A REVIEW OF Hib EPIDEMIOLOGY IN ASIA

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Abstract. Meningitis due to an invasive *Haemophilus influenzae* type b (Hib) infection, has been previously perceived to be relatively uncommon in Asia. However, the incidence of disease and its impact may have been underestimated. In addition to a lack of microbiological facilities in some hospitals, difficulties in culturing the organism and the widespread use of antibiotics may have hidden the true incidence of the disease in some countries. Furthermore, the reported disease burden probably underestimates the incidence of Hib pneumonia.

The epidemiology of invasive Hib disease for various Asian nations is reviewed in this paper. Hospital-based studies show that Hib is a major cause of bacterial meningitis and/or pneumonia in the Philippines, India, Thailand, Malaysia, Indonesia and Vietnam. Singapore and Hong Kong have a low incidence of infection compared with Western and other Asian nations. This low incidence is not due to a higher level of natural protective antibodies, but may be related to an interaction between environmental and genetic factors. Therefore the widespread belief that Hib infection is unimportant in Asia does not refer to Asia as a whole and possibly to Chinese patients only, and failure to recognize this has serious implications.

The inclusion of Hib vaccine in the routine infant immunization schedule in many industrialized nations has significantly reduced the incidence of invasive disease. Recent studies have shown Hib vaccination is also effective in preventing invasive disease in children in developing countries.

While population-based data may be required to confirm the need for public-funded infant Hib immunization in Asia, its introduction in countries with a high incidence of Hib meningitis and/or pneumonia has the potential to significantly improve pediatric health and survival.

INTRODUCTION

Haemophilus influenzae type b (Hib) causes serious bacterial infections in children including meningitis, pneumonia, epiglottitis, septicemia and septic arthritis (Shapiro and Ward, 1991). Before the introduction of Hib vaccines, it was the major cause of childhood bacterial meningitis in industrialized nations.

The epidemiology and the clinical pattern of Hib disease in developing countries differs from that seen in industrialized nations. Developing countries have a higher incidence of the disease, and it occurs in younger children, with most cases occurring before 12 months of age (Funkhouser *et al*, 1991; Bijlmer, 1994). In developing countries, pneumonia is a more common manifestation of infection than meningitis (Greenwood, 1992).

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QUADRENNIAL REVIEW

Epidemiology and prophylaxis of viral hepatitis: A global perspective

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Abstract

Background: Viral hepatitis with various forms of acute and chronic liver disease as potential and ultimately fatal sequelae presents a public health problem worldwide.

Methods: Recent published reports on the global epidemiology and prophylaxis of viral hepatitis were reviewed.

Results: With the advances in novel technologies, eight distinct types of hepatitis virus have been described: Hepatitis A, B, C, D, E, G, TT and SEN viruses. Hepatitis A and E viruses are transmitted by the fecal-oral route and do not induce a chronic carrier state. Due to major changes in epidemiology of hepatitis A virus their significance is more pronounced in areas of intermediate endemicity. Since the available hepatitis A vaccine is rather expensive, cost-benefit studies should be performed with emphasis on the area under consideration or specialized vulnerable groups. Parenterally transmitted hepatitis B and C viruses are major causes of chronic liver disease, including cirrhosis, hepatocellular carcinoma and end-stage liver failure. Hepatitis D virus is unable to replicate on its own, it requires an established hepatitis B virus infection to be able to replicate. Since its introduction, hepatitis B vaccine has been widely used leading to a significant decrease in HBV infection in countries with universal vaccination. Hepatitis G and TT viruses have been characterized within the latter part of the past decade but their significance as to the causation of human liver disease has yet to be elucidated. Likewise, the precise impact of the most recently described SEN virus isolated from patients with post-transfusion hepatitis awaits further studies.

Conclusions: In the course of this review, we present the situation and focus on research activities emphasizing epidemiology and prevention of the various forms of viral hepatitis.

Keywords: epidemiology, prophylaxis, hepatitis virus, prevention, vaccine, global.

INTRODUCTION

Viral hepatitis has remained a major public health problem worldwide. With the advance in technologies, eight distinct types of hepatitis viruses have been described so far: Hepatitis A, B, C, D, E, G, TT and SEN viruses. In this article, we review the present situation on epidemiology and prophylaxis of various forms of viral hepatitis in the global perspective.

HEPATITIS A

Hepatitis A virus (HAV) is a small, nonenveloped RNA virus of the genus Hepatovirus, family picornaviridae, usually transmitted by the fecal-oral route through interpersonal contact. Hepatitis A virus is thermostable and acid resistant. Since the virus lacks a lipid envelope, it is also resistant to bile lysis. This latter capacity allows efficient fecal-oral transmission. The likelihood of showing symptoms related to HAV infection is related to the age of the patient. Most infections in children below the age of 6 years are asymptomatic, whereas

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Infection with hepatitis C virus among intravenous-drug users: prevalence, genotypes and risk-factor-associated behaviour patterns in Thailand

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Hepatitis C virus (HCV) infection, a major problem worldwide, is usually transmitted parenterally or by use of contaminated needles among intravenous-drug users (IVDU). In a cross-sectional study, demographic data were collected and behaviour patterns investigated in interviews with 453, consenting IVDU. Blood samples were collected from each interviewee and checked for anti-HCV antibodies and, in a PCR-based assay, for the RNA of HCV. Almost all (92.5%) of the IVDU investigated were found positive for anti-HCV and/or the viral RNA. Most (73.5%) of those positive for HCV RNA were found to be infected with genotype 3a alone, the rest being infected with 1b (17.9%), 6a (3.5%), 3b (1.4%), 1a (1.0%), or both 3a and 6a (2.1%) or having non-typable infections (0.6%). Curiously, 26.0% of those who appeared seronegative for anti-HCV were positive for HCV RNA. The longer an interviewee had been using intravenous drugs, the more likely he or she was to be infected with HCV. Among the IVDU, the sharing of needles, syringes and/or other drug-related paraphernalia appeared to be the behaviour carrying the highest risk of HCV infection, giving an adjusted odds ratio and (95% confidence interval) of 4.84 (1.88–12.43). Programmes of needle and syringe exchange should probably be implemented among IVDU in Thailand and elsewhere, to slow the transmission of HCV.

Hepatitis C virus (HCV) is a single-stranded RNA virus of the family *Flaviviridae* and closely related to the viruses causing dengue, Japanese B encephalitis and yellow fever. It has been estimated that 170 million people — 3% of the global population — are infected with HCV (WHO, 1997). The results of phylogenetic-tree analysis (Simmonds *et al.*, 1994) indicate that HCV has at least six main genotypes and three of these (genotypes 1b, 3a and 6) are prevalent in Thailand (Theamboonlers *et al.*, 2000). HCV is transmitted parenterally or by direct, percutaneous exposure to infectious materials, such as through blood transfusion from infectious donors (a particular problem in countries where blood donations are not screened for HCV; Chinchai *et al.*, 2001), the sharing of contaminated needles among intravenous-drug abusers (Alter and Moyer, 1998), and needle-stick injuries among healthcare workers (Kiyosawa *et al.*, 1991). Approximately 2.3 million–4.7 million HCV infections may occur each year as the result of the use

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THE PREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION AMONG DIFFERENT GROUPS OF DRUG ADDICT AND BLOOD DONORS

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Hepatitis C virus infection, which can cause chronic liver diseases, cirrhosis and hepatocellular carcinoma, is still a major problem worldwide, with approximately 170 million people already infected. In order to understand the epidemiology and route of transmission, we examined the prevalence and genotypes of hepatitis C virus among different groups of drug addicts and blood donors in Thailand. There were intravenous drug users (n=134), methamphetamine users (n=100), inhalation drug users (n = 19) and alcoholics (n=50). The prevalence of hepatitis C in blood donors was determined by screening among new donors (n=66,340) in 2000. One hundred and seventy nine blood donors with positive antiHCV were randomly selected for HCV-RNA and genotyping. HCV-RNA was performed by nested RT-PCR from the 5' noncoding region. The genotypes assay was based upon analysis of amplified sequences from the 5' NCR and RFLP (Davidson et al. 1995 and Mellor et al 1996). The results showed that there was a higher rate of HCV infection in the IVDU group (70%) compared to the other drug addict groups (12-21%) and new blood donors (0.98%).
Based on our data and considering that the virus is blood borne, the risk factor for HCV transmission in the IVDU group is contaminated blood transmitted from person to person by the sharing of needles. HCV genotype 3a, 1b and 6a are the predominated genotypes in Thailand.

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A 7-YEAR LOOK-BACK INVESTIGATION ON THE RISK OF PROVIDER-TO-PATIENT TRANSMISSION OF HEPATITIS C VIRUS

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Context: Currently, it is not known how often the hepatitis C virus (HCV) is transmitted from infected health-care workers to patients during medical care and what is the potential public health impact of these incidents.
Objective: To determine the rate of provider-to-patient transmission of HCV among former patients of an HCV positive gynecologist, who infected one of his patients during a caesarean section.
Design: Retrospective epidemiological study supported by molecular virological analyses.
Setting: A department of gynecology and obstetrics in a German Academic Teaching Hospital.
Patients: A total of 2,907 woman, who had been operated on by the HCV infected gynecologist between July 1993 and March 2000.
Results and conclusions: Of the 2,907 women affected, 78.6% could be screened for markers of HCV infection. Seven of these former patients were found to be HCV infected. Phylogenetic analysis of HCV sequences from the gynecologist and the women did not indicate that the virus strains were linked. Therefore, no further iatrogenic HCV infections caused by the gynecologist could be detected during the look-back investigation. The resulting overall HCV transmission rate was 0.04% (1/2,286). The observed HCV transmission rate was within the range predicted previously by model-based probability calculations. Although further look-backs among former patients of other surgical specialities are needed, our findings support the notion that provider-to-patient transmission of HCV is a relatively rare event and might provide a factual basis for future recommendations on the management of HCV-infected health care workers.

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Management of Chronic Hepatitis B and C Virus Infections

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Abstract. Hepatitis B and C virus (HBV and HCV) infections present an important health problem causing significant morbidity and mortality on a worldwide scale. The younger the subjects infected, the higher the risk predisposing to progression towards chronic infection. Treatment of chronic HBV and HCV infections is aimed at reducing hepatic inflammation and thus improving the symptoms, decreasing the likelihood of long-term sequelae such as hepatocellular carcinoma, and increasing the survival rate. Interferon accelerates the spontaneous course of chronic HBV infection in children with greater disease activity and lower levels of replication. There is limited information on the use of lamivudine and its long-term benefit in children with chronic HBV infection. The response of combination therapy with IFN and ribavirin in children with chronic HCV infection is still under investigation. The long-term clinical and virological effects of various drugs used in chronic HBV and HCV infections on children remain to be evaluated. **[Indian J Pediatr 2002; 69 (2) : 149-154]**

Key word : *Chronic hepatitis; HBV; HCV; Children*

Hepatitis B and C virus infections can cause chronic liver diseases such as chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Those infected with either virus in early childhood are at risk of developing virus related HCC at a younger age than those infected in adulthood.

HEPATITIS B VIRUS

Hepatitis B virus (HBV) is a DNA-containing 42-nm Hepadnavirus. HBV infection presents an important health problem causing significant morbidity and mortality on a worldwide scale. There are at least 350 million carriers of HBV worldwide.[1] Approximately 25-30% of these carriers who have acquired HBV infection either during infancy or early childhood will eventually succumb to one or other of the potentially ensuing fatal sequelae, such as liver failure, chronic hepatitis, cirrhosis, and HCC. At least 1 million chronically HBV infected individuals die each year from those sequelae.[2] The global prevalence of chronic HBV infection varies from high (≥8%) in Africa, Asia, and the Western Pacific to intermediate (2-7%) in Southern and Eastern Europe, and low (<2%) in Western Europe, North America, and Australia. Around 75% of chronic HBV carriers are populations of Asian and the Western Pacific regions.[3]

Modes of HBV transmission include vertical (perinatal) and horizontal ones, such as transmission by multiple use of needles or instruments not properly sterilized, transfusion of unscreened blood, or sexual contact. However the most significant route of transmission in Asian countries is vertically transmission, which in most cases leads to be a chronic carrier stage.

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NATURAL HISTORY OF CHRONIC HBV INFECTION

The course of chronic HBV infection can be divided into three phases based on virus-host interactions resulting in a variety of hepatic lesions. The first phase, the so-called 'immuno-tolerant phase' is characterized by high levels of viral DNA in serum, expression of HBeAg, and no or minimal hepatic inflammation and hence, an asymptomatic course of the disease. This phase may persist for two or three decades.

The second, so-called immune clearance or 'active' phase is characterized by intermittent or continuous hepatitis of varying degrees of severity. Exacerbations are the result of intermittent efforts by the host's cell-mediated immune response to eliminate replicating HBV. Seroconversion to anti-HBe expression may occur during this phase. In Southeast Asia, this second phase is usually observed in individuals between 20-40 years of age. In the course of the first two phases, HBV is actively replicating. The third phase is the 'inactive' phase, during which viral concentrations are low and thus, there is minimal hepatic inflammation. During this phase, although some of the infected hepatocytes have been eliminated, HBV DNA can become integrated into the host genome and hepatocytes containing HBsAg gene continue to express the antigen. The severity of symptoms during this phase depends on the previous phase.

The risk of chronic infection is related to two major factors including the age at which infection occurs and the capacity of the host's immune response to eliminate the virus from the hepatocytes. As a consequence of immune

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SHORT COMMUNICATION

Declining Hepatitis A Seroprevalence Among Medical Students in Bangkok, Thailand, 1981-2001

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SUMMARY The severity of clinical symptoms following hepatitis A virus (HAV) infection is age dependent. Hepatitis A in children is mostly an asymptomatic disease while adolescents and adults usually show symptoms of clinical hepatitis. Improved personal hygiene and environmental sanitation has led to a decline in natural immunity acquired in childhood, creating a population of susceptible adults. In the past decade, the incidence and prevalence of hepatitis A disease in Thailand have decreased significantly. In this study, we used enzyme-linked immunosorbent assay to determine the prevalence of anti-HAV antibodies among medical students at two different time points in 1996 and 2001. We then compared these results with data from previous studies in 1981 and 1992. The seroprevalence was 73.01%, 30.23%, 16.67% and 6.67% in 1981, 1992, 1996 and 2001, respectively. A significant decline has happened over the past two decades ($p < 0.001$). Considering the decreasing immunity to HAV in the younger generations, more cases of symptomatic HAV infection could be anticipated. Further seroprevalence studies in other adolescence groups from different socioeconomic status are needed to elucidate the current situation of HAV infection in the young generation more comprehensively and to develop an appropriate prevention program.

Hepatitis A virus (HAV) is a small, non-enveloped RNA virus of the Hepatovirus genus, in the *Picornaviridae* family. Transmission happens usually via the fecal-oral route through contaminated food and water. Because the virus has no lipid envelope, it resists biliary lysis, therefore, allowing efficient fecal-oral transmission. The likelihood of developing clinical hepatitis after HAV infection depends on the age of the patient. Most infections in children aged under 6 years are asymptomatic, whereas those in older children and adults are usually symptomatic, with jaundice occurring with increasing age.[1,2] However, the hepatitis A virus has just a single serotype and long-lasting immunity develops after infection. Improvement of personal hygiene and environmental sanitation has led to a decline in the number of persons with natural immunity against the virus. This leads to an increasing number of susceptible adults within the population.

In the past decade, the incidence and prevalence of HAV infection in Thailand have significantly decreased. Over the past 25 years, there has been a shift in the epidemiology of hepatitis A in Thailand, from high to moderate endemicity. The prevalence of HAV infection has fallen especially among the younger age groups.[3] This resulted in a growing population of susceptible adolescents and adults, which are the groups more likely to become symptomatic when infected with HAV.

Medical students represent a group within the younger generation. The data on hepatitis A seroprevalence in this group can help reflect the status of HAV infection in adolescents and young adults. In 1982 Viranuvatti *et al.*[4] reported that anti-HAV antibodies were

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Research article

Serological evidence of herpesvirus infection in gibbons

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Abstract

Background: Herpesviruses are not only infectious agents of worldwide distribution in humans, but have also been demonstrated in various non-human primates as well. Seventy-eight gibbons were subjected to serological tests by ELISA for herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), Epstein-Barr virus (EBV) and cytomegalovirus (CMV).

Results: The prevalence of IgG antibodies against HSV-1, HSV-2, EBV and CMV was 28.2%, 28.2%, 14.1% and 17.9%, respectively.

Conclusions: Antigenic cross-reactivity is expected to exist between the human herpesviruses and gibbon herpesviruses. Gibbons have antibodies to human herpesviruses that may reflect zoonotic infection with human herpesviruses or infection with indigenous gibbon herpesviruses. Therefore, it is difficult to draw concrete conclusions from serological studies alone. Identification should be based on further isolation and molecular characterization of viruses from seropositive animals.

Background

Gibbons (*Hylobates spp.*) have become valuable animals for zoological, medical and psychological research. Their small size (the smallest of the anthropoids), ease of handling and maintenance in captivity and their close phylogenetic relationship to humans represent only a few of their desirable characteristics as laboratory animals. Gibbons are found throughout the tropical rainforest of South and Southeast Asia, including Thailand, Malaysia and Indonesia. Illegal pet trade is the main cause of the decreasing gibbon population in Thailand. Since gibbons were categorized as a conserved species in Thailand, hundreds of appropriated and abandoned animals have been handed over to the authorities of the Royal Forest Department (RFD). An infectious disease screening process is necessary to interrupt the spread of diseases, including herpesvirus infection. Little is known regarding natural or experimental herpesvirus infections in this interesting arboreal primate and reports of outbreaks or natural disease are still limited. Hence, screening is required to prevent the spread of infectious diseases, including herpesvirus.

Herpesviruses have been isolated from a wide variety of mammalian and non-mammalian species. The eight human herpesviruses, herpes simplex virus type 1 and 2 (HSV-1 and -2), varicella-zoster virus (VZV), Epstein-Barr

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ORIGINAL ARTICLE

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Plasma endothelin-1 levels in patients with biliary atresia: possible role in development of portal hypertension

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Abstract ***Background***: Biliary atresia (BA) is a severe neonatal liver disease characterized by progressive extrahepatic biliary tract and intrahepatic inflammatory process. Hepatic fibrosis and portal hypertension (PH) still occur despite the disappearance of jaundice following successful hepatic portoenterostomy. Endothelin-1 (ET-1) is a potent vasoconstrictor and has been reported to stimulate hepatic collagen synthesis. The aim of this study was to demonstrate the potential role of ET-1 in the pathogenesis of the progressive inflammation, fibrosis and PH in BA. ***Methods***: Thirty pediatric patients with biliary atresia post-hepatic portoenterostomy and 12 healthy children were examined. The ET-1 level was determined by commercially available enzyme-linked immunosorbent assay kits. ***Results***: Endothelin-1 levels were elevated in the patients compared with those of the controls (5.45 ± 3.34 vs. 2.74 ± 2.17 pg/ml, $P = 0.01$). Moreover, patients with PH also had greater levels of ET-1 than those without PH (6.73 ± 3.27 vs. 3.26 ± 2.2 pg/ml, $P = 0.004$). Patients with abnormal transaminase enzymes had significantly higher ET-1 levels than those with normal enzymes (6.43 ± 3.33 vs. 3.17 ± 2.1 pg/ml, $P = 0.01$). In the jaundice-free group, endothelin-1 levels were elevated in the patients with PH compared with those without PH (5.93 ± 2.15 vs. 2.88 ± 2.1 pg/ml, $P = 0.02$). ***Conclusions***: Our findings showed elevation of plasma ET-1 levels in patients with BA, especially in those with PH. ET-1 levels were also higher in patients with elevated transminase enzymes as well as in the jaundice-free group with PH. ET-1 might play a role in the pathogenesis of the progressive inflammation, fibrosis and PH in BA.

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Keywords Endothelin-1 · Biliary atresia · Portal hypertension

Introduction

Biliary atresia (BA) is a severe neonatal liver disease resulting from a sclerosing cholangiopathy of unknown etiology. Hepatic fibrosis and portal hypertension (PH) still occur despite the disappearance of jaundice following successful hepatic portoenterostomy. The mechanisms responsible for increased collagen production and hepatic fibrosis in BA are still obscure. Ramm and colleagues demonstrated that hepatic stellate cells (HSCs) played roles in the production of type I collagen leading to hepatic fibrosis in patients with BA [1]. They also showed that the hyperplastic bile duct epithelial cells, HSCs, and hepatocytes were the source of the profibrogenic cytokine, transforming growth factor- β_1 (TGF- β_1). However, the mechanisms involved in HSCs activation and bile duct proliferation are still elusive. The increased portal pressure results from an increase in vascular resistance and an elevated portal inflow. The elevated portal pressure leads to the formation of portosystemic collaterals and esophageal varices in an effort to decompress the portal venous system.

The endothelium can initiate vasoconstriction through the release of diffusible vasoconstrictor substances. The stimuli for endothelial-dependent contractions include hypoxia, several neurohumoral mediators (ADP, serotonin and acetylcholine) and physical stimuli (pressure and stretch) [2, 3, 4, 5]. Endothelial-derived contracting factors such as endothelin (ET) are powerful vasoconstrictor substances released from the endothelium and can affect both vascular smooth muscle cell tone and structure [6, 7, 8]. Endothelins consist of a family of contractile peptides (ET-1, ET-2 and ET-3) made up of 21 amino acids [9]. ET-2 and ET-3 differ from ET-1 at the two and six amino acid residue positions, respectively. Endothelins are produced by

RESEARCH NOTE

PREVALENCE AND GENOTYPES OF HEPATITIS C VIRUS INFECTION AMONG DRUG ADDICTS AND BLOOD DONORS IN THAILAND

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Abstract. Hepatitis C virus (HCV) is an infectious agent that has the potential to cause chronic liver disease, cirrhosis and hepatocellular carcinoma. We determined the prevalence and genotypes of HCV infection among groups of drug addicts: intravenous drug users (n = 134), methamphetamine users (n = 100), inhaled-drugs users (n = 19) and alcoholics (n = 50); a group of blood donors acted as a control. The control group consisted of 179 randomly-selected anti-HCV positive samples : these were subjected to HCV RNA screening and genotyping. The anti-HCV test was performed by ELISA; HCV RNA screening was by nested RT-PCR that employed primers from the 5′ noncoding region. The genotype assay was based upon analysis of the 5′ NCR amplified sequences and RFLP. Hepatitis C virus was highly prevalent among all groups of drug addicts (12-70%). In 2000, among the new blood donors (n = 66,340) at the National Blood Center, Thai Red Cross, anti-HCV prevalence amounted to 0.98%. The HCV genotype distribution showed that the most prevalent genotype was 3a, followed by 1b and 6a. Our data demonstrated the very high prevalence of HCV infection in IVDUs, a finding that is consistent with the blood-borne nature of the virus. In order to curb HCV infection, a determined effort to educate both the general population and high-risk groups is required; such a program of education would address both general and particular methods of transmission, especially the use of non-sterile needles etc.

Hepatitis C virus (HCV) infection, which can cause chronic liver diseases, cirrhosis, and hepatocellular carcinoma, is a major problem worldwide: approximately 170 million people are already infected and 3-4 million cases of new infection are expected every year (World Health Organization, 1999). HCV is transmitted parenterally or by direct percutaneous exposure to infectious materials, such as contaminated blood products. This is especially true in countries where donated blood is not screened for HCV and the sharing of contaminated needles by intravenous drug users (IVDVs) is common. In order to understand the epidemiology and route of transmission of HCV, we studied the prevalence and genotypes of hepatitis C virus among groups of drug addicts and blood donors in Thailand. HCV can be classified by phylogenetic tree analysis as at least 6 genotypes, of which 1b, 3a and 6 are prevalent in Thailand (Simmonds *et al*, 1993; Mellor *et al*, 1995).

The study protocol was approved by the Ethics Committee of the Ministry of Public

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Reactogenicity and immunogenicity of reduced antigen content diphtheria–tetanus–acellular pertussis vaccine (dTpa) administered as a booster to 4–6 year-old children primed with four doses of whole-cell pertussis vaccine

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Abstract

A trial to compare the reactogenicity and immunogenicity of a reduced antigen content diphtheria–tetanus–acellular pertussis (dTpa) vaccine with diphtheria–tetanus–whole-cell pertussis (DTPw) vaccine was conducted in Thailand. Three hundred and thirty children aged 4–6 years, primed with four doses of DTPw, received a single injection of either dTpa or DTPw. There was a significantly lower incidence of local and general reactions following dTpa than DTPw ($P < 0.001$). One month after vaccination, 99.4 and 100% of all subjects had protective anti-diphtheria and -tetanus titers, respectively. The vaccine response rate to pertussis antigens was similar in both groups, with 96.9% versus 92.5% for anti-pertussis toxin (PT), 96.9% versus 97.5% for anti-filamentous hemagglutinin (FHA) and 95.1% versus 90.8% for anti-pertactin (PRN) in the dTpa and DTPw groups, respectively. For anti-BPT, the vaccine response in the dTpa group was 29.6% versus 94.4% for DTPw. In conclusion, the dTpa vaccine was as immunogenic and significantly better tolerated than DTPw. The new dTpa vaccine could improve coverage for routine booster vaccination in children and provide a good replacement for DTP vaccines at 4–6 years of age.

Keywords: Immunogenicity; Reactogenicity; Booster; DTPa

1. Introduction

The World Health Organization (WHO) has implemented childhood immunization programs against diphtheria, tetanus and pertussis world-wide since 1974 [1]. However, concerns have been raised about the frequency of local and systemic reactions, as well as the potential relationship between the whole-cell pertussis vaccine and encephalopathy in countries such as Japan, Germany, Italy, Sweden and UK [2–9]. This has led to the development of acellular pertussis vaccines that have been demonstrated to be less reactogenic than whole-cell vaccines [10–12]. Rare and serious adverse events associated with pertussis vaccination are also less frequent after administration of diphtheria–tetanus–acellular pertussis (DTPa). Furthermore, a number of DTPa vaccines have shown to be highly efficacious against the disease [13–15]. Presently, acellular pertussis vaccines are licensed for infant immunization in many countries such as the United States, most European countries, Australia, Latin America and the Asia–Pacific region [16–18].

DTP vaccines display a somewhat increasing reactogenicity with subsequent doses and age [13,19–21]. Therefore, a vaccine with reduced antigen content (dTpa) has been investigated to enable booster vaccination against diphtheria, tetanus and pertussis in one combination for children older than 10 years, adolescents and adults rather than using dT boosters alone. The purpose of this study was to compare the reactogenicity and immunogenicity of reduced antigen content vaccine (dTpa) with a dose of

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Prevalence of canine coronavirus and parvovirus infections in dogs with gastroenteritis in Thailand

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ABSTRACT: Canine coronavirus (CCV) and canine parvovirus type 2 (CPV-2) are the causative agents of gastroenteritis in dogs. Seventy fecal samples from dogs with signs of gastroenteritis (vomiting and diarrhea), twenty-five fecal samples from healthy dogs and one CPV-2 vaccine strain were amplified by semi-nested polymerase chain reaction (PCR) and semi-nested reverse transcriptase polymerase chain reaction (RT-PCR), aimed at specifically studying the gene encoding the most abundant capsid protein VP2 of CPV-2 and spike protein of CCV. The specificity of the CCV RT-PCR product was evaluated by sequencing. Positive specimens comprised 44 samples (62.8%) and 9 samples (12.8%) for CPV-2 and CCV, respectively. In nine CCV positive samples, seven displayed co-infection between CCV and CPV-2. Our CCV sequence (AF482001) showed a 94.9% nucleotide identity to CCV reported in GenBank accession number D13096. High prevalence of CCV and CPV-2 infections was found in 1–2 month- and 3–6 month-old dogs, respectively. Molecular biology of these viruses is important primarily for epidemic control and preventative measures.

Keywords: canine coronavirus; canine parvovirus type 2; gastroenteritis; PCR

Canine viral enteritis should be suspected in dogs with an acute onset of vomiting and diarrhea, especially in puppies and where several animals are affected simultaneously. To date, four viruses have been identified as the essential causes of severe enteritis in dogs: Canine Parvovirus, Canine Coronavirus, Canine Rotavirus and Canine Distemper Virus (Pollock and Carmichael, 1983). Electron microscopic (EM) examination of fecal suspensions or isolation in tissue cultures are the most commonly used techniques for diagnosis of the infection in dogs. Recently, polymerase chain reaction (PCR) has increasingly been employed for detection of pathogens, especially when present at very low titers. The PCR is characterized by high sensitivity, specificity, and rapidity, thus becoming widely used for detecting various microorganisms.

Initially, the only prophylactic intervention available against canine parvovirus type 2 (CPV 2) comprised inactivated or live attenuated feline panleukopenia virus vaccines which proved largely ineffective. At a later, vaccines derived from live attenuated CPV-2 become available. Vaccination with an inactivated canine coronavirus (CCV) vaccine can significantly reduce not only viral replication, but the occurrence of clinical disease following a virulent CCV infection (Fulker *et al.*, 1995). In Thailand, the data available on CCV and CPV-2 infection are limited. The objective of the present study is to evaluate the prevalence of CCV and CPV-2 infections in gastroenteritic dogs by using semi-nested RT-PCR and semi-nested PCR to detect CCV RNA and CPV-2 DNA, respectively, in fecal specimens derived from gastroenteritic dogs and healthy dogs.

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Plasma Endothelin-1 in Infants and Young Children with Acute Bronchiolitis and Viral Pneumonia

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SUMMARY Respiratory syncytial virus (RSV) infections that occur during the first three years of life have been demonstrated to be associated with the development of childhood asthma. The mechanism of virus-triggered airway inflammation is not fully understood. Endothelin-1 is a potent bronchoconstrictor involved in many diseases including respiratory tract infections. Infants and young children diagnosed with either viral pneumonia or acute bronchiolitis, their age ranging between 2 months and 3 years, were recruited into this study. Nasopharyngeal aspirates were taken for detection of respiratory virus by antigen immunofluorescence stain, RT-PCR analysis and viral culture. Plasma endothelin-1 (ET-1) was measured by using a commercially available enzyme-linked immunosorbent assay (ELISA). Ten of the nineteen infants and children (52%) were positive for RSV infection, one co-infected with Influenza A. Nine infants (90%) were positive for RSV subtype A. There was only one infant with subtype B. One of the RSV negative individuals was positive for Influenza A. In addition, we recruited 10 patients without chronic underlying or respiratory tract illness as controls. ET-1 levels were significantly increased in RSV infection compared to the controls (3.6 ± 1.2 and 1.2 ± 1 pg/ml, respectively ($p < 0.05$). In conclusion, infants and young children who are infected with RSV have an increase in circulating plasma endothelin-1. This in turn may contribute to the subsequent development of childhood asthma.

Acute bronchiolitis and viral pneumonia consume a substantial amount of resources of primary healthcare systems throughout the world.[1-3] Respiratory syncytial virus (RSV), a major pathogen within the paramyxovirus family, causes severe lung disease in young children as well as immunocompromised individuals. It is the most frequent cause of acute bronchiolitis and pneumonia in infants and young children requiring hospitalization.[4-5]

Studies done during the past few decades have expanded our knowledge extensively regarding the specific mechanisms involved in the pathogenesis of RSV bronchiolitis and subsequent chronic obstructive airway disease. It is known that RSV bronchiolitis and subsequent development of asthma may be triggered by Th2-type cytokines.[6] The airway's epithelial cells are the primary target cells for RSV infection. A growing body of evidence suggests that the epithelium is not only a physical barrier, but also has the potential to synthesize a variety of cytokines, e.g. interleukin-8 (IL-8), granulocyte macrophage-colony stimulating factor (GM-CSF) and transforming growth factor (TGF). During acute RSV infection the immune response may induce long-lasting detrimental effects, thereby contributing to post bronchiolitis wheezing.[7-9]

Endothelin-1 (ET-1) is another important cytokine with a major impact on asthmatic patients. It is an endothelial regulatory peptide present in pulmonary tissue where it exerts several biological effects both on bronchial and vascular smooth

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Oral Medicine

Hepatitis C virus infection in Thai patients with oral lichen planus

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OBJECTIVE: Many studies focusing on the association between hepatitis C virus (HCV) infection and oral lichen planus (OLP) have been conducted. Diversities of geographical locations could be a major factor influencing the prevalence of HCV. This study was aimed to define whether there was a relationship between the OLP and HCV infection in Thailand.

MATERIALS AND METHODS: Serum samples of 60 patients (with OLP) and 60 controls (without OLP), whose age and gender were matched, were respectively screened for anti-HCV by ELISA (third generation), and reverse transcription polymerase chain reaction (RT-PCR) for HCV-RNA.

RESULTS: We found five patients (8.33%) with OLP infected with HCV: three patients were positive for both anti-HCV and HCV-RNA; one patient was only positive for anti- HCV; and one patient was only positive for HCV-RNA; whereas all the controls were negative for both anti-HCV and HCV-RNA ($P = 0.029$). Three of five cases of OLP with HCV infection had histories of blood transfusions over 10 years ago.

CONCLUSION: The present study reports a small, but statistically significant high prevalence of HCV infection among patients with OLP, although the underlying mechanism still remains unknown.

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Keywords: hepatitis C virus; oral lichen planus; anti-HCV; HCV-RNA

Introduction

Lichen planus (LP) is relatively a common, chronic mucocutaneous disease of unknown etiology (Regezi and Sciubba, 1993). During the last two decades, several reports proposed a possible association between LP and chronic liver disease (CLD) (Ayala *et al*, 1986; Cottoni *et al*, 1988; Gruppo Italiano Studi Epidemiologici in Dermatologia (GISED), 1990; Rebora, Robert and Rongioletti, 1992), especially in primary biliary cirrhosis (PBC) (Graham-Brown, Sarkany and Sherlock, 1982; Powell, Rogers and Dickson, 1983) and in chronic active hepatitis (CAH) (Rebora *et al*, 1978; Rebora, Rongioletti and Canepa, 1982; Rebora and Rongioletti, 1984). Recently, it was reported that there was a high prevalence of hepatitis C virus (HCV) infection in patients with LP. The first report documented in 1991 suggested a possible relation between HCV infection and LP (Mokni *et al*, 1991), and it might explain some aspects of the association between LP and CLD. The prevalence of anti-HCV antibodies in patients with cutaneous lichen planus (CLP) and/or oral lichen planus (OLP) ranged from 3.8 to 65% (Divano, Parodi and Rebora, 1992; Rebora *et al*, 1992; Bagan *et al*, 1994; Cribier *et al*, 1994; Gandolfo *et al*, 1994; Nagao *et al*, 1995; Tanei, Watanabe and Nishiyama, 1995; Carrozzo *et al*, 1996; Sanchez-Perez *et al*, 1996; Chosidow *et al*, 1997; Bagan *et al*, 1998; Dupond *et al*, 1998; Mignogna *et al*, 1998). The majority of the studies were conducted in countries where there were high prevalences of HCV infection in general populations, especially southern Europe and Japan. However, the studies from the UK (Ingafou *et al*, 1998) and the Netherlands (van der Meij and van der Waal, 2000), where the prevalences of HCV infection was low, did not find any serological evidence of antibodies to HCV. Besides, two studies from Germany where the prevalence of HCV infection was also low found conflicting results: Imhof *et al* (1997) in a controlled study found a significant association between LP and HCV while Grote *et al* (1998) did not apparently find the association in an uncontrolled study. In the USA all but one of the performed studies reported a significant association between LP and HCV infection (Bellman, Reddy and Falanga, 1995; Chuang *et al*, 1999; Beaird *et al*, 2001; Chainani-Wu *et al*, 2001; Eisen,

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SEN virus infection in patients with chronic liver disease and hepatocellular carcinoma in Thailand

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Background. SEN virus (SENV) has been recently identified as a candidate agent of non-A-E hepatitis virus. However, the exact role of this novel virus in the pathogenesis of chronic liver disease, including chronic hepatitis and cirrhosis, and the development of hepatocellular carcinoma (HCC) remains to be established. ***Methods.*** Using seminested polymerase chain reaction (PCR) amplification to detect SENV-D and SENV-H strains in serum, we investigated SENV infection in voluntary blood donors and in patients with chronic liver disease and HCC. ***Results.*** SENV was detected in 5 of 100 blood donors (5%), in 15 of 60 patients with chronic liver disease (25%), and in 25 of 60 patients with HCC (42%). The prevalence of SENV in patients with HCC was higher than that in patients with chronic liver disease ($P = 0.05$) and in blood donors ($P < 0.001$). An age-specific prevalence of SENV was found at high levels among individuals aged 21–40 years, but was not detected among individuals in the lower age group. No differences between SENV-infected and non-infected patients were demonstrated with respect to demographic data, assumed source of infection, biochemical abnormalities, and severity of chronic liver disease and HCC. Moreover, SENV infection had no apparent effect on the survival of patients with HCC. ***Conclusions.*** Our data suggest that SENV infection is frequent among patients with chronic liver disease and HCC. However, pathogenic effects associated with SENV infection in chronic liver disease and HCC need further investigation.

Key words: hepatitis virus, SEN virus, chronic liver disease, hepatocellular carcinoma, Thailand

Introduction

Chronic liver disease (including chronic hepatitis and cirrhosis) and hepatocellular carcinoma (HCC) are common in Thailand and have been known to be associated with chronic hepatitis B virus (HBV) and chronic hepatitis C virus (HCV) infection.[1] Nonetheless, there is still a significant proportion of cases in which the etiology is unknown, which is suggestive of the existence of additional causative agents.[2] With the advent of sophisticated molecular biological techniques, two novel hepatitis virus candidates, designated hepatitis G virus (HGV) and TT virus (TTV) were recently identified, in 1995 and 1997, respectively.[3–5] Despite the worldwide distribution of HGV and TTV, the association of these viruses with liver disease has not clearly emerged. Most recent studies indicate that HGV and TTV are relatively harmless to the liver and are not the etiological agents in the majority of patients with cryptogenic chronic liver disease and HCC.[6–8]

SEN virus (SENV) represents the latest hepatitis virus, which was first isolated from the serum of an intravenous drug user (IVDU) infected with human immunodeficiency virus (HIV).[7,9] SENV was initially described as a single-stranded DNA virus of approximately 3600–3900 nucleotides and possessing at least three open reading frames.[7,9] Subsequent genomic and molecular evolutionary analyses have demonstrated that it is a small single-stranded circular virus that belongs to the superfamily of TTV-related viruses.[10] To date, eight distinct strains of SENV (A-H) have been identified. Among them, it has been shown that two SENV strains (SENV-D and SENV-H) are significantly associated with transfusion-associated non-A-E hepatitis.[11] SENV-D and SENV-H were also detected more frequently in patients with chronic liver disease and HCC than in healthy adults.[12,13] Despite its high prevalence among patients with liver disease, the exact role of SENV infection regarding the etiology of chronic liver

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Interspousal transmission of hepatitis C in Thailand

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Background. Previous studies evaluating the possibility of interspousal sexual transmission of hepatitis C virus (HCV) have yielded many conflicting results. Our study was carried out to determine the exact potential and risk factors of interspousal HCV transmission. ***Methods.*** The spouses (54 men and 106 women; mean age ±SD, 48 ± 8 years) of 160 patients with HCV infection (106 men and 54 women) were serologically tested for HCV using a third-generation enzyme-linked immunosorbent assay (ELISA). Positive results were confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). For positive couples, the cluster nucleotides of the HCV gene and genotypes were compared on the basis of restriction fragment length polymorphism (RFLP), Innogenetic Line Probe Assay (INNO-LiPA), and direct sequencing. Similarly, phylogenetic tree and sequence homology analysis was performed in order to precisely verify interspousal transmission. Risk factors promoting interspousal HCV transmission were also identified. ***Results.*** Throughout a mean duration of exposure of 23 + 5 years, most of the 160 partners had their usual and unprotected sexual relationships with the index patients. HCV-associated antibodies and HCV-RNA were detected in only 3 (1.88%) of the 160 spouses. Furthermore, homology and phylogenetic tree analysis could not clearly demonstrate that any one of these 3 positive spouses was infected with the same strain of HCV as that identified in the index cases. Because a positive group remained elusive, risk factors of interspousal HCV transmission could not be determined in this study. ***Conclusions.*** According to this study, interspousal transmission of HCV seems to be very rare. HCV-positive spouses should be firmly reassured that they can maintain their normal marital life.

Key words: hepatitis C virus, transmission, RFLP, risk factors

Introduction

Hepatitis C virus (HCV), the principal cause of non-A, non-B hepatitis,[1] is an RNA virus belonging to the family of *flaviviridae*; an estimated 170 million people, or about 3% of the global population, have been infected with HCV.[2] The virus can be grouped into at least six genotypes in different geographic areas.[3] Infection with different genotypes may affect the clinical outcome and response to treatment.[4–7]

Hepatitis C virus is transmitted by direct percutaneous exposure to infected blood products, such as the transfusion of various derivatives of blood products, and by intravenous drug abuse, which are well-established causes.[8,9]

To prove the mode of transmission, the identification of common strains of HCV can be performed by various methods, e.g., genotyping and polymorphism analysis,[10] direct sequencing of the genome, and phylogenetic analysis.[11]

Although sexual contact has been implicated as a route of transmission, the results are still controversial.[12–18] Infrequent sexual transmission of HCV has been shown in studies performed in Western countries, but many studies originating from Asia suggest that interspousal transmission may be crucial for the interfamilial spread of HCV, with a longer duration of marriage as the most evident risk factor.[17,18] Sexual transmission was documented in the presence of coexistent HIV infection, and this suggested the co-transmission of HCV and HIV to be more efficient than HCV transmission alone.[19]

To determine more accurately the epidemiology of interspousal HCV-transmission and possible risk

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Molecular epidemiology of gibbon hepatitis B virus transmission

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Although transmission of human hepatitis B virus (HBV) variants to nonhuman primates is well documented, it remains to be elucidated whether nonhuman primate HBV is transmissible to humans. The prevalence and transmission routes of gibbon HBV were analysed in 101 captive gibbons in Thailand. Approximately 40 % of these animals showed at least one marker of HBV infection; 19 animals were chronic HBV carriers, characterized by elevated levels of alanine amino transferase and the presence of HBV DNA. Some of the chronic animals were found to be anti-HBc (HBV core antigen) negative (4 of 19), while precore promoter point mutations (nt 1762 or 1764) were determined in four animals by RFLP analysis. Phylogenetic tree analysis of the complete surface gene sequences revealed that gibbon viruses clustered separately from hepadnaviruses of other hosts. Evidence for horizontal and vertical transmission in captive gibbons was obtained. HBV DNA was also detected in the saliva of HBV carrier gibbons. Although some of the animal caretakers at the Krabok Koo Wildlife Breeding Centre were found to be chronic HBV carriers, genotype and sequence analysis did not reveal any evidence for zoonotic disease transmission.

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INTRODUCTION

Hepatitis B virus (HBV), a small double-shelled virus that contains a partially double-stranded DNA genome of approximately 3200 bases (White & Fenner, 1994), is found in several species, including woodchuck, ground squirrel, a range of bird species, such as duck, goose and grey heron (Mason *et al.*, 1980; Marion *et al.*, 1980; Summers *et al.*, 1978), and nonhuman primates like chimpanzee (*Pan troglodytes*), woolly monkey (*Lagothrix lagothrica*), orang-utan (*Pongo pygmaeus*) and gorilla (*Gorilla gorilla*) (Grethe *et al.*, 2000; Lanford *et al.*, 1998; Vaudin *et al.*, 1988; Warren *et al.*, 1999). HBV was also isolated from a gibbon, infected in the wild and housed at the CDC for 2 years (Mimms *et al.*, 1993). Phylogenetic analysis of the complete genome revealed that gibbon HBV represents a unique group when compared to HBV genotypes described previously. Remarkably, a 33 bp deletion after the start codon of preS1, the most divergent part of the genome, was observed (Norder *et al.*, 1996). Nonrecognition of an anti-preS1 monoclonal antibody at aa 27–35 to gibbon virus particles confirmed that the gibbon HBV surface protein conformation is different from that of human HBV (Mimms *et al.*, 1993).

Phylogenetically, HBV isolated from gibbons and chimpanzees share an early lineage, indicating that these viruses were indigenous to their respective hosts (Norder *et al.*, 1996). On the other hand, infection of chimpanzees with human and gibbon HBV can be accomplished (Gallagher *et al.*, 1991). Experimental transmission of human HBV to gibbons by exposure to human saliva containing HBV has been reported also (Bancroft *et al.*, 1977; Scott *et al.*, 1980). Replication of human HBV in the respective animals supported the close relation of these hosts and may indicate natural HBV cross-transmission. On the other hand, no evidence has been obtained thus far for HBV transmission from gibbon or chimpanzee to human. HBV is present at levels as high as 1×10^{13} virions ml^{-1} in the blood of HBV e antigen (HBeAg)-positive patients but virus particles have also been found in other body fluids, including saliva/nasopharyngeal fluids, semen, cervical secretions and

The GenBank accession numbers of the sequences reported in this paper are AF274495–96, AF274499, AF275378, AF477482–94, AY077735–36 and AF529308–09.

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Human Herpesvirus Infection in Children with Fever and Maculopapular Rash

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SUMMARY Fever with maculopapular rash is a common problem in children. Infection with human herpesviruses is one of the common etiologies in fever with rash. The aim of this study has been to examine patients presenting with fever and maculopapular rash without respiratory symptoms for human herpesviruses infection by using multiplex nested-polymerase chain reaction. A descriptive and prospective study was conducted at King Chulalongkorn Memorial Hospital, Bangkok, Thailand from June 2000 to December 2001. One hundred patients, 43 boys and 57 girls, aged between 2 months and 14 years were recruited. Human herpesvirus 6 (HHV6) was the most common (24%) whereas HHV7, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were present in 9%, 3% and 2% of the patients, respectively. Four percent of the patients simultaneously harbored HHV6 and HHV7. Only one patient had CMV, HHV6 and HHV7. Patients with HHV7 had a mean age of 4.5 years, whereas those with HHV6 had a mean age of 1.6 years. HHV6 and HHV7 were commonly found as causes of fever and maculopapular rash without respiratory symptoms. Co-infection with different herpesviruses can be found in the same patient.

Fever with maculopapular rash without a localized sign is a common problem in children. It may be due to viral infection, bacterial infection, or drug allergy.[1,2] Viral infections commonly causing maculopapular rash are attributed to cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus (HHV)-6, HHV-7, enterovirus and parvovirus B-19.[1-6]

Human herpesviruses (HHVs) can cause primary infection, which may proceed towards latency.[7,8] They are responsible for acute febrile illnesses in children. Viral reactivation in immunocompromised patients has been linked to a number of diseases and causes significant morbidity and mortality, especially in transplant recipients and HIV patients.[9,11]

Serologic evidence, such as a four-fold or greater increase of the IgG titer, does not differentiate between primary or reactivated latent infection.[12-14] The detection of HHV6 and HHV7 in peripheral blood leucocytes (PBL) only is of limited significance since viral DNA persists in the PBL of healthy persons. Viral DNA detection in a cell free body fluid such as plasma has been shown to correlate with active viral replication.[15,16] Plasma polymerase chain reaction has demonstrated diagnostic accuracy in detecting primary HHV6 infection in immunocompetent children with a sensitivity and specificity amounting to 90% and 100%, respectively.[12,17] Polymerase chain reaction (PCR) has become one of the most widely used techniques in human virology diagnostic. Multiplex nested-PCR represents a modification of the original PCR protocol applied to simultaneously amplify DNA originating from different pathogens using several primer pairs in the same reaction.[18] Based on a technique described previously,[18] we applied a sensitive multiplex nested-PCR to determine the prevalence of human lymphotropic herpesviruses in children with fever and maculopapular rash.

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DESIGN OF DEGENERATE PRIMERS FOR MULTIPLEX NESTED-PCR DETECTION OF HUMAN LYMPHOTROPIC HERPESVIRUSES

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Abstract. To develop the rapid diagnosis and typing of human lymphotropic herpesviruses by using multiplex nested-PCR, the primary PCR (1° PCR) primers were redesigned as degenerate primers based on a highly conserved sequences of each DNA polymerase gene of EBV, CMV, HHV-6, HHV-7 and HHV-8. The forward degenerate primer (HHV/1+) contained 12 different sequences, whereas there were 8 different sequences in the reverse degenerate primer (HHV/1-). Optimization of multiplex nested-PCR assay conditions were performed to search for the appropriate amount of degenerate primers, dNTP, *Taq* DNA polymerase, template of secondary PCR (2° PCR) and annealing temperature used in 1° PCR reaction. Detection sensitivity was the same as described in previous report (approximately 10-100 genome copies). To ensure a true negative result, PCR detection of hepatitis B virus genome was used as internal control. Our presented results, the designed degenerate primers could be used to detect various types of HHV by multiplex nested-PCR.

INTRODUCTION

Epstein-Barr virus (EBV, HHV-4), human cytomegalovirus (CMV, HHV-5), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7) and human herpesvirus 8 (HHV-8, Kaposi's sarcoma associated herpesvirus) are known human lymphotropic herpesvisus (HHVs) whose natural host is human. Although the viruses are different from one another, regarding their biological behavior and genomic arrangements, they share the same ability to establish latency after primary infection (Roizmann *et al*, 1992). Recurrent or reactivated HHVs infection are commonly found as opportunistic diseases in HIV-infected person (Fabio *et al*, 1997; Schulz, 1998; Clark, 2000) or in immunosuppressed patients following bone marrow, kidney, liver, or heart transplantation (Chan *et al*, 1997; Osman *et al*, 1997; Clark, 2000). HHV-6 and HHV-7 have also been associated with febrile illness and childhood diseases, exanthem subitum (roseola infantum) (Yamanishi *et al*, 1988; Tanaka *et al*, 1994). Nevertheless, EBV appear to be an important etiological factor for nasopharyngeal carcinoma. The use of serum/plasma EBV DNA as a reliable tumor marker prior to, during, and after treatment of the cancer was reported (Liebowitz *et al*, 1994; Shotelersuk *et al*, 2000).

Recently, PCR-based assays have been recognized as sensitive and specific method for molecular detection and identification of HHVs (Wakefield *et al*, 1992; Tenorio *et al*, 1993; Vandevanter *et al*, 1996; Clark *et al*, 1997; Kidd *et al*, 1998; Minjolle *et al*, 1999; Pozo and Tenorio, 1999; Johnson *et al*, 2000; Kessler *et al*, 2000; Kearns *et al*, 2001). A multiplex nested-PCR for simultaneous detection and typing of HHV4 (EBV), HHV-5 (CMV), HHV-6, HHV-7 and HHV-8 was developed by Francisco Pozo and Antonio Tenorio in 1999. Two sets of specific primers, designed for amplification of a highly con-

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