[5]. Previous studies had reported an incidence of CMV infection range of 30-58% in acute leukemia patients and an overall increase during the past two decades. High dose of antiviral drugs and increased patient age was the major factors for CMV associated mortality in these patients that ranged from 30-58%[6].

CMV reactivation rate among leukemia patients are low and dependent on the type of disease and therapy used. Higher rates were observed in lymphoid derived leukemia as compared to myeloid derived leukemia (13.6% vs. 3.9%) with the highest incidence reported in those with chronic lymphocytic leukemia[7]. The most frequent test use for the diagnosis of CMV infection is the detection of antigen pp65 (antigenemia assay). Viral isolation by either tissue culture or shell vial is the most specific diagnostic test and until now was regarded as the gold standard, but it is labor intensive, costly and the availability is limited with delayed results. Molecular methods for the detection of CMV DNA from various

samples are also being widely used. PCR is a highly sensitive method because of its ability to detect small amounts of nucleic acid in various clinical samples. In this study PCR was used to detect the CMV DNA in leukemia patients which were on chemotherapy and thought to be immunocompromised[11].

MATERIALS AND METHODS

All type of leukemia Patients that were receiving chemotherapy and blood transfusion were selected for analysis. Five ml of peripheral blood was drawn after taking the written informed consent from all patients. The study was approved by the ethics committee of Gulab Devi chest Hospital. Half of the blood sample was sent to the pathology laboratory for CBC and Peripheral smear.

DNA extraction was done according to the kit protocol (Thermo Scientific Catalog no. k0512). Two ml of whole blood was used for DNA extraction. The protocol followed was briefly described as 300µl of sample was mixed with 400µl of lysis solution and incubated at 65 ^{0}C for 30 minutes. 600µl of chloroform was added immediately, gently emulsify by inversion for 3-5 times and the sample was centrifuged at 10,000 rpm for 2 min. Precipitation solution was prepared by mixing 720µl of sterile de-ionized water with 80µl of supplied 10X concentrated precipitation solution. The upper aqueous phase containing DNA was transferred to a new tube and 800µl of freshly prepared precipitation solution was added, the solution was mixed gently by several inversions at room temperature for 1-2 min and centrifuged at 10,000 rpm for 2 min. Supernatant was removed completely and DNA pallet was dissolved in 100µl of NaCl solution by gentle vortexing. 300µl of isopropanol was then added in the solution and placed at -20ºC for 20 min then centrifuged at 10,000 rpm for 3-4 min. After that, isopropanol was removed and pallet was dissolved in 50µl of sterile de-ionized water by gentle vortexing.

Beta action was used as internal control to validate the DNA extraction. A total of 8pmoles of each primer was used in the reaction of 20μl along with PCR buffer, $MgCl_2$ (1.5mM), dNTPs (250μM) and TAQ DNA Polymerase (1U). Forward and reverse primer has the sequence GCAACCTTGGGAACAATACG and CCACGTTGTCCATGAAGAGG respectively. The temperature profile used was initial denaturation at 95C^0 for 5 min followed by 35 cycles of denaturation at 95C^0 for 30 seconds, annealing at 54C^0 for 30 seconds, extention at 72C^0 for 30 seconds and final extention at 72C^0 for 5 minutes. The band corresponding to the 110bp size marker was considered as positive.

PCR amplification for CMV was done with the CMV PCR detection kit (CinnaGen Inc. Catalog no. PR7836C) following the manufacturer's protocol. The product was resolved on the 2% agarose gel. The bands corresponding to the 222bp size marker and positive control were considered as positive.

DATA ANALYSIS

The data analysis was done by using SPSS version 16. The qualitative data were presented in the form of tables along with its percentage. The quantitative data were presented in the form of mean, range and standard deviation by the simple descriptive analysis.

RESULTS

Mean Age of the study population was 36 ± 16 years. Out of 16 patients 10 (62.50%) were males and 6 (37.50%) were females. The mean level of hemoglobin was 8.69±3.02 g/dl in these patients. The mean level of TLC was 36.75±94.8/μl among 16 patients. Mean level of Lymphocytes were 37±20% among the studied population (table I). The mean level of Platelets was 164±146.5/μl. In this study 9 (56.25%) patients had low platelet level. 9 (56.25%) patients were presented with ALL. 6 (37.50%) patients were AML and 1 (6.25%) patient with CML. In these 16 patients, 3 (18.75%) patients were presented with interstitial pneumonia and 13 (81.25%) patients were presented without interstitial pneumonia. 15 (93.75%) patients were presented with History of blood transfusion and 1 (6.25%) patient was presented with no history

of blood transfusion. 14 (87.50%) patients have signs of retinitis and 2 (12.50%) patients have no signs and symptoms of retinitis. 3 (18.75%) patients were presented with Esophagitis and 13 (81.25%) patients were presented with no history of Esophagitis. In this study 5 (31.25%) patients were presented with clinical symptoms of colitis and 11 (68.75%) patients were presented without clinical symptoms of colitis (table II). In this study 1 (6.25%) patient having ALL was positive for CMV DNA and remaining 15 (93.75%) patients were negative (Table-III).

Descriptive Statistics	Total number	Minimum	Maximum	Mean	S.D
Age	16	18	70	36.94	16.9
Hb	16	3.10	13.90	8.69	3.02
TLC	16	2.30	389.00	36.75	94.8
Lymphocyte	16	9	71	37	20.0
Neutrophils	16	15	89	53	25
Platelets	16	19	523	164	146.5

Table-I. Descriptive statistics of peripheral smear

Descriptive Statistics	Total Number	Present	Not Present
Interstitial Pneumonitis	16	03	13
History of Blood Transfusion	16	15	01
Retinitis	16	14	02
Esophagitis	16	03	13
Colitis	16	05	11

Table-II. Descriptive statistics of risk factors

		CMV PCR		
		Detected	Not Detected	Total
Type of Disease	ALL	1	8	9
	AML	0	6	6
	CML	0	1	1
	Total	1	15	16

Table-III. Result of CMV PCR

DISCUSSION

CMV may cause a variety of clinical syndromes in immune-compromised patients including those with lymphoproliferative disorder and thus considered important. Even in immuno-competent patients, it had been reported more frequently in T cell lymphoma than B cell disease[8]. In this study 1 (6.25%) patient having ALL was positive for CMV PCR and remaining 15 (93.75%) patients were negative (table III).

The incidence of CMV infection is generally low but there is a trend of increasing incidence over time in clinical reports of both lymphoma and leukemia. The higher incidence may be the result of more serious immunosuppresion due to the increased treatment intensity of chemotherapies.

The study conducted in Taiwan in 2010 had reported an incidence of CMV infection 2.7%[9]. *In the report of Nguyen et al., CMV infection caused an overall mortality of 57% in leukemia patients[10]. Hemoglobin level and TLC count are important for leukemia patients. Initially TLC count raises in acute leukemia patient and then declines in the course of the disease due to the bone marrow suppression after chemotherapy. In this study only 2 (12.50%) patients had hemoglobin level above the upper limit and only 7* (43.75%) *patients* had TLC level above normal limit (Table-II).

CONCLUSIONS

CMV infection occurs at various ages and with various underlying hematologic diseases, including AML, ALL and CML. We did not identify any unique risk factor of CMV infection for patients with AML, ALL and CLL due to multiple immuno chemotherapies and small sample size. Interstitial Pneumonitis, retinitis, Esophagitis and colitis are the possible risk factors of CMV infection. Blood

screening for CMV should be done prior to transfusion to patients on chemotherapies due to possible immune suppression.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
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2	Mughees Ahmed	Data collection	
3	Dr. Imran Hanif	Proof reading