HUMAN HERPESVIRUS 6 ANTIBODIES IN β-THALASSEMIA/ HEMOGLOBIN E PEDIATRIC PATIENTS

P Bhattarakosol[1], V Wiwanitkit[2], C Boonchalermvichian[2] and I Nuchprayoon[3]

[1]Division of Medical Virology, Faculty of Medicine, Chulalongkorn University;
[2]Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University;
[3]Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract. Human herpesvirus 6 (HHV-6) is a viral pathogen that causes exanthem subitum in children. It has also been identified as the cause of life-threatening illness in immunocompromised pediatric patients and transplant recipients. We undertook a serological study of HHV-6 IgM and IgG antibody among 29 children (12 females and 17 males) with β-thalassemia/ HbE disease. The rate of infection was 86.2%; the rates of early recent infection (IgM positive only), recent infection (both IgM and IgG positive) and past infection (IgG positive only) were 13.8%, 41.4% and 31.0%, respectively. The geometric means of the IgM and IgG titers of the splenectomy group (9 cases) were 10.15 units and 11.18 units, respectively. The geometric means of the IgM and IgG titers of the non-splenectomy group (20 cases) were 10.10 units and 12.84 units, respectively. According to this study, the prevalence of HHV6 infection among pediatric patients with β-thalassemia/HbE is very high; morever, the significantly higher titer among these patients may imply a high risk for further possible bone marrow transplantation. Increased awareness of HHV-6 infection among this population is necessary.

INTRODUCTION

Human herpes virus type 6 (HHV- 6) is a member of the herpes virus group. It was first isolated in 1985 and was included in the Herpesviridae family and the beta-herpes virinae subfamily, mainly on the basis of its genomic similarities with the human cytomegalovirus (HCMV). HHV-6 is widely distributed in the population. Seroconversion takes place very early, most often before three years of age (Tirdei *et al*, 1994).

The majority of infections are self-limiting, asymptomatic, and occur before 2 years of age. Symptomatic infections include exanthem subitum. Seroprevalence in adults may reach 100%. A recent study suggests that there are clinical syndromes associated with the presence of anti-HHV-6 IgM, in which serological screening for evidence of acute HHV-6 infection may be useful (Irving *et al*, 1990). More severe primary infections may include hepatitis, meningoencephalitis, interstitial pneumonitis, and mononucleosis-like syndrome (Irving *et al*, 1990; Liedtke *et al*, 1995). In immunosuppressed patients (such as transplant recipient), primary HHV-6 infection or reactivation may induce a rejection of transplanted organs which may prove fatal (Brion *et al*, 1995).

Correspondence: Chaiyaporn Boonchalermvichian, Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10300, Thailand.

We conducted a seroprevalence survey of HHV-6 serology among children with β-thalassemia/ HbE disease, a common congenital hematological disorder in Southeast Asia. This group can be classified as being at risk of infection. Most of these patients require frequent blood transfusions, which puts them at risk of acquiring blood-borne infections. In addition, the virus replicates in the salivary glands, is secreted in saliva, which is a proven source of infection. Other possible modes of transmission warrant investigations, such as the transfusion of blood products, especially as HHV-6 infection in immunocompromised hosts can lead to severe complications and even death (Crawford, 1993; Sayers, 1994; Campadelli-Fiume, 2000).

MATERIALS AND METHODS

Subjects

Twenty-nine children (aged 0 to 15 years) with β-thalassemia/HbE disease who visited the Pediatric Hematology Clinic, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University between April and December 2000 were enroled in the study. All cases were transfusion-dependent with regular follow-up at the clinic; none had a past history of HIV or viral hepatitis. Informed consent was obtained from each subject's parents before the collection of blood samples.

Laboratory investigation

Three milliliters of clotted blood were collected from each subject. All samples were allowed to clot at room temperature and then centrifuged. The sera

were separated and stored frozen. Hemolytic or lipemic sera were excluded. HHV-6 antibody assay was by an ELISA (PanBio, Australia). Each assay was conducted in duplicate.

Serum antibodies of the IgG or IgM class, when present, combined with an HHV-6 antigen that was attached to the polystyrene surface of the microwells. Residual serum was removed by washing and peroxidase conjugated anti-human IgG or IgM was added. The microwells were washed and a colorless substrate system, tetramethylbenzidine/hydrogen peroxide (TMB/H_2O_2) was added. The substrate was hydrolyzed by the enzyme and the chromogen changed to a blue color. After stopping the reaction with acid, the TMB became yellow. The color intensity was directly related to the concentration of HHV-6 IgG or IgM antibodies in the test sample. The cut-off for a positive value in this test was 10 units.

Statistical analysis

All data were collected and analyzed. Descriptive statistics were used as appropriate.

RESULTS

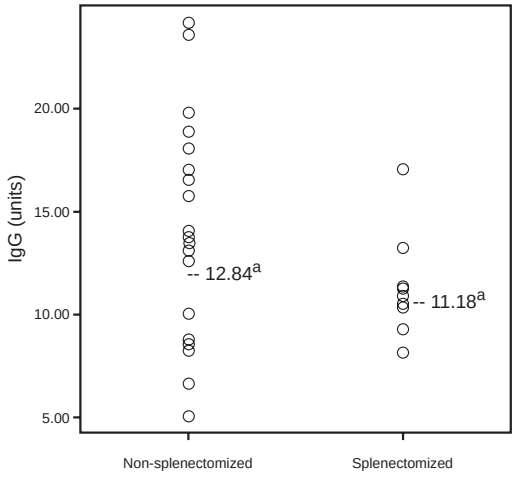
Twenty-nine children (12 females and 17 males) with β-thalassemia/ HbE disease were studied. The rate of infection was 86.2%; the rates of early recent infection (IgM positive only), recent infection (both IgM and IgG positive), and past infection (IgG positive only) were 13.8%, 41.4% and 31.0% respectively.

Among 9 splenectomized subjects, the geometric means of the IgM and IgG titers were 10.15 units and 11.18 units respectively. The geometric means of the IgM and IgG titers in the non-splenectomy group (20 cases) were 10.10 units and 12.84 units respectively (Figs 1 and 2).

DISCUSSION

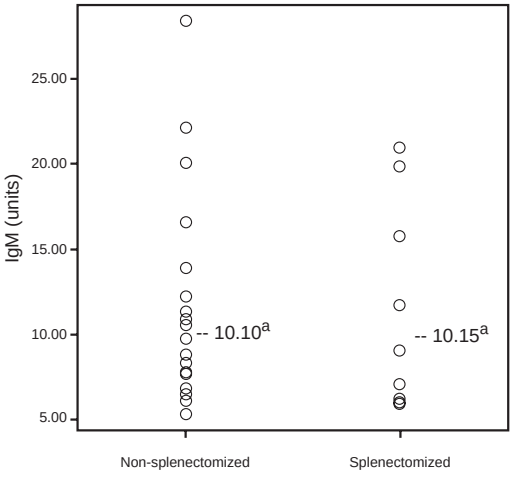
Human herpesvirus 6 is a large, enveloped virus with an icosahedral nucleocapsid and a linear, double-stranded DNA genome of 160-170kb (Campadelli-Fiume, 2000). Recently two strains, variants A and B, have been identified. Although both variants may be seen in latent and reactivated infections in adults, all the isolates examined from infants with primary infection have been variant B only. Several infant isolates that were phenotypical of variant A, turned out, upon sequencing of their genome, to be A mixture of A and B (Dewhurst *et al*, 1993).

Seroepidemiological surveys have showed that anti-HHV-6 IgG antibodies were present in more than 90% of the adult population (Irving *et al*, 1990) and 84% of Thai children (≤2 years old) (Kositanont *et al*, 1995). At present, much is known about the *in vitro* cultivation of the virus, the viral genome and proteins, the epidemiology of the infection, and the etiopathogenic relationship of the virus and exanthem subitum. However, knowledge about this virus and its effects on immunocompromised hosts, especially those with HIV and those undergoing transplantation, is far



[a] Geometric mean IgG level.

Fig 1- Scattergram comparing the IgG level of non-splenectomized children with β-thalassemia/HbE with that of splenectomized children with β-thalassemia/ HbE.



[a] Geometric mean IgG level.

Fig 2- Scattergram comparing the IgM level of non-splenectomized children with β-thalassemia/HbE with that of splenectomized children with β-thalassemia/ HbE.

Table 1
Seroprevalence studies of HHV-6 among pediatric subjects.

Study	Setting	Prevalence (%)
Romagnoli *et al*, 2000	Argentina; ethnic subjects	75
de Freitas and Linhares,1997	Brazil;ethnic subjects	24.2
Our study, 2002	Bangkok; β-thalassemia/HbE subjects	86.2

from adequate.

This study is the first to report on the seroprevalence of HHV-6 among patients with β-thalassemia/ HbE disease. This group of patients, especially those that have been splenectomized can be considered as susceptible to infection. Furthermore, given the use of bone marrow transplantation as a treatment for thalassemia, the HHV-6 serology of these patients is of interest.

According to this study, the prevalence of HHV-6 infection among those with β-thalassemia/HbE is very high. Compared to data from a seroprevalence survey of the same age group, a significantly higher prevalence of HHV-6 is evident (Table 1) (de Freitas and Linhares, 1997; Romagnoli *et al*, 2000).

In addition, our study determined the average antibody titer, a feature that was not considered in other studies. A significantly higher titer among these patients may imply a high risk for further possible bone marrow transplantation; increased awareness of HHV-6 infection among this population is necessary. We did not study the possible correlation between the prevalence and titer of HHV-6 and transfusion history; further research in this area is recommended.

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