ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2007) 25: 16-174

The Prevalence and Persistence of Human Parvovirus B19 Infection in Thalassemic Patients

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SUMMARY Human parvovirus B19 infection was studied in 60 thalassemic patients in Thailand. Seroprevalence, persistence of parvovirus B19 and their genotypes were identified in blood samples. Prevalence of anti-parvovirus B19 IgG and DNA found in thalassemic patients were 38% and 13%, respectively. Anti-parvovirus B19 IgM could be detected in 4% of these positive anti-parvovirus B19 IgG patients. The seroprevalence and parvovirus B19 DNA in patients with a history of blood transfusion were not significantly higher than those without such a history (44% *vs*. 34% and 20% *vs*. 9%, respectively). Phylogenetic analysis of NS1 nucleotide sequences of three parvovirus B19 samples revealed that they were parvovirus B19 genotype 1. They showed low genetic diversity from prototype (Au) strain. We concluded that acute and chronic persistent parvovirus B19 infection were found in the thalassemic Thai patients. Chronic persistence of parvovirus B19 infection might play important clinical role in thalassemic patients because of the high prevalence of parvovirus B19 DNA. Blood transfusion had no significant influence to increase the prevalence of parvovirus B19 infection in thalassemic patients.

Human parvovirus B19 has been classified in genus *Erythrovirus*, Family *Parvoviridae*. Parvovirus B19 contains single-stranded DNA of approximately 5.6 kb in length and replicates autonomously in erythroid progenitor cells. Nonstructural protein (NS1) is known as a transactivator which influences the activity of viral and cellular promoters.[1] VP1 and VP2, viral capsid proteins, act as neutralizing epitopes.[2] The nucleotide sequence of parvovirus B19 was originally established by sequencing a viral isolate obtained from the serum of a child with homozygous sickle cell disease. Entirely or a part of parvovirus B19 genome isolates have been sequenced. Among most isolates the NS1 gene is well conserved, while the VP1 and VP2 regions may occasionally show a greater variability.[3] To date, erythrovirus sequences available in GenBank indicate that the erythrovirus group can be divided into three well-individualized genotypes, with parvovirus B19–related virus corresponding to genotype 1 (prototype strain Au), and V9-related viruses being divided into genotype 2 (prototype strain Lali) and genotype 3 (prototype strain V9).[4] The nucleotide distances observed between Au-V9, Au-Lali and V9-

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Lali were 10.5 to 12%, 7.6 to 8.9% and 6.4 to 7.9%, respectively. Virus transmission generally occurs by the respiratory route. Moreover, parvovirus B19 can be transmitted from mother to fetus through the transplacental route and parenterally via blood or blood products.[5,6] The most common manifestation of human parvovirus B19 infections is erythema infectiosum.[7] Human parvovirus B19 infections can cause severe disease in some individuals, such as infection in pregnant women can lead to spontaneous abortions and hydrops fetalis.[5] Persistent parvovirus B19 infections are associated with chronic clinical manifestations, such as chronic pure red cell aplasia, chronic arthropathy, and chronic anemia.[8,9] Thalassemia disease is a common chronic hemolytic anemia in Thailand. Transient aplastic crisis can be found in chronic parvovirus B19 infection of sickle cell anemia patients and other chronic hemolytic disorders, such as thalassemia and autoimmune hemolytic anemia.[10,11] Objectives of this study are to determine the prevalence of parvovirus B19 IgG and IgM antibodies and the persistence of parvovirus B19 genome in blood samples of thalassemic patients and to identify the genotype of the parvovirus B19 in 3 plasma samples by analyzing nucleotide sequences of NS1 region.

Table 1 Demographic data of thalassemic patients

Thalassemic patients (n = 60)	**Demographic data**
Age (years)	
Range	15-53
Mean ± SD	31 ± 10
Median	32
Age distribution	
≤ 20	13 (21.7%)
21-30	16 (26.7%)
31-40	18 (30.0%)
≥ 41	13 (21.6%)
Gender	
Female	39 (65%)
Male	21 (35%)
History of blood transfusion	
Yes	25 (42%)
No	35 (58%)

MATERIALS AND METHODS

The studied subjects were 60 patients with β-thalassemia disease. They visited thalassemia clinic, Siriraj Hospital, Thailand, between June 2001 and May 2002. Demography of subjects is shown in Table 1. The median age was 32 years and 65% of patients were females. There were 25 thalassemic patients who had a history of multiple blood transfusions. The samples of EDTA-blood were collected and processed to obtain plasma and peripheral blood mononuclear cells (PBMC). Anti-parvovirus B19 IgG was determined by using indirect ELISA kit (Genzyme Virotech GmbH, Germany). NS1 region of parvovirus B19 DNA in the plasma and in PBMC samples were amplified by nested PCR. The two pairs of primer sequences for amplification of NS1 regions were 5'GCC GCC AAG TAG AGG AA-3', 5'CCA CGA TGC AGC TAC AA -3' and 5'AAT GCG TGG AAG TGT AGC TGT G-3', 5'TCA CCA CCA TG CTG ATA C-3' (given by Dr. Neal S. Young and Dr. Kevin E. Brown, Virus Discovery Group, Hematology Branch, National Heart, Lung and Blood Institute, NIH, USA). Plasma and PBMC samples were extracted using QIAamp DNA Blood Mini kit (CA, USA) and 5 μl were used for amplification. The PCR was processed by an initial denaturation step of 94ºC for 1 minute and followed by 30 cycles of amplification at 92ºC for 40 seconds, 60ºC for 40 seconds and 75ºC for 1.5 minutes. Known human parvovirus B19 DNA serum was used as a positive control (PC). The amplified products sized 350 bps of three samples and the PC were purified and sequenced by the Bioservice Unit, National Science and Technology Development Agency (NSTDA), Thailand. Genotypes were identified by phylogenetic analysis, nucleotide sequences were multiple aligned by using the program CLUSTAL_X (version 1.83). Alignments were then fed into phylogenetic trees that were constructed for each subalignment by using the neighbor- joining methods implemented by the MEGA program. The statistical validity of the neighbor-joining methods was assessed by bootstrap re-sampling with 1,000 replicates. The sequences of parvovirus B19 from GenBank were used as reference genotypes: accession number AF162273, M13178 (Au), Z70560, Z70528, Z70599, Z68146 for genotype 1; AY044266 (Lali), AY903437, AY064475, AY064476 for genotype 2; AX003421 (V9), AJ249437, DQ234769, DQ234775 for genotype 3.

RESULTS

The prevalence of parvovirus infection determined by anti-parvovirus B19 IgG was found in 23/60 (38%) of thalassemic patients. Anti-parvovirus B19 IgM were detected in 1 of 23 (4%) positive anti-parvovirus B19 IgG patients. The prevalence of anti–parvovirus B19 IgG in multi-transfused patients was not significantly higher than in the non-transfused patients (44% *vs.* 34%, $p = 0.45$). Anti-parvovirus B19 IgG was found in 41% and 33% of female and male patients, respectively ($p = 0.58$). Increase of anti-parvovirus B19 IgG prevalence in patients aged ≥ 41 years old is shown in Table 2**.**

Parvovirus B19 DNA was found in 8 of 60 (13%) plasma samples (Table 3). Of 8 positive parvovirus B19 DNA samples, 3 were anti-parvovirus B19 IgG positive. One of 8 (13%) patients showed parvovirus B19 DNA both in plasma and PBMC samples. Of 8 DNA positive samples, 5 had history of blood transfusion. The prevalence of parvovirus B19 DNA in patients with history of blood transfusion was not significantly higher than those without blood transfusion (20% *vs.* 9%, $p = 0.2$).

The nucleotide sequences of NS1 region of parvovirus B19 DNA in 3 plasma samples of patients (codes BT002, BT056 and BT058) and the positive control were sequenced. The nucleotide sequences were compared to reference strains of Au, LaLi and V9 in GenBank (Fig. 1). All analyzed sequences showed similarity and displayed higher degree of similarity relative to the reference sequence of Au strain (99.6%) than to the reference sequence of LaLi (91.3%) and V9 strains (90.6%). The analyzed sequences showed nucleotide change at position 1931, from A to C, when compared with the sequence of Au strain. The NS1 parvovirus B19 sequences obtained from BT 002, BT 056 and BT058 and PC were submitted to GenBank under accession numbers EF590139, EF590138, EF590137, and EF590140, respectively. The sequences were aligned with those known parvovirus genotypes and subjected to phylogenetic analysis. Sequences of parvovirus B19 in 3 studied samples and PC were clus-

Table 2 Anti-parvovirus B19 IgG found in each age group of thalassemic patients

Age (years)	**No. of patients investigated**	**No. of positive anti-parvovirus B19 IgG patients** (%)
≤ 20	13	5 (38.5)
21-30	16	5 (31.3)
31-40	18	6 (33.3)
≥ 41	13	7 (53.8)

Table 3 Serological status of 8 parvovirus B19 DNA positive thalassemic patients

Patients code	**Sex, age** (years)	**Blood transfusion**	**Anti-parvovius B19 IgG**	**Anti-parvovirus B19 IgM**	**Parvovius B19 DNA detected in**	
					Plasma	**PBMC**
BT002	Female, 15	Yes	negative	not tested	positive	negative
BT006	Male, 34	Yes	negative	not tested	positive	negative
BT011	Female, 20	Yes	positive	negative	positive	negative
BT037	Female, 39	No	positive	negative	positive	negative
BT044	Male, 28	Yes	negative	not tested	positive	negative
BT053	Female, 53	Yes	positive	negative	positive	positive
BT056	Male, 31	No	negative	not tested	positive	negative
BT058	Female, 20	No	negative	not tested	positive	negative

tered in genotype 1 (Fig. 2).

DISCUSSION

Parvovirus B19 infection in humans is distributed worldwide. Seroepidemiologic studies of several countries showed that prevalence of parvovirus B19 infection varied among countries and populations, and increased with age.[12] The prevalence of parvovirus B19 infection demonstrated by anti-parvovirus B19 IgG found in thalassemic patients is similar to those cases of chronic hemolytic anemia in Brazil.[13] High seroprevalence of anti-parvovirus B19 IgG of patients with hematological disorders, including thalassemia disease were reported in Taiwan and Sweden.[14,15] In our study, acute and persistent chronic parvovirus B19 infection occurred in thalassemic patients. During acute infection, viremia and hematological changes occurred.[16] IgM antibody appears during the second week and persists for months. IgG antibody appears on the third week and persists for years. Persons who cannot control the infection can develop a chronic block in red blood cell production, causing severe persistent anemia. Parvovirus B19 DNA are found in blood of patients with acute and chronic infection. Prevalence of parvovirus B19 DNA found in plasma of thalassemic patients in our study was higher when compared with α and β thalassemic patients in Hong Kong.[17] Parvovirus B19 DNA were found in both plasma and PBMC sample only in one patient, suggesting a low number of parvovirus B19 DNA copies might exist in PBMC. In fact, it has been suggested that parvovirus B19 DNA persists in PBMC after the disappearance of parvovirus B19 DNA and specific IgM from serum.[18] Chronic parvovirus B19 infection was demonstrated by the presence of parvovirus B19 DNA and anti-parvovirus B19 IgG in patients' plasma, while lacking of anti-parvovirus B19 IgM. Thalassemic patients who had parvovirus B19 DNA in their blood plasma but had no anti-parvovirus B19 IgG might be in an early phase of acute parvovirus

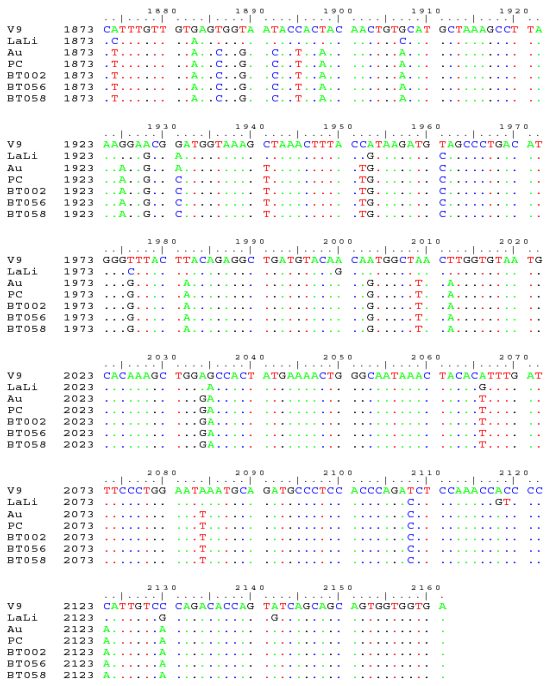

Fig. 1 Nucleotide sequences of parvovirus B19 NS1 region of positive control (PC) and thalassemic patients (BT 002, BT 056 and BT 058) compared to reference sequences of Au, LaLi and V9 strains (GenBank accession number M13178, AY044266 and AX003421, respectively). Nucleotide sequences identical to those strains are indicated with a dot.

B19 infection.[3]

In general, transmission of parvovirus B19 is via the respiratory route. Parvovirus B19 infection may be transmitted by blood transfusion. The transfused thalassemic patients may receive packed red cells containing parvovirus B19.[6] Increased seropositive rates of parvovirus infections associated with blood transfusion are reported in Taiwan and in Hong Kong.[19] However, the prevalence of anti–parvovirus B19 IgG and DNA in multi-transfused thalassemic patients was not higher than in the non-transfused patients in our study. This might be due to low prevalence of parvovirus B19 infection among the blood donor group. Prevalence of parvovirus B19 infection of the general populations in Thailand including adult blood donors was low compared to Western country.[20] The frequency of parvovirus B19 viremia in voluntary blood donors has been estimated to be low. In addition, there was no evidence of virus transmission from blood donor who had low levels of parvovirus B19 DNA.[21] However, study of parvovirus B19 DNA quantity in plasma of thalassemic patients may show significant role of multiblood transfusions of parovirus infection.

The sequence of parvovirus B19 found in the thalassemic group was genotype 1. Genotype 1 and 2 are parvovirus B19 when isolated from serum of patients during aplastic anemia.[22,23] NS1 gene is highly conserved among parvovirus B19 species. The nucleotide sequence of three isolates displayed a higher degree of similarity relative to the reference sequence of Au strain. There was one common nucleotide change at position 1931, from A to C. The genetic diversity among parvovirus B19 isolates has been reported to be very low, with less than 2% nucleotide divergence in the whole genome.[22] Divergence has been reported as low as 0% to 0.6% base substitutions among parvovirus B19 isolates obtained from a single community-wide outbreak and only slightly greater among isolates obtained from distinct epidemiological settings and geographical areas, ranging between 0.5 and 4.8% for the most distant isolates.[24] However, some isolates obtained from patients with persistent infection exhibit a higher degree of variability in some parts of the genome.[25] No correlation between specific disease symptoms and parvovirus B19 genotype has been observed. In our study the three NS1 sequences of parvovirus B19 in Thalassemic group were in genotype 1 which their nucleotide sequences were similar and displayed a higher degree of similarity relative to the reference sequence of Au strain.

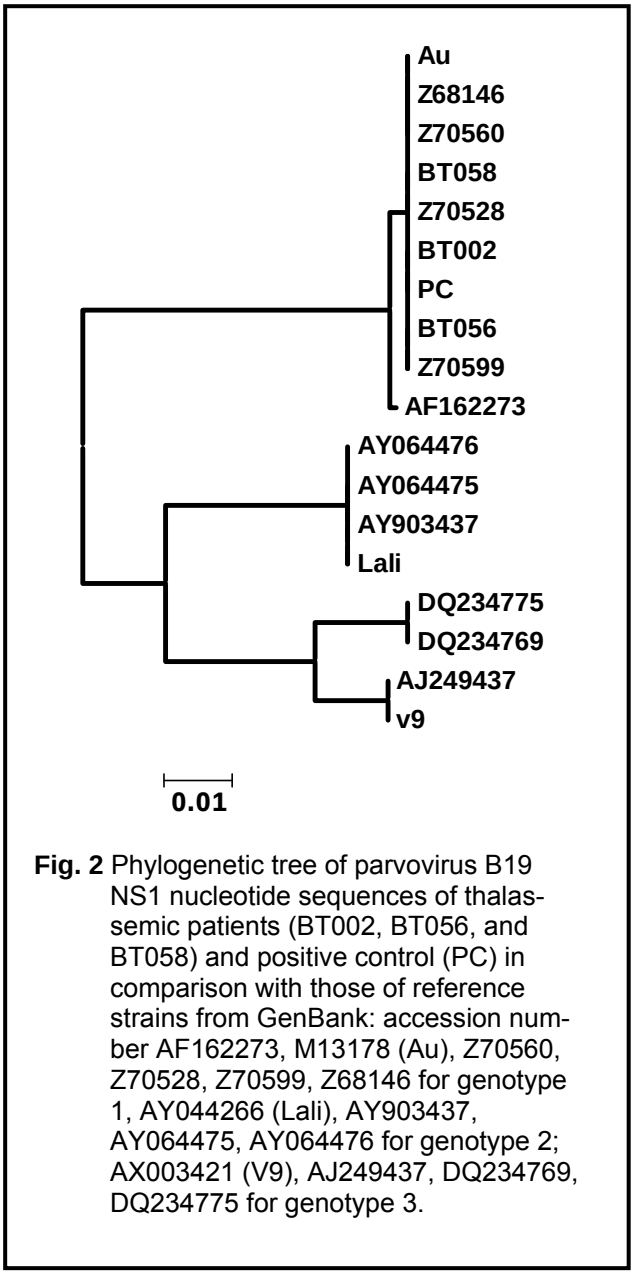

Fig. 2 Phylogenetic tree of parvovirus B19 NS1 nucleotide sequences of thalassemic patients (BT002, BT056, and BT058) and positive control (PC) in comparison with those of reference strains from GenBank: accession number AF162273, M13178 (Au), Z70560, Z70528, Z70599, Z68146 for genotype 1, AY044266 (Lali), AY903437, AY064475, AY064476 for genotype 2; AX003421 (V9), AJ249437, DQ234769, DQ234775 for genotype 3.

ACKNOWLEDGEMENTS

This work was supported by Japanese Foundation for AIDS Prevention (JFAP). The authors acknowledge the kind editorial assistance of Mr. Peter Hall at the Language Centre, Mahidol University, Bangkok, Thailand.

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