ORIGINAL ARTICLE

Seroprevalence of Human Parvovirus B19 Infection in an Urban Population in Malaysia

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Summary

A seroepidemiological study carried out on 800 stored serum samples collected between January 1999 to December 2000 derived from an urban population in Malaysia showed that the overall seropositive rate of human parvovirus B19 infection was 37.6%, with an overall geometric mean titre (GMT) of 18.3 IU. The seropositive rates of B19 among the male and female populations were 39.0% and 36.3% respectively. The seropositve rates among the racial groups were 37.2%, 38.2%, 38.1% and 29.4% respectively for the Malays, Chinese, Indians and other races. There was no statistical significant gender and racial differences in the B19 seropositive rates.

When compared with the seroprevalence of B19 infection in other Asian countries, the seropositive rate of B19 in Malaysia was low in the younger age group and increased steadily with age. The unusual finding in this study was the presence of a high seropositive rate in those between six months to five years of age, especially in children in the one year old age group.

Key Words: Parvovirus B19, Seroprevalence, Malaysian population

Introduction

Parvoviruses are amongst the smallest of the animal DNA viruses. The virion has a diameter of 18 to 26 nm and is composed entirely of protein and DNA. The parvovirus virion has a relatively simple structure composed of only three proteins and a linear, single-strand DNA molecule of about 5,000 nucleotides. The capsid enclosing the viral genome is composed of 60 protein subunits, consisting primarily of VP2 (major structural protein, composing 90% of the capsid)[1]. The main structure motif of VP2 is an 8-stranded β-barrel protein and contains the dominant antigenic determinants. Parvoviruses replicate in the nuclei of infected cells. Of all the DNA viruses, they seem to be most dependent on cellular function for their replication. The autonomous parvoviruses require the cell to go through S phase in order to replicate[2].

Human parvovirus B19 was discovered by Cossart *et al.* in 1975, when they were developing the laboratory assays for hepatitis B[3]. The virus's peculiar name, incidentally, is derived from the blood bank code of one of the viraemic blood donors whose serum was number 19 in row B of the panel. B19 is the only

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parvovirus known to be pathogenic in man. It has a remarkable narrow cellular and tissue tropism, and productive infection has only been demonstrated in human erythroid progenitor cells. Viral tropism is the result of expression of its cellular receptor, erythrocyte P antigen or globoside, in only a few human tissues[4].

The pattern of disease that follows parvovirus infection is the result of a balance between virus, marrow target cells and the immune response. Hence, human diseases associated with parvovirus B19 present with a wide range of clinical manifestations such as erythema infectiosum in children[5], acute arthritis in adults[6,7], and aplastic crisis in patients with chronic haemolytic anemia[8-11]. Parvovirus B19 may also cause chronic infection in immunocompromised patients leading to persistent anemia[12]. The virus has also been implicated as a cause of hydrops fetalis and foetal loss during pregnancy[13].

Parvovirus B19 is a common infection in humans. Specific IgG antibody which appears in the first two weeks after infection and persists for life, is the most convenient marker of past exposure. Serosurveys for B19 antibodies have been reported for Japan[14], USA[15], West Germany[16], England and Wales[17], Brazil[18], Hong Kong[19] and Taiwan[20]. Antibody prevalence ranged from 20% to 80%, depending on the simultaneous occurrence of B19 outbreaks during the study. In western countries, about 50% of adults have IgG antibody to B19 and the proportion increases to more than 90% in the elderly. An annual seroconversion rate of 1.5% was estimated from studies of serial samples from women of childbearing age. Thus, most individuals acquire immunity during childhood but susceptibility continues for others throughout adult life. However, in Asia, lower prevalence rate was recorded in young adults (Taiwan, 23.5%; Japan, <20% and Hong Kong, 9.5%) which is different from those in the western countries. The prevalence of B19 virus infection and its associated risk factors in Malaysia have never been reported. We undertake this study to determine the seroepidemiology of B19 virus infection in an urban population in Malaysia.

Materials and Methods

Serum samples kept frozen at -20°C collected between January 1999 to December 2000 from the following categories of population were included in the serosurvey:

i) healthy donors of blood platelets and potential organ donors (inclusive of bone marrow);
ii) undergraduate students seen in the student health clinic of the University of Malaya, Kuala Lumpur; and
iii) patients treated in the University Hospital, Kuala Lumpur for various surgical, obstetrical, medical, opthalmological and ENT conditions.

Patients on any form of immunosuppressive therapy or with any form of neoplastic or autoimmune disorders were excluded to ensure specificity of the test. The study population, selected randomly and consisted of various races, was divided into eight age groups. Each group consisted of 50 males and 50 females. A total of 800 serum samples were screened for the presence of parvovirus B19 specific IgG.

The Biotrin (Dublin, Ireland) Parvovirus B19 IgG Enzyme-linked Immunoassay (ELISA) Kit was used for this seroprevalence study. The test system is based on a sandwich enzyme immunoassay for the detection of human IgG class antibodies that will bind to purified parvovirus B19 recombinant VP 2 protein coated on the microtitre wells, followed by detection using peroxidase-labelled rabbit anti-human IgG and enzyme substrate. The procedures for performing the test and quality control were followed strictly according to the attached protocol in the commercial kit. Briefly, all the reagents and sera for testing were first allowed to equilibrate to room temperature. The wash solution and sample diluent were prepared as specified in the protocol. The test sera were

diluted a hundred fold (i.e. 1:100 dilution) using the sample diluent together with the high, low and negative standard controls supplied with the kit and five standard serum samples of known concentration of parvovirus B19 specific IgG in international units (IU) supplied by the company. One hundred microlitre of each diluted sample and control was added to the respective wells of the microtitre plate with plain sample diluent added into the first well of the plate as blank control. After incubation at room temperature for an hour, the wells were washed four times with wash solution using a semi-automated ELISA washer (Dynex AM60, USA), after which the plate was tapped against an absorbent paper towel to drain away remaining wash solution. One hundred microlitre of diluted peroxidase-labelled rabbit anti-human IgG was then added to each well. After 30 minutes of incubation at room temperature, the process of washing was repeated as above and 100µl of substrate (3,3',5,5'-tetramethyl benzidine) solution was subsequently added to each well. After 10 minutes of incubation for the colour reaction to develop, 100µl of stop solution was added to each well and the absorbance value for each was immediately read using a microplate ELISA reader (Dynatech MR5000, USA). The validity of each test run and the cut-off value for determining the seropositivity of each serum sample for the presence of parvovirus B19 specific IgG was calculated based on the guideline stated in the protocol. A standard graph was plotted using the derived absorbance values of the five serum samples of known concentration. The concentration of parvovirus B19 specific IgG of each positive sample in term of IU was determined using the plotted graph.

Statistical analysis was performed using the Epi Info 6, a word processing, database and statistical programme for public health, Centers for Disease Control and Prevention (CDC), USA and SPSS version 7. The results of the study were subjected to chi-square test and log regression test for any statistical significant association. A p-value of 0.05 or less was taken as the level of significance association for each ordinal variable with the relevant adjusting variables.

Results

Of the 800 serum samples from all age groups tested, 301 samples were positive for parvovirus B19 specific IgG, giving an overall seropositive rate of 37.6%. The seropositive rates for males and females were 39.0% (156/400) and 36.3% (145/400) respectively (Table I). There was no significant gender difference in seropositive rate (x^2 = 0.53, p = 0.4655). The overall B19 seropositive rates among Malays, Chinese, Indians and other races were 37.2% (106/285). 38.2% (129/338), 38.1% (61/160) and 29.4% (5/17) respectively (Table I). There was also no significant race difference in the B19 seropositive rate (x^2 = 0.57, df = 3, p = 0.9032).

Table I
The Seropositive Rates of Human Parvovirus B19 by Gender and Race

Variable	Group	No. tested	No. positive (%)	X^2	p-value
Gender	Male	400	156 (39.0)		
	Female	400	145 (36.3)		
	Total	800	301 (37.6)	0.53	0.4655
Race	Malays	285	106 (37.2)		
	Chinese	338	129 (38.2)		
	Indians	160	61 (38.1)		
	Others	17	5 (29.4)		
	Total	800	301 (37.6)	0.57	0.9032

Table II
The Seropositive Rates of Human Parvovirus B19 by Age-group

Age-group	Male		Female		Total		X^2	p-value
(year)	No. tested	No. +ve (%)*	No. tested	No. +ve (%)	No. tested	No. +ve (%)		
>0.5 - 5	50	26 (52)	50	26 (52)	100	52 (52)	0.04	0.8414
6 - 10	50	12 (24)	50	7 (14)	100	19 (19)	1.04	0.3079
11 - 15	50	6 (12)	50	5 (10)	100	11 (11)	0.00	1.0000
16 - 20	50	12 (24)	50	11 (22)	100	23 (23)	0.00	1.0000
21 - 30	50	16 (32)	50	16 (32)	100	32 (32)	0.05	0.8303
31 - 40	50	22 (44)	50	21 (42)	100	43 (43)	0.00	1.0000
41 - 50	50	36 (72)	50	30 (60)	100	66 (66)	1.11	0.2912
51 and above	50	26 (52)	50	29 (58)	100	55 (55)	0.16	0.6877

No. +ve (%) = Number of positive (percent positive)*

The seropositive rates for each of the eight age groups are shown in Table II. The seropositive rates was high (52%) in the age-group six months to five years, decreased quite drastically to 11% in the age-group 11 to 15 years before gradually increasing at the rate of 0.9% to 2.3% per year to reach the highest seroprevalence rate of 66% in the age-group 41 to 50 years, then declined to 55% for the age-group 51 years and above (81 years old was the upper limit of age). There was a statistical significant difference in the seropositive rate among the different age-groups in this study (x^2 = 112.66, df = 7, p < 0.0001). As with the finding in the overall seropositive rate among different sexes, there was no significant gender difference in the seropositive rate within each age-group (Table II).

The overall geometric mean titre (GMT) of parvovirus B19 IgG antibody was 18.3 IU/ml. The geometric mean titre of B19 IgG antibody level with respect to gender, race and age-group are shown in Table III. The GMT was lower at the extreme age-groups (9.4 IU and 16.5 IU) and highest (28.9 IU) in the age-group 16 to 20 years old (Figure 1). Statistically, there was a significant difference in geometric mean antibody titre with respect to different age groups with a quadratic pattern of distribution (R = 0.287, p < 0.0001).

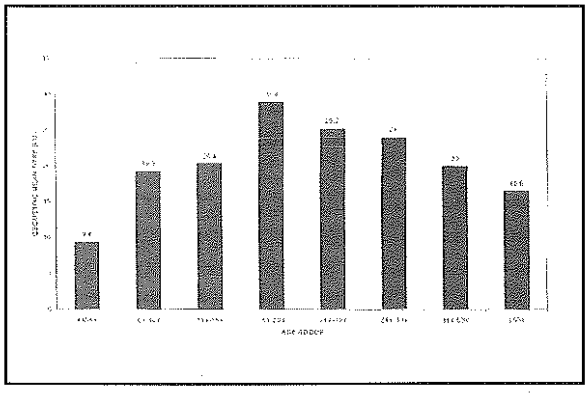

Fig. 1: The geometric mean titre of human parvovirus B19 IgG antibody by age-group.

Further analysis of the age-specific seroprevalence rate by year and the GMT between the two age-groups, the six months and above to five years and the 6 to 10 years, showed that the seropositive rate of B19 was highest in the one year old category (71.4%) whereas the GMT was highest in the 8 year old category (37.4IU/ml) (Table IV). In the analysis of this subset data, there was a statistical significant

Table III
Geometric Mean Titre (GMT) of Human Parvovirus B19 IgG Antibodies by Gender, Race and Age-group

Variable	Group	GMT (IU)*
Gender	Male	17.3
	Female	19.4
Race	Malays	17.5
	Chinese	15.9
	Indians	26.3
	Others	17.8
Age-group (year)	>0.5 - 5	9.4
	6 - 10	19.3
	11 - 15	20.4
	16 - 20	28.9
	21 - 30	25.2
	31 - 40	24.0
	41 - 50	20.1
	51 and above	16.5

**IU = International unit*

Table IV
The Seropositive Rates of Human Parvovirus B19 by Year for the Age Groups 6 Months to 5 years and 6 to 10 years

Age (year)	No. tested	No. positive (%)	GMT (IU)*
>0.5 to <1	10	7 (70.0)	5.4
1	28	20 (71.4)	8.9
2	25	13 (52.0)	14.9
3	17	7 (41.2)	6.1
4	8	3 (37.5)	17.7
5	12	2 (16.7)	9.1
6	17	3 (17.6)	37.2
7	19	2 (10.5)	13.3
8	18	2 (11.1)	37.4
9	26	6 (23.1)	9.3
10	20	6 (30.0)	26.7

**GMT = geometric mean titre, IU = International unit*

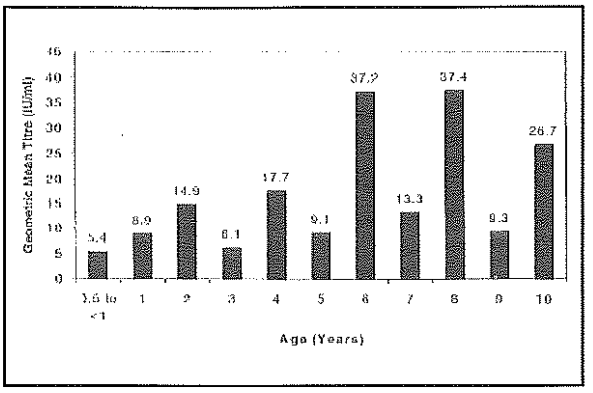

Fig. 2: Geometric mean titres of human parvovirus B19 IgG from 6 months to 10 years.

difference in the seropositive rate among the different ages from 1 to 10 years old (x^2 = 36.58, df = 9, p < 0.0001). There was also significant difference in the GMT with respect to different ages with a linear pattern of distribution (R = 0.121, p = 0.0364) (Figure 2).

Discussion

Human parvovirus B19 was first identified from serum samples that were assayed for hepatitis B virus. Subsequently it has been identified to be associated with a number of diseases such as erythema infectiosum, aplastic crisis in patients with chronic haemolytic anemia, stillbirths, arthritis and chronic anemia due to persistent infection in immunosuppressed patients[5-12]. It has also been associated with contamination of blood products[21].

B19 infection has been reported in many countries around the world. Studies in Western countries showed the infection occurred earlier in life than those in Asian countries. In England and Wales, the seropositive rate was 5 - 15% in those between one to five years and reached 60% in the 16 to 20 years age group[17]. In Japan and Taiwan,

the seropositive rate was also low at the young age group of less than 15 years and only reached about 20% and 30% for age groups under 20 and under 30 years respectively[14,20]. The findings in this study showed that the seropositive rate of B19 for the age groups under 20 and under 30 years in Malaysia is in concordance with the findings in Japan and Taiwan. The seropositive rates for the age groups 16 - 20 years and 21 - 30 years in this study are 23% and 32% respectively. After the age of 20, the seropositive rate increased in the range of 1.1 to 2.3 % per year till the highest rate of 66% in the age group 41 to 50 years, then declined to 55% in the age group 51 years and above. This increasing seropositive rate with increasing age is consistent with those reported in other countries[16-20, 22-25].

The unusual finding in this study, which is not in concordance with the findings in all the other seroprevalence studies, is the presence of high seropositive rate in the age group of six months to five years (52%). Traditionally, it is an accepted fact that maternal IgG antibodies should have reduced to an undetectable level by six months of age. However, it has been shown that certain sensitive ELISA kit can even detect the maternal IgG antibodies beyond the expected six months of age. The possibility that the high B19 seropositive rate in this age group was due to the presence of maternal IgG, especially with the finding of low geometric mean titre in this age group, has not been excluded. To determine the contributing role of maternal IgG to the finding of high seropositive rate in the 6 months to 5 years age group, a subset data consisting of sera from this age group together with the 6 to 10 years age group was re-analysed separately with respect to the seropositive rate per year of age as shown in Table IV. Though the sample size was small in the subset data, the seropositive rate was still high in the age one and two years (71.4% and 52.0% respectively). The geometric mean titres (GMT) in these two ages were also low (8.9 IU and 14.9 IU). Thus, the low GMT in this very young age group was probably due to the relatively immature immune system not being able to mount an aggressive immune response. Dwindling immune response in the elderly, together with the natural decay of antibodies with time, probably account for the lower GMT seen in the age group 51 years and above. This probably accounts for the lower seropositive rate seen in this age group as the antibodies could have decreased to a level beneath the detection limit of the test system used here.

In conclusion, the findings in this study confirmed the presence of human parvovirus B19 in Malaysia and the seroprevalence rate is comparable to the rates found in Asian countries except for the first three years of life. The study also showed that a proportion of adults of childbearing age was still susceptible to the virus and serological data also showed evidence of infection occurring in this age group. It will be of interest for the obstetricians in this country to note the role of B19 in relation to the extent of stillbirths and hydrops foetalis and to if there is any need for a vaccine to reduce foetal wastage.

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