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Annals of Virology and Research

Research Article

The Relationship between Cytomegalovirus Viral Load in Peripheral Blood and Clinical Characteristics of Pneumonia Related to Cytomegalovirus Infection in Children

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Submitted: 26 July 2017

Accepted: 05 April 2017

Published: 06 April 2017

Keywords

- Cytomegalovirus
- Pneumonia
- Correlation
- Clinical
- Viral load
- Children

Abstract

Background: Pneumonia is a common disease among children with especially high morbidity and mortality rate for children under 5 years of age. Literature on CMV Among pediatric pneumonia cases, those with viral etiology occupied about 50-70%. Cytomegalovirus (CMV) infection, particularly in immune compromised hosts, and is associated with high mortality. While CMV infection in immune competent individuals has traditionally been considered a benign and self-limited disease, a few studies have reported the clinical manifestations and treatment of CMV infection among immune competent patients. However, little is known about the relationship between CMV viral load in peripheral blood and clinical features in pneumonia related to CMV infection in children.

Objective: To investigate the relationship between CMV viral load and clinical characteristics in CMV infected pneumonia in children.

Method: From January 2010 to December 2012, in patients with pneumonia at Respiratory Department at National Pediatric Hospital, Vietnam were prospectively evaluated. All patients have submitted one blood sample for CMV load testing by real time PCR assay.

Result: Among 246 pediatric pneumoniae having CMV infection, the age from 2 to 6 months were most commonly found (60.98%). CMV viral concentration showed decrease as patients' age increased. The increased CMV load was observed in the group of patients having slow growth rate ($p < 0, 05$), The CMV load was also found increased in patients with fever, low SpO2, wetral on lung auscultation, high white blood cell count, interstitial changes on chest X ray, extended treatment duration, longer oxygen supplement duration requirement. The higher death rate was observed in patients with CMV viral load increased ($p < 0.05$).

Conclusions: The increasing of CMV load was correlated with clinical severity in pneumonia related to CMV infection in children.

ABBREVIATIONS

CMV: Cytomegalovirus; NHP: National Hospital of Pediatrics; IQR: Interquartile Ranges; Hb: Hemoglobin level: WBC: White Blood Cell Count

INTRODUCTION

Pneumonia is a common disease worldwide with highest morbidity and mortality in infectious diseases in children. According to data of the World Health Organization (WHO), the annual rate of children deaths from pneumonia accounts for nearly one fifth of deaths worldwide. In Europe, annually the rate of pneumonia accounted for between 30 to 40 cases / 1,000 children and higher morbility and mortality have been reported among young children under 5 years of age [1]. The viral etiology was thought to be attributed to 50-70% of pathogens causing pediatric pneumoniae [2]. The accurate diagnosis of viral pneumonia is crucial for treatment and prognosis [3]

Respiratory Syncitial Virus (RSV), Influenza, Adenovirus are common viruses cause pneumonia [3]. CMV is also common virus that can infect almost anyone. Most people don't know they have CMV because it rarely causes symptoms [4]. However, pneumonia associated with Cytomegalovirus (CMV) infections have been reported in only few case series studies among immune competent patients [5-9]. In Vietnam, studies on CMV were very limited, especially pediatric pneumonia which is associated to CMV infection [10]. The lack of well understanding of CMV infection role in pediatric pneumoniae results to the absence of standardization in diagnosis and treatment, causing the difficulties for clinical decision for pediatric pneumoniae infected CMV. Therefore, the increasing the knowledge on CMV

infected pediatric pneumonia regarding to clinical presentation and viral load would contribute to establish the optimal diagnosis and treatment regimen.

In this study, we performed real-time PCR to detect CMV viremia in patients having pneumonia. The aim of this study is to investigate the relationship between CMV load and clinical characteristics in CMV infected pneumonia.

PATIENTS AND METHODS

Patient enrolment and study procedure

The study was carried out during 1st January 2010 and 30th December 2012 at the Respiratory Department of National Hospital of Pediatrics, Hanoi, Vietnam. All patients diagnosed having pneumonia according to WHO guideline [11], were eligible into the study. The criteria for pneumonia in children included cough with sputum/phlegm, fast breathing, severe chest in-drawing, intercostals recession, lung auscultation and abnormal chest X ray [12]. The patients were enrolled into the study if they were met the pnemoniae criteria and provided informed consents. The children who were younger than one month, having HIV infection, previous underwent transplantation or chemotherapy or absence of informs consents were excluded from the study. Each enrolled patient submitted one blood sample for CMV DNA detection. The demographic and clinical characteristics and treatment outcome of patients were prospectively collected. All patients were followed up until discharge.

CMV infection

CMV infection was defined as the detection of CMV DNA in blood specimen.

Diagnosis of pneumonia related to CMV infection

The CMV infection related pneumonia was defined as the detection of CMV DNA in blood from patients having pneumonia [13].

CMV DNA detection

DTA treated blood sample was used for the detection of CMV-DNA using the real-time PCR method which was described by Nystro¨m K et al. [14]. Briefly, 200 ul plasma or TA was used for total acid nucleic extraction. The extraction procedure was performed by MagNAPure LC 2.0 system (Roche Molecular Systems, Mannheim, Germany). The PCR amplification was performed in a total volume of 25 µL in the presence of TaqMan Universal PCR master mix (2X) (Qiagen, TaqMan MGB Probe, Germany), with each primer and TaqMan probe. PCR was performed with the IQ5 real-time PCR system (Bio-Rad, Hercules, CA) under the following conditions: 2 minutes at 46°C, 10 minutes at 95°C, 45 cycles of 95°C for 15 seconds and 58°C for 1 minute. All testing procedures were conducted at the Molecular Biology Laboratory, Microbiology Department, NHP.

Statistical analysis

Data were entered in to Epidata 3.1 (EpiData Association, Odense, Denmark), and then transferred into SPSS 10.0 (IBM SPSS, Armonk, NY, USA) for analysis.

To identify factors related to inflammation of the lungs are infected with CMV, testing the OR and 95% of OR are used to determine the factors related to the elements or not. To determine the impact of these characteristics to CMV infection, the method of multivariate regression analysis used logistic with OR and 95% confidence intervals. When comparing the quantitative results in the two intervention groups, such as length of hospital stay, duration of use, numbers of days of oxygen therapy in both groups are allowed ... T-student test was used. In the case of T-test did not satisfy the condition of normal distribution, the Wilcoxon signed rank test-test was used and all are allowed to check captions at the bottom of the results table. The p value less than 0.05 was considered as statistically significant.

Ethical considerations

The Ethical Committee of the NHP approved the study. Written informed consent was obtained from the parent or legal guardians of the patients.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Patients and general characteristics

During the study period, there were 427 patients admitted to the National Hospital of Pediatrics with an initial diagnosis of pneumonia, 57% (246/427) were detected having CMV infection. The proportion of male versus female was 2:1. The average age of all patients on admission was 2.5 months (SD= 2.2). Children from age of 2 to 6 months were most frequently observed (60.98%). The distribution of patients by age group is shown in Figure (1).

The relationship between viral load and clinical factors

Table (1) shows the characteristics of the CMV load of the patients. Among 246 patient whose had CMV DNA in the plasma, one hundred and ninety three patients had more than 4 log copies/ml CMV in the blood (78,45%). We further analyzed the relationship between viral load in blood versus age group (Table

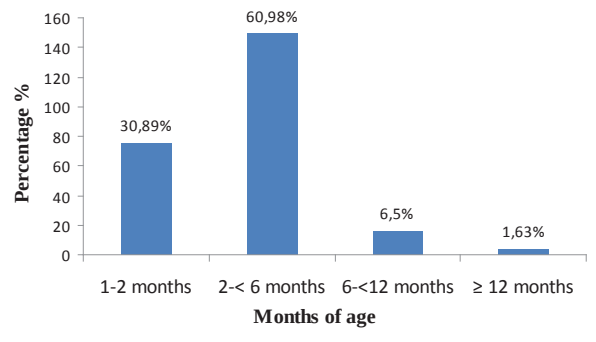

Figure 1 Distribution by age groups of study subjects (n=246) The most populated age group was children from 2 to 6 months old, 60.98%.

Table 1: Characteristics of viral load distribution.

CMV VL log in plasma	Patient number	Percentage (%)
< 3.7 log copies/ml	29	11,79
3.7 log copies/ml-<4 log copies/ml	24	9,76
≥ 4 log copies/ml	193	78,45

Table 2: The average viral load of patients according to the disease and the age group.

Age group	< 2 months	2-<6 months	6-<12 months	>12 months
CMV VL in plasma (log copies/ml, median (IQR)	4.74 (4.2-5.21)	4.65 (4.15-5.08)	4,31 (3.91-4.81)	4.47 (4.0-4.95)
CMV VL in BAL (log copies/ml, median (IQR)	5.11 (4.21-6.05)	5.34 (4.48-5.9)	5.1 (4.70-5.42)	5.11 (4.12-6)

2). We found that the younger age group had high median viral load in blood in comparison to that in the older age group. The CMV detection also performed the respiratory fluid specimen and clearly showed the higher concentration than that in the blood samples from the same patients.

We also conducted subanalyses of the relationship between viral load and clinical factors. The viral load was classified into 3 categories: less than 3.7 log copies/ml, from 3.7 log to 4 log copies/ml and more than 3.7 log copies/ml. The characteristics in each group are shown in Table 3. There was no statistical difference among groups in regard of gender, average age, hepatomegaly, and splenomegaly of patients. The growth rate of patient (weight gain in the first three months) decreased with increasing viral load (p=0.0012; 0.004; 0.036). Fever symptoms occur more frequently in patients with increased viral load (p=0.017). Saturation of oxygen in the blood reduces when the viral load gradually increased (p=0.038). The percentage of patients with wet ral occurred more frequently in patients with increased viral load (p=0.013) (Table 3).

The increased peripheral blood leukocytes were found in the group with increased viral load ($p < 0.05$). The average platelet count decreases when ascending viral load ($p < 0.05$). The number of patient who had interstitial change image on chest x ray increases when viral load increases ($p < 0.05$). The average duration of treatment increases as gradually increasing viral load ($p < 0.05$). Number of patients and deaths concentrated in the group of patients with high viral load ($p < 0.05$) (Table 4).

DISCUSSION

In this study, we present the relationship between CMV viral load in peripheral blood and clinical characteristics of pneumonia related to CMV infection in children. Among pneumonia cases, approximate 60% of children with CMV infection were under 6 months. This findings is consistent with the findings by the other studies [5], in which CMV infection can occur in all ages, but the CMV disease are more frequent in the young children [4]. Another study by Avila - Aguero ML et al. showed the mean age of CMV infected patients was 11.5 months (0.3-132 months) [5]. Liu Z et al., also found that the common age having CMV infection was less than 6 months (82.91%) [15]. According to the authors, the rate Ivanov IS patients with CMV infection increases with age [16]. However, the author Adewuyi OA as opposed to the result of CMV infection is not related to the age of the patient [17]. In the studies which investigated the CMV pneumonia, patients were often selectively enrolled and their median age had variation by studies.

In this study, we found that approximate 78% patients with pnemonia had more than 10^4 copies/ml of CMV in the blood. This result has been reported in other studies [18].

The correlation of CMV viral load and characteristics of patients with pneumoniae have been reported in several studies [6,19]. In our study, the viral load had found higher in patients who had slow growth rate. This result is consistent with the study's author Restrepo-Gualteros SM et al. [18]. The authors also found that there was a relationship of CMV infection with clinical symptoms of patients with pneumonia. Clinical symptoms include: cough (100%), lack of oxygen (100%), wet ran (100%)

Table 3: Relationship between viral load and clinical factors.

Clinical factor	<3.7 log copies/ml	3.7 log -<4 log copies/ml	≥4 log copies/ml	p
Number of patients (male/female)	29 (20/9)	24(17/7)	193(133/60)	0,99
The average age (day)	70(46-119)	62,4(42-90,5)	57(44-79)	0,2
Birth weight (g)	2700(2000-3100)	2950(1900-3100)	2900(2200-3200)	0,76
Weight gain in the first month (g)	1050(800-1200)	850(650-1050)	700(500-1000)	0,0012
Weight gain in second month (g)	800(700-1000)	800(650-950)	600(400-800)	0,0004
Weight gain in third month (g)	700(600-800)	400(350-750)	500(400-700)	0,036
Body temperature (^{0}C)	36,9(36,5-37,7)	37(36,6-37,05)	37(36,5-37,5)	0,99
Fever(>37,5^{0}C)	10/116	9/116	97/116	0,017
SpO2 (%)	92(90-97)	90(83,5-96,5)	89(85-94)	0,038
Wet ral	24/232	22/232	186/232	0,013
Hepatomegaly	6/78	5/78	67/78	0,17
Splenomegaly	3/42	3/42	35/42	0,57

Table 4: The relationship between viral load and a number of sub-clinical indicators.

Laboratory factor	<3.7 log copies/ml	3.7 log -<4 log copies/ml	≥ 4 log copies/ml	p
WBC (cells/mL)	12280(8820-15510)	10190(8660-13000)	13500(10190-18440)	0,011
Lymphocyte (%)	53(39,3-63,9)	51,45(34,5-61,75)	51,6(37,4-62,7)	0,75
Platelet (10^3 cells/mL)	410(297-515)	374(298-465,5)	314(228-415)	0,0124
Aspartate aminotransferase (AST), UI/L, Median (IQR)	42,25(30-57,6)	55,1(48,5-73,7)	58,1(39,05-83,8)	0,1
Alanine aminotransferase (ALT), UI/L, Median (IQR)	20,2(11,9-38,5)	28,2(18,1-53,9)	32,65(22,1-56,9)	0,058
Interstitial change on chest X ray	17	14	163	0,007
Oxygen treatment duration , day, Median (IQR)	6(4-8)	8(5-15)	9(5-15)	0,052
Treatment duration , Median, day, (IQR)	13(9-29)	16(11-20,5)	20(14-30)	0,01
Treatment result Cured Healthy when discharged Died	 13 15 1	 12 12 0	 140 45 8	0,004
Time antiretroviral therapy (day)	6 15(7-16)	6 13(11-14)	13,3 14(10-17)	0,82

and 93% breathing difficulty [18]. Fever occur more frequently in patients with increased viral load (p <0.05), blood oxygen saturation decreases when ascending viral load (p <0.05), the proportion of patients with wet ran in patients with increased viral load (p <0.05). This result is consistent with the study by Avila - Aguero ML et al., the clinical symptoms such as fever, anemia, splenomegaly common in patients with CMV infection [5]. Author Liu Z clinical symptoms of CMV infection are usually not specific for cough, asthma, fever, dyspnea [15].

Author Avila - ML Aguero also found that elevated liver enzymes, thrombocytopenia common in patients with CMV infection and the severity of the disease increased when the CMV viral load elevated [5]. Restrepo-Gualteros SM et al., found that an association of CMV infection with clinical symptoms of patients with pneumonia. Laboratory characteristic: 80% of patients with diffuse lung injury or freezing frosted glass form. CMV can be found by PCR in bronchoalveolar lavage (93%) or surgical techniques or immune diseases (100%). CMV is the cause of 47% of respiratory failure and death was 13.3%. The authors concluded: CMV can cause pneumonia deaths of children not infected with HIV. The suspect clinical symptoms such as lack of oxygen in clinical, x-ray infiltration may be suggestive symptoms of CMV infection in children. Antiretroviral therapy early is for good clinical results [18].

According to the authors Zanpoli M CMV infection is severe prognostic factors of patients with or without CMV infection [20]. Rafailidis found that CMV infection is not a rare disease and can cause life threatening pneumonia patients [21]. Kim AE found that diffuse interstitial lung lesions common in patients with CMV infection [22]. Author Liu Z also noticed that common chest X ray images of those patients were interstitial change [15].

CONCLUSION

The detection of CMV DNA in peripheral blood correlated with the clinical severity in Pneumonia related to Cytomegalovirus infection patients.

AUTHORS CONTRIBUTIONS

Thanh Doan designed the study, treated the patients, collected data, did the statistical analysis and drafted the manuscript. Son Pham performed the statistical analysis and Hanh Thi Hong Le helped to draft the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We would like to greatly thank Vice Professor Dao Minh Tuan (head of respiratory department) for his expert support of this study.

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Cite this article

Mai Doan TT, Pham SH, Hong Le HT (2017) The Relationship between Cytomegalovirus Viral Load in Peripheral Blood and Clinical Characteristics of Pneumonia Related to Cytomegalovirus Infection in Children. Ann Virol Res 3(1): 1028.