

HCMV among Pediatrics Acute Lymphoblastic Leukemia patients in South Egypt Cancer Institute

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Abstract: Background: Objective: Human Cytomegalovirus (HCMV) is one of the causes of morbidity and mortality in pediatric cancer patients. **Patients and methods:** Out of 48 newly diagnosed ALL pediatric cancer patients (age range 2 to 13 years); treated with Total XIII Chemotherapy protocol in SECI were studied for HCMV Seropositivity (IgM/IgG) and viremia in blood plasma via PCR at diagnosis (day 0) and on the end of Chemotherapy Induction phase (day 36). **Results:** Shown that at diagnosis (Day 0) IgG was positive in 21/48 (43.8%), equivocal in 10/48 (20.8%) and Negative in 17/48 (35.4%) of patients while IgM and plasma PCR were negative in 48/48 (100%) of cases and upon repeating the same panel at the end of Induction phase (day 36) we observed complete Seroconversion with 48/48 (100%) of patients with negative IgG, IgM and PCR were also Negative and when reviewing the BMA results at the end of induction we have found that only 2/21 (9.5%) of patients failed to reach Bone Marrow Remission; both were HCMV IgG positive. **Conclusion:** No observation of Acute CMV Infection either recent before cancer diagnosis, Hospital Acquired or even reactivation through chemotherapy induction phase treatment; further studies are to be made to assure HCMV impact on cancer treatment outcome in SECI

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1. Introduction

Leukemias are considered to be one of the most common malignant neoplasms in childhood, where it accounts about 30% of all pediatric malignancies(1). Children with leukemia are significantly immunocompromised; either due to their disease condition or through active chemotherapy. Infection with and/or reactivation of Human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and Adenovirus (ADV) are frequent in those patients (1).

CMV is as a member of Herpesviridae family which is icosahedral, double-stranded DNA virus and is very common in the human population, seroprevalence is highly affected by the socioeconomic class and geographic location, with an overall range from 30–70% (2). Usually transmitted via contact with body fluids, blood transfusion, venereal, oral, respiratory routes or vertically from the mother to her fetus via placental transfer causing major complications including pneumonia, hepatitis, and colitis (3)

Aggressive acute leukemia induction chemotherapy appears to increase the risk of CMV infection and disease where Wade and his coworkers in 2006 reported an overall increase in CMV gastritis, and pneumonitis occurs among patients with acute leukemia and with mortality ranged from 30% to 57% (4)

2. Patient and Methods:

Two blood plasma samples (at diagnosis and on day 36 of Induction) collected from 48 newly diagnosed ALL pediatric cancer patients (age range 2 to 13 years) enrolled from July 2015 to August 2016 treated with Total XIII Chemotherapy protocol in South Egypt Cancer Institute.

HCMV Serology

Patients were studied for HCMV IgG and IgM Seropositivity via kite from Calbiotech, Inc (USA).

Viremia Study via PCR

CMV DNA Extraction: was performed by the RTP® DNA/ RNA Virus Mini Kit from STRATEC Molecular, Roche, Germany, **Internal control (IC) / Extraction control:** from the DNA Technology Inc. (Russia) was used as extraction controls added after finalization of the lysis step, **the quantitative-PCR Kit:** DNA-Technology Real-Time PCR detection Kits, **Device used:** The Thermo Scientific™ 24-well PikoReal™ Real-Time PCR System (Cat. no. TCR0024) (Made in Finland) **Software:** Thermo Scientific™ PikoReal™ Software Rev. 2.2, (Cat. No. N12076) (Made in Finland)

Response to chemotherapy induction treatment

Multiple groups have shown the prognostic importance of the rapidity of clearance of blasts (in peripheral blood and bone marrow) during induction therapy(5), Bone marrow aspirate was performed at

baseline and at the end of Induction phase to assess remission status of patients(6).

Data Analysis

Statistical tool used for results analysis present in this study was IBM SPSS Statistics 24.0 (2016).

3. Results

The study sample included 23/48 (47.9%) boys and 25/48 (52.1%) girls with 34/48 (70.8%) age range from 1 to 9 years of old and 14/48 (29.2%) above 9 years old.

All patients were presented with Bone Marrow Aspirate (BMA) report with blast count above 25%, and the Total Leucocytic Count (TLC) was below 10,000 cell/ul in 26/48 (54.2%), in-between 10,000 – 49,000 k/uL in 16/48 (33.3%) and above 50,000 K/uL in 6/48 (12.5%) of patients.

The Hemoglobin level was less than 7fL in 21/48 (43.8%) and in-between 7-11 fL in 27/48 (56.2%) of patients at diagnosis as described in Table (1).

Virology studies at Diagnosis (D0)

At diagnosis, HCMV IgG was positive in 21/48 (43.8%), equivocal in 10/48 (20.8%) and was negative

in 17/48 (35.4%) of patients, while 48/48 (100%) of patients were IgM and PCR negative.

A significant distribution of positive HCMV IgG at diagnosis among gender (*P-Value* = **0.001**) and in respect to different age groups (*P-Value* = **0.001**) where 17/21(81%) of girls and only 4/21 (19%) in boys and 14/21 (66.70%) is below 9 years old and 7/21(33.30%) above 9 years old respectively while no significant disease-related infection was recorded as shown in table (2).

When the same panel was repeated at the end of induction (D36) the results were 48/48 (100%) negative for HCMV IgM, IgG and PCR altogether. The distribution of BMA report at end of induction (D36) was significantly distributed among HCMV IgG positive result at where 13/21 (61.90%) in below 5% blasts count and 6/21(28.60%) in between 5-25% blast count and 2/21 (9.5%) above 25% blast count, while the equivocal results were in 3/10 (30%), 7/10 (70%) and 0/10 (0%) and the negative results was 14/17 (82.4%), 3/17 (17.6%) and 0/17 (0%) respectively with *P-Value* = **0.029** as shown in table (3).

Table (1) Patient demographics and laboratory at diagnosis.

		Frequency/Total # of Patients	Percent
Gender	Boys	23/48	47.90%
	Girls	25/48	52.10%
Age	1 to 9 years' old	34/48	70.80%
	More than 9 years' old	14/48	29.20%
BMA D0 Blasts	above 25%	48/48	100%
TLC D0*	Below 10,000 cell/ul	26/48	54.20%
	from 10,000-49,000 cell/ul	16/48	33.30%
	More than 50,000 cell/ul	6/48	12.50%
PLT D0*	Below 20,000cell/ul	6/48	12.50%
	20,000-99,000 cell/ul	23/48	47.90%
	Above 100,000 cell/ul	19/48	39.60%
Hb D0**	Less than 7	21/48	43.80%
	More than 7 & less than 11	27/48	56.30%

*TLC and PLT in cell/ul, **Hb in fL

Table (2) HCMV IgG-status at diagnosis in relation to gender

	Boys	Girls	<i>P-Value</i>	
CMV IgG positive D0 (21/48)	Gender	4 (19%)	17 (81%)	0.001
	Age Category	Age 1- 9	Age 9-18	0.001
		14 (66.7%)	7 (33.3%)	
		B Line	T Line	0.182
ALL Linage	12 (57.1%)	9 (42.9%)		

Table (3) HCMV IgG-status at diagnosis in relation BMA report at end of induction (D36)

CMV IgG status D0	BMA D36 Blasts			Total
	Below 5	5-25	above 25	
Positive	13 (61.9%)	6 (28.6%)	2 (9.5%)	21 (100.0%)
Equivocal	3 (30.0%)	7 (70.0%)	0 (0.0%)	10 (100.0%)
Negative	14 (82.4%)	3 (17.6%)	0 (0.0%)	17 (100.0%)

P-Value = 0.029

4. Discussion

The serology results of HCMV IgG was positive 43.8% and negative in 35.4% of patients which was reported earlier in 2006, through a prospective surveillance study by the University of Maryland Cancer Center showing an incidence of CMV infection among patients with acute leukemia ranged from 32% to 58% (4) on the contrary an Egyptian study performed in the National Cancer Institute by Loutfy and her coworkers showed 100% IgG positivity(7).

All of the patients at diagnosis were HCMV IgM and PCRnegative similar to the study presented by Loutfy and her coworkers in 2006showing100% negativity in CMV IgM and only 2/68 (2.9%) of patients were CMV PCR positive (7).

Another study was performed by Leng and his coworkers in 2011 in Johns Hopkins University showed that not all CMV IgG-seropositive individuals had a detectable CMV DNA where out of almost 70 samples tested showing IgM negative results, IgG was positive and PCR results were Negative (8)

Among the conventional methods for the diagnosis of CMV infection/disease including viral isolation by viral culture, serology detection for CMV specific antigen and antibody, molecular method for detection of viral DNA from blood and clinical specimens(9)

Although serology is sensitive and specific; studies were performed to access its reliability upon studying human herpes viruses, especially in immunocompromised patients since their defective ability to produce IgM, which excludes it from being a tool of diagnosis of active infection (9, 10)

The negative results for PCR was justified by Lotfy and Coworkers in their research on another lymphotropic virus HHV-6 that could be attributed to several reasons: Where the virus could be active at any specific site rather than peripheral blood, Variant viruses' improper hybridization with the used primers and low amount of circulating viral DNA accompanied with sever neutropenia can raise false negative results. (11)

The significant distribution of positive HCMV IgG at diagnosis among gender was presented but in a smaller difference in Loutfy and coworker in NCI-Egypt among pediatric lymphoma patients where CMV positivity was found in (17%) females compared to (13%) males respectively(11).

And the significant distribution of positive HCMV IgG at diagnosis among different age groups was similar to the high incidences presented in Jain and coworkers in ALL children diagnosed with CMV infection and disease where >90% of children having acquired infection by the age of 5 years (12)

When the same panel was repeated at the end of ALL induction phase (D36) the results were 48/48 (100%) negative for HCMV IgG; and this was described by Pizzo where the cytotoxic effect of chemotherapy could cause depletion of circulating lymphocyte, including profound depletion of circulating B cells resulting in diminished circulating Immunoglobulin levels, and substantial qualitative and quantitative defects in antibody response. (5)

In this study, 2/21 (9.5%) of HCMV Seropositive patients failed to reach remission by more than 25% blasts (M3 marrow), while equivocal and negative didn't show any M3 marrow significantly with ***P-Value= 0.029*** similar to that reported by Rahbarimanesh and coworkers in his study on CMV disease in children with Acute Lymphoblastic Leukemia in the nontransplant setting where three patients had at least one relapse during the course of Therapy(13)

5. Conclusion

Even though the little research and data concerning CMV in Cancer patients in Egypt; still CMV represents a significant part of our "virome" and was observed in 43.8% of newly diagnosed pediatric ALL cancer patients in South Egypt Cancer Institute.

There was no evidence of Acute CMV Infection either recent before cancer diagnosis, or Hospital Acquired during induction or even reactivation after induction treatment in SECI, so further studies are crucial to investigate the Complications that may face CMV positive cancer patients and implement proper management.

Ethical statement

The Institution Review Board of the National Cancer Institute, Cairo University of Egypt which is constituted and operates according to ICH-GCP guidelines met on 23/12/2014 and reviewed the study protocol.

Organization No. IORG0003381, IRB NoIRB00004025, FWANo. FWA00007284.

Me and All authors whose names are listed in this research certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter and materials discussed in this manuscript.

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