

Clinical manifestations and quantitative analysis of virus load in Taiwanese children with Epstein-Barr virus-associated infectious mononucleosis

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Background and Purpose: To delineate the clinical manifestations in different age groups and to define the viral load in patients with Epstein-Barr virus-associated infectious mononucleosis (EBV-associated IM).

Methods: We reviewed data on 69 children with EBV-associated IM from November 2001 to October 2005. Clinical features were evaluated among four age groups: <3 years, 3 to 5 years, 6 to 9 years and 10 to 18 years. EBV viral load was measured by quantitative real-time polymerase chain reaction (PCR) in 13 patients with 15 specimens.

Results: Majority of the children were younger than 7 years of age (76.8%) and the male-to-female ratio was 1.6:1. The symptoms and signs included fever (91.3%), tonsillopharyngitis (88.4%), lymphadenopathy (78.3%) and hepatitis (75.4%). The younger age group had higher monocyte count, lower occurrence of hepatitis, and lower glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels than the older age group. The median (range) EBV viral load of peripheral blood mononuclear cells (PBMCs) and plasma in IM patients was 738 (0-7455) copies/ μ g DNA and 51 (0-957) copies/mL plasma, respectively. The PBMC detection rate was high in the early (within 10 days after onset) and late phase (>10 days after onset) [90-100%]. The plasma detection rate in the early phase (66.7%) was higher than that in the late phase (40%).

Conclusions: The younger age group of EBV-associated IM patients had higher monocyte count, lower occurrence of hepatitis, and lower GOT and GPT levels than the older age group. The PBMC detection rate was almost equally high in both the early and late phases, while the plasma detection rate was higher in the early phase. Quantitative real-time PCR of EBV DNA is useful for diagnosing and monitoring EBV-associated IM, especially in younger children.

Key words: Epstein-Barr virus infections; Infectious mononucleosis; Signs and symptoms; Viral load

Introduction

Epstein-Barr virus (EBV) is a gamma-herpesvirus and ubiquitous in humans [1,2]. Fifty to ninety percent of individuals become seropositive for EBV by young adult life worldwide [3]. While most of the primary EBV infections in childhood were usually asymptomatic, some children and young adults manifested infectious mononucleosis (IM), an illness characterized by fever, pharyngitis, lymphadenopathy,

hepatosplenomegaly and malaise [4]. Malignancy or infection with adenoviruses, *Toxoplasma gondii*, rubella virus, human immunodeficiency virus, and hepatitis A virus also may produce a mononucleosis-like syndrome. EBV is the leading causative agent of IM in children. The prognosis of EBV-associated IM is favorable, although it occasionally causes serious complications such as central nervous system involvement, upper airway obstruction, rupture of spleen, hepatic failure and pneumonitis.

EBV-associated IM was often confirmed by clinical manifestations and EBV serology. However, serology study, although specific for EBV infection, was on occasion relatively insensitive and unreliable for

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diagnosis, especially in younger children with relative immature immunity [5]. Eighty to ninety percent of 1- to 16-year-old children with EBV-associated IM were antiviral capsid antigen immunoglobulin M (VCA IgM) antibodies-positive, and only 10% had a 4-fold increase in antiviral capsid antigen immunoglobulin G antibodies [6].

Recently, quantitative polymerase chain reaction (PCR) has been used to detect EBV DNA in patients with EBV-associated IM. According to previous reports, the EBV viral load in serum or plasma is highly sensitive in young children [5,7]. The EBV viral load level during the EBV-associated IM course has not been reported in Taiwan.

This study evaluated clinical manifestations of EBV-associated IM among four age groups of <3 years, 3 to 5 years, 6 to 9 years and 10 to 18 years due to the different manifestations noted in different age groups, as previously reported [8].

Also, in order to define the detection rate and the viral load level during EBV-associated IM, we analyzed peripheral blood mononuclear cell (PBMC) and plasma EBV viral load by using quantitative real-time PCR and compared them in different age groups.

Methods

Patients and case definition

We retrospectively collected cases younger than 18 years old with documented EBV-associated IM in the inpatient, outpatient and emergency departments at National Taiwan University Hospital, a referral tertiary hospital in Taiwan, during a 4-year period from November 2001 to October 2005. Demographic and clinical information was reviewed. Sixty nine children (1 to 18 years; median, 5 years) were enrolled. EBV-associated IM was defined by: 1) presence of at least 3 of the following symptoms or signs: fever, tonsillopharyngitis, cervical lymphadenopathy, hepatomegaly or splenomegaly; and 2) serology profile with primary EBV infection positive for VCA IgM [9]. Patients with immunodeficiency or immunosuppression were excluded. EBV-associated IM course was divided into two periods, early (<10 days) and late periods (>10 days) following disease onset.

Sample preparation, DNA extraction

PBMCs were isolated from sodium citrate anticoagulated blood samples. The PBMCs were separated from plasma by centrifugation. DNA was extracted

by use of the QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the protocol described previously [10].

Quantitative real-time PCR assay

The quantitative real-time PCR assay was performed using a LightCycler (Roche, Mannheim, Germany) and a Model 7700 Sequence Detector, as previously described [11]. Real-time PCR was based on the continuous optical monitoring of the progress of a fluorogenic PCR reaction [12]. Epstein-Barr nuclear antigen (EBNA)-1 region was detected with the forward primer EBNA-1162F (5'-TCATCATCATCCG GGTCTCC-3'), reverse primer EBNA-1229R (5'-CCTACAGGGTGGAAAAATGGC-3') and the dual-labeled fluorescent probe EBNA-1186T (5'-[FAM] CGCAGGCCCCCTCCAGGTAGAA [TAMRA]-3') [quantitative real-time PCR for the beta-*globin* gene consisted of primers and probes used as a control for the amplification of plasma DNA]. The calibration curve and amplification data analysis were done as in the previously described protocol [10,11]. The system was sensitive enough to detect 5 copies of EBV DNA [10].

Statistical analysis

Kruskal-Wallis H test for continuous variables and chi-squared test for categorical variables were performed to test the trend of each clinical characteristic among different age groups (Table 1 and Table 2). A *p* value of <0.05 was significant. All statistical computations were two-tailed and were performed with Statistical Package for the Social Sciences (SPSS) for Windows, Version 12.0 (SPSS, Chicago, IL, USA).

Results

Epidemiology and demographic features

Sixty nine children met the study criteria. The median age was 5 years (range, 1-18 years) and the majority were younger than 7 years (76.8%) [Fig. 1]. The peak incidence was at 2 to 5 years. Males were more likely to suffer from IM (male-to-female ratio, 1.6:1). No seasonal or yearly variation in the incidence of EBV-associated IM was observed.

The symptoms and signs included fever (91.3%), tonsillopharyngitis (88.4%), lymphadenopathy (78.3%), hepatitis (75.4%), hepatomegaly (66.7%), splenomegaly (47.8%), gastrointestinal discomfort (44.9%), and jaundice (21.4 %) [Table 1].

Table 1. Clinical features (No. [%]) among different age groups of patients with Epstein-Barr virus-associated infectious mononucleosis^a

Characteristic	Age group					<i>p</i>
	Total (<i>n</i> = 69)	<3 years (<i>n</i> = 17)	3-5 years (<i>n</i> = 25)	6-9 years (<i>n</i> = 15)	10-18 years (<i>n</i> = 12)	
Male gender	42 (60.9)	9 (52.9)	15 (60.0)	10 (66.7)	8 (66.7)	0.840
Fever	63 (91.3)	16 (94.1)	21 (84.0)	14 (93.3)	12 (100.0)	0.381
Tonsillopharyngitis	61 (88.4)	17 (100.0)	19 (76.0)	13 (86.7)	12 (100.0)	0.055
GI discomfort	31 (44.9)	6 (35.3)	10 (40.0)	8 (53.3)	7 (58.3)	0.535
Lymphadenopathy	54 (78.3)	11 (64.7)	20 (80.0)	14 (93.3)	9 (75.0)	0.266
Skin rash	12 (17.4)	4 (23.5)	5 (20.0)	2 (13.3)	1 (8.3)	0.701
Hepatomegaly	46 (66.7)	11 (64.7)	18 (72.0)	11 (73.3)	6 (50.0)	0.542
Splenomegaly	33 (47.8)	8 (47.1)	14 (56.0)	8 (53.3)	3 (25.0)	0.339
Hepatitis	52 (75.4)	8 (47.1)	19 (76.0)	13 (86.7)	12 (100.0)	0.006
Jaundice	6 (8.7)	0 (0)	1 (4.0)	1 (6.7)	4 (33.3)	0.117

Abbreviation: GI = gastrointestinal

^aComparative data were analyzed with chi-squared test.

Clinical characteristics and laboratory findings

The clinical features and laboratory tests on admission among different age groups are shown in Table 1 and Table 2, respectively. The younger age group had higher monocyte count ($p < 0.01$), lower occurrence of hepatic impairment ($p < 0.01$), and lower aspartate aminotransferase ($p < 0.05$) and alanine aminotransferase ($p < 0.01$) than the older age group.

Quantitative PCR assay

Thirteen patients with 15 specimens were obtained for quantitative viral load measurements. The viral load varied widely in both PBMC and plasma samples. The median (range) EBV viral load of PBMC and plasma in IM patients were 738 (0-7455) copies/ μ g DNA and 51 (0-957) copies/mL plasma, respectively.

PBMC and plasma viral load had no difference among the four age groups [Table 2]. In this early period of IM, 9/10 (90%) of PBMC and 6/9 (66.7%) of plasma samples had detectable EBV DNA. In the early period, a median of 1155 ($10^{3.06}$) copies/ μ g DNA and 130 ($10^{2.11}$) copies/mL plasma were detected in PBMC and plasma samples, respectively [Table 3]. In the late period, 10/10 (100%) of PBMC and 2/5 (40%) of plasma samples had detectable EBV DNA. In this late period, a median of 44 ($10^{1.64}$) copies/ μ g DNA and no copies were detected in PBMC and plasma samples, respectively. The PBMC detection rate was almost equally high in both early and late phases, while the plasma detection rate was higher in the early phase. The median copy number of PBMC and plasma EBV DNA in the early phase were relatively higher than those in

the late phase of IM, although the difference was not statistically significant.

Discussion

In developing countries, 80% to 100% of children were seropositive by 3 to 6 years of age. In developed countries, primary infection occurred later in life, often between the ages of 10 and 30 years [1]. In Tsai et al's report, the seroprevalence rate was more than 90% after 4 years of age in Taipei in 1984 [12]. The differences of seroprevalence may be associated with public hygiene status. In our study, 76.8% of EBV-associated IM patients were younger than 7 years, similar to the recent study in Taiwan in 1998 to 2002 [13]. The peak incidence was at 2 to 5 years, and the phenomenon correlated with the rapid rise in the seroprevalence of EBV in early childhood in Tsai et al's report [12]. No infant younger than 1 year old presented with IM in our series, reflecting the protection by maternal antibodies [14].

Four patients showed positive EBNA, indicating the early emergence of anti-EBNA antibodies in the acute phase sera in some patients, as reported in previous literature [6]. It seemed that the older children had higher frequencies of hepatic impairment and jaundice in our report, rising to 100% in adolescent patients, similar to another study [15]. IM-associated hepatitis may implicate differences in the host immunity against EBV among different age groups. In our study, monocyte count was significant higher in younger children, which was rarely mentioned in previous reports [8]. Henke et al mentioned that hematologic changes in

Table 2. Laboratory findings (median [range]) among different age groups of patients with Epstein-Barr virus-associated infectious mononucleosis^a

Variable	Age group					p
	Total (n = 69)	<3 years (n = 17)	3-5 years (n = 25)	6-9 years (n = 15)	10-18 years (n = 12)	
Peak BT (°C)	39.1 (38-41)	39.4 (39-40)	39.6 (38-41)	39.0 (38-40)	39.0 (38-40)	0.130
Fever (days)	8 (0-14)	10 (0-11)	6 (0-11)	6 (0-14)	7 (1-14)	0.265
WBC (/μL)	15,420 (3560-47,370)	15,880 (7900-32,960)	16,320 (7060-47,370)	14,700 (5140-24,630)	13,240 (3560-19,920)	0.351
Platelet (k/μL)	181 (64-443)	206 (91-418)	182 (101-228)	162 (70-443)	137 (64-295)	0.088
Lymphocyte (/μL)	7337 (1433-23,140)	8723 (3516-1768)	6943 (1433-23,140)	6615 (2190-15,024)	6206 (1780-10,401)	0.461
Atypical lymph (/μL)	1243 (0-8918)	1267 (0-7520)	1933 (0-8918)	769 (0-4557)	1071 (71-4385)	0.110
Monocyte (/μL)	1089 (0-3102)	1288 (476-1957)	1184 (0-2998)	923 (164-3102)	507 (183-2777)	0.006
AST (U/L)	107 (28-720)	51 (28-720)	158 (31-697)	98 (34-451)	157 (28-348)	0.029
ALT (U/L)	164 (12-678)	58 (12-608)	279 (28-678)	159 (55-608)	233 (48-449)	0.006
PBMC load (copies/μg DNA)	738 (0-7455)	1911 (0-3822)	956 (2-3301)	4403 (26-7455)	104 (7-200)	0.315
Plasma load (copies/mL plasma)	51 (0-957)	0 (0-0)	86 (0-169)	169 (0-957)	190 (16-365)	0.380

Abbreviations: BT = body temperature; WBC = white blood cell; AST = aspartate aminotransferase; ALT = alanine aminotransferase; PBMC = peripheral blood mononuclear cell

^aData evaluated with Kruskal-Wallis H test.

children were more frequent than those in adults [16], and these differences could explain the observation in our study. Airway obstruction, splenomegaly, hepatomegaly and rash (the majority were maculopapular rash

and not associated with antibiotic administration) occurred more frequently in younger children [8], and the trend was the same in our experience. The correlation between amoxicillin prescription and the subsequent

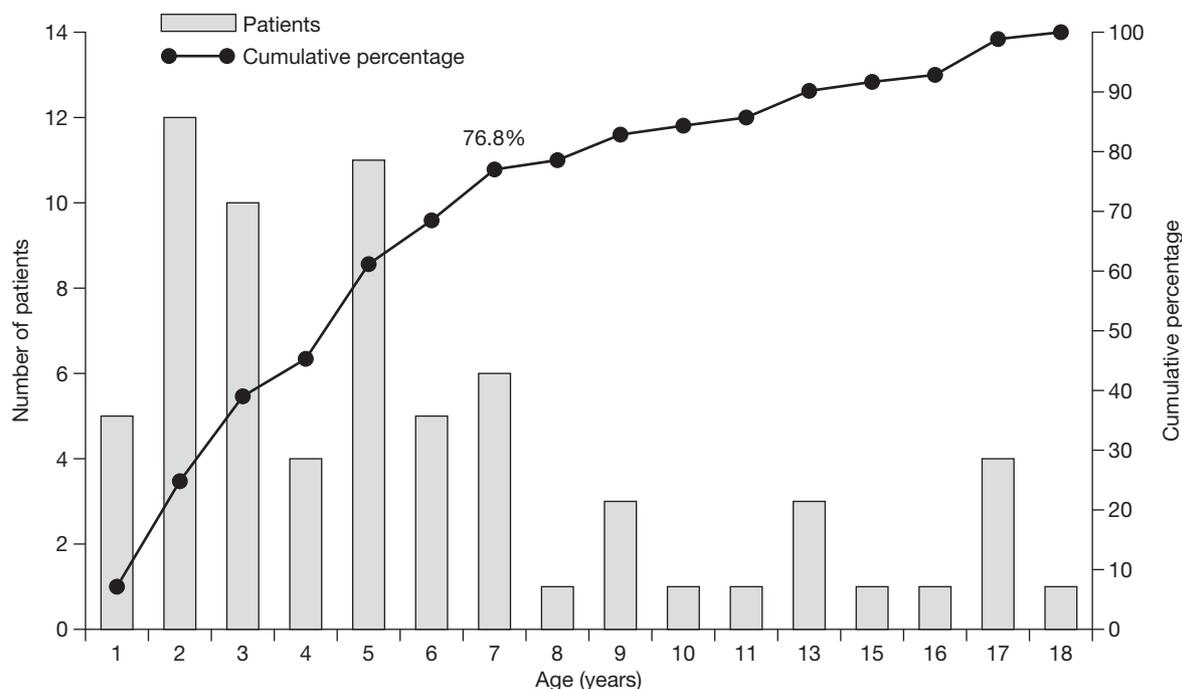
**Fig. 1.** Age distribution of patients with Epstein-Barr virus-associated infectious mononucleosis.

Table 3. Detection and copy numbers of Epstein-Barr virus (EBV)-DNA in peripheral blood mononuclear cells (PBMCs) and plasma of EBV-associated infectious mononucleosis (IM) patients^a

Duration of IM	Number of samples positive/total number No. (%)		Copies of EBV Median (range)	
	PBMC	Plasma	PBMC copies/ μ g DNA	Plasma copies/mL plasma
0-10 days	9/10 (90.0)	6/9 (66.7)	1155.04 (0-7455)	130.00 (0-957)
>10 days	5/5 (100)	2/5 (40.0)	44.00 (2-6323)	0 (0-455)

^aNot significant (Mann-Whitney *U* test).

development of rash in young adults was not observed in our study [8].

Our findings of EBV DNA in plasma suggested that viremia occurred during IM. The PBMC detection rate was high in the early and late phase. However, the plasma detection rate in the early phase was higher than that in the late phase, as were PBMC and plasma EBV DNA. The finding was consistent with previous studies in Japan [4,7]. Yamamoto et al reported that plasma samples were positive for EBV DNA in all patients (100%) in the acute phase and in 44% of the patients in the convalescent phase [7]. It also revealed that plasma from patients with IM contained the highest amount of virus DNA within 7 days following the onset of disease. EBV viral load could be a more sensitive method in rapid identification of EBV-associated IM in the early course of illness, as the serologic response may not begin in the first few days. One study revealed that among the primary EBV infection cases, those with detectable virus tended to have evidence of lymphadenopathy, and higher mean absolute atypical lymphocyte count, absolute lymphocyte count, and aspartate aminotransferase level than those without detectable virus [5].

It was unclear why one PBMC and some plasma samples were negative for EBV DNA in this study. Some other studies also found that not all IM patients had a positive EBV DNA [17,18]. The sample might have had too low an EBV DNA for detection, or a lack of viremia or the timing of sampling might have been the reason for this anomaly [7]. However, the possibility of false-positive VCA IgM antibody test results cannot be excluded. A prospective study with an independent standard is necessary to determine the relative sensitivities and specificities of PCR and IgM serology.

In summary, the younger age group of EBV-associated IM patients had higher monocyte count, lower occurrence of hepatitis, and lower glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase levels than the older age group.

The median (range) EBV viral load of PBMC and plasma in IM patients was 738 (0-7455) copies/ μ g DNA

and 51 (0-957) copies/mL plasma, respectively. The PBMC detection rate was almost equally high in both the early and late phases, while the plasma detection rate was higher in the early phase. The median copy number of PBMC and plasma EBV DNA in the early phase was relatively higher than in the late phase of IM, although the differences were not statistically significant.

Thus, quantitative real-time PCR of EBV DNA is useful for diagnosing and monitoring EBV-associated IM, especially in younger children.

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