# SEROEPIDEMIOLOGY OF HUMAN HERPESVIRUS 6 IN A POPULATION SEEN IN THE UNIVERSITY HOSPITAL, KUALA LUMPUR, MALAYSIA

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**Abstract.** Sera from healthy donors and patients stored over a period of 2 years, aged 1 to 83 years, were examined for reactivity to human herpes virus 6 (HHV-6) by the standard indirect immunofluorescence assay (IFA). Of the 600 serum specimens screened, 502 showed positive reactivity to HHV-6. This gives an overall seropositive rate of 83.7 %. There is no significant difference in the overall positive rate between the ethnic groups (Chinese, Malays, Indians) ($\chi^2 = 0.35$ df = 2 $p > 0.05$). However, there is significant difference in the positive rates at the extreme age groups of 1 year as well as 61 years and above. From birth up to below 1 year of age, the seroprevalence rate was 82%. At one year of age the positive rate decreased to 66% before gradually rising so that the percentage seropositivity of 6 to 10 years old becomes similar to that in older children and adults (11 to 40 years). The positive rate then starts to decline after 40 years of age. Using a standardized scoring system, the corresponding antibody titer was found to be high in the very young population and starts to decline after the age of 15 years. This suggests that in our population group, primary infection occurs mainly in the pediatric age group. It also accounts for the low positive rate in the age group of 61 years and above, as by then the titer had fallen to the level below the detection limits of the assay system.

## INTRODUCTION

Human herpesvirus - 6 (HHV-6) was first isolated from patients with various lymphoproliferative and immunosuppressive disorders (Salahuddin *et al*, 1986). Since then, extensive studies have been carried out in many laboratories using a variety of techniques to investigate its role inhuman diseases. Seroepidemiological studies of HHV-6 have utilized an indirect immunofluorescence assay (IFA) as the 'gold standard' since the first HHV-6 serology reports used an assay based on the techniques applied to the study of EBV (Henle and Henle, 1966). A number of studies have shown that there is no cross - reactivity with other herpesviruses in the HHV-6 IFA, in that the HSB-2 cell line used for the HHV-6 assay is free of EBV as well as other human herpesviruses and retroviruses (Ablashi *et al*, 1988).

Though IFA is not as sensitive as other assays, such as the enzyme - linked immunosorbent assay (ELISA) (Halprin *et al*, 1986; Saxinger *et al*, 1988), it has a particular advantage in the area of specificity. An experienced reader using appropriate positive and negative controls can readily determine if the pattern of cells reacting with the test serum is comparable to the pattern of cells known to be infected with HHV-6. In this report, we have determined the age related, sex related and race related seroprevalence of HHV-6 antibodies in a population seen in the University Hospital, Kuala Lumpur, Malaysia using a commercially available IFA.

## MATERIALS AND METHODS

Serum frozen at -20°C kept in the Department of Medical Microbiology, Faculty of Medicine, University of Malaya from January 1993 to December 1994 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.

iii) Patients treated in the University Hospital, Kuala Lumpur for various surgical, obstetrical, medical, opthalmological and ENT conditions.

iv) Patients on any form of immunosuppressive therapies 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 12 age groups. Each group consisted of 25 males and 25 females. A total of 600 serum samples were screened for antibody against HHV-6.

The Stellar Bio-System's Indirect Immunofluorescence Assay (IFA) for HHV-6 IgG antibody was used for this seroprevalence study. Briefly, each teflon coated slide consisted of 10 wells. Each well contained fixed HSB-2 cells infected by group A - GS strain of HHV-6 (broadly reacting with group A and group B strains) and non - infected cells as negative control. 5 µl of serum was diluted with 95 µl of phosphate buffered saline (PBS) (1 : 20 dilution). 50 sera were tested in each batch. Each batch test run included one positive control, one negative control and one blank (PBS) control. 20 µl of the diluted serum sample was applied to each well. The slides with the applied test sera were incubated in a moist chamber for 30 minutes at 37°C. They were initially rinsed off with PBS before being subsequently soaked for a further 10 minutes in PBS solution. The slide was blotted dry and probed with 20 µl of fluorescein conjugated goat anti - human IgG with counter stain. The slides were then further incubated for 30 minutes at 37°C in a moist chamber. The same process of washing and drying was again carried out after incubation. Following this, the slides were mounted with the supplied mounting fluid and examined under U-V fluorescence microscope using 400 x magnification. The degree of positivity for each test was assigned 1 + to 4+ using the following criteria:

1+ : at least 5 infected cells showed discernible fluorescence in the test well (1 : 20 to less than 1 : 40 dilution).

2+ : more than 5 infected cell had fairly bright fluorescence (1 : 80 to 1 : 160 dilution).

3+ : brilliant fluorescence but did not cover the whole of the infected cells (*ie* counter stain on the infected cells can still be easily seen) (1 : 160 to 1 : 320 dilution).

4+ : brilliant fluorescence (equivalent to strong positive control, 1 : 640 dilution and above).

The grading was to reflect the antibody titer of each test serum. Semi-quantitatively, 4+ degree of positivity reflects a high antibody titer whereas 1+ degree of positivity reflects a low antibody titer. The results of the test obtained were subjected to the chi-square test for staistical significance.

## RESULTS

Of 600 serum samples from all age groups screened at 1 : 20 dilution, 502 samples were positive for HHV-6, giving an overall seropositive rate of 83.7%. The data obtained was further analyzed according to ethnic group, sex, age groups and degree of positivity using the scoring criteria set to reflect titer at various age groups. Table 1 shows the HHV-6 seropositive rate according to various ethnic groups from all age groups. There was no significant difference in the seropositive rate among the ethnic groups (Chinese, Malays and Indians) ($\chi^2 = 0.35$ df = 2 $p > 0.05$). The 100% positive rate noted in the other races may not reflect the actual positive rate as the number (15) tested in this category was too small to be of statistical significance (Fisher's test $p > 0.05$). There was also no significant difference in the positive rates between the

Table 1

The seropositive rates against HHV-6 based on ethnicity.

| Race | No. of sera tested | No. of positive sera | Positive rate (%) |
|---|---|---|---|
| Chinese | 247 | 203 | 82.2 |
| Malays | 226 | 190 | 84.1 |
| Indians | 112 | 94 | 83.9 |
| Others | 15 | 15 | 100 |

sexes ($\chi^2 = 0.60$ $p > 0.05$) though there was slightly higher positive rate in the females (85.0%) as compared to that of the males (82.3%) (Table 2). The lowest positive rate (66%) was noted in the age group of one year and highest positive rate (92%) in the age group of 31 to 35 years (Table 3, Fig 1).

Fig 2 shows the comparative degree of positivity as reflected by our scoring system for the different age groups. In the less than one year age group, the majority of the seropositive specimens (51.2%) were seen to be having low score reactive level. This could be attributed to the presence of maternal antibody against HHV-6. The proportion of sera having 4+ degree of positivity were seen mainly in the 1 to 10 years old suggesting that, may be in our population, this is the age group that accounts for most of the primary infections. These findings correlate with the epidemiological observations of the age of occurrence of exanthem subitum (Yamanishi *et al*, 1988; Koudo *et al*, 1990).

As seen in Fig 2 and Table 4, the antibody titers start to decline with increasing age with zero percent having the 4+ degree of positivity for the age group of 31 to 35 years old and above. The antibody titers with respect to various age groups in percentage are portrayed in Fig 3.

## DISCUSSION

The results of our study showed that HHV-6 infection is ubiquitous in our population in that

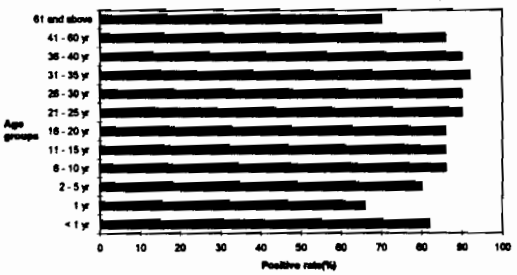


Fig 1–Seroprevalence of HHV-6 by age group.

Table 2

The seropositive rates against HHV-6 based on sex.

| Sex | No. of sera tested | No. of positive sera | Positive rate (%) |
|---|---|---|---|
| Male | 300 | 247 | 82.3 |
| Female | 300 | 255 | 85.0 |

Table 3

Seroprevalence of HHV-6 by age groups.

| Age group (year) | No. of serum tested | No. of positive | Positive rate (%) |
|---|---|---|---|
| < 1 | 50 | 41 | 82 |
| 1 | 50 | 33 | 66 |
| 2-5 | 50 | 40 | 80 |
| 6-10 | 50 | 43 | 86 |
| 11-15 | 50 | 43 | 86 |
| 16-20 | 50 | 43 | 86 |
| 21-25 | 50 | 45 | 90 |
| 26-30 | 50 | 45 | 90 |
| 31-35 | 50 | 46 | 92 |
| 36-40 | 50 | 45 | 90 |
| 41-60 | 50 | 43 | 86 |
| 61 and above | 50 | 35 | 70 |
| Total | 600 | 502 | 83.7 |

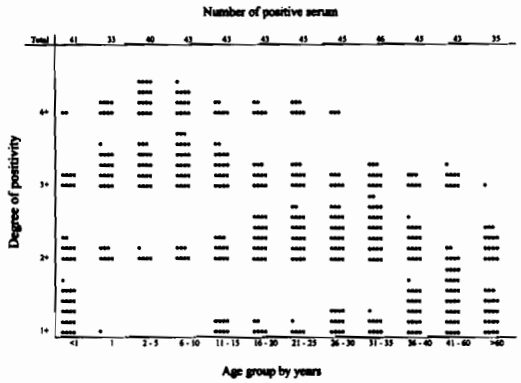


Fig 2–Degree of seropositivity against HHV-6 by age groups.

92% of the population have already seroconverted by 35 years of age. The high positive rate in our study population is in accordance with the seroprevalence rate of HHV-6 in the Caucasian and African populations (Levy *et al*, 1990b). This also corresponds with the studies carried out in Japan (Okuno *et al*, 1989). The high positive rate in our present study was not in accordance with the previous study carried out by Yadav *et al* (1990). This discrepancy could be attributed to the difference in the antigen used in the assay system. However, like the previous study, our study also shows no significant difference in the seropositive rate among the races and sex.

Futher analysis of the results in this study (Table 3 and Fig 2), shows that there is high maternal

Table 4

Percentage of the degree of the seropositivity against HHV-6 by age group.

| Age group (year) | Percentage of the degree seropositivity | | | |
|---|---|---|---|---|
| | 4+ | 3+ | 2+ | 1+ |
| < 1 | 4.9 | 19.5 | 24.4 | 51.2 |
| 1 | 24.2 | 51.5 | 21.2 | 3.0 |
| 2-5 | 40.0 | 47.5 | 12.5 | 0 |
| 6-10 | 30.2 | 53.5 | 16.3 | 0 |
| 11-15 | 14.0 | 41.9 | 25.6 | 18.6 |
| 16-20 | 14.0 | 25.6 | 46.5 | 13.9 |
| 21-25 | 15.6 | 24.4 | 48.9 | 11.1 |
| 26-30 | 6.7 | 15.6 | 51.1 | 26.7 |
| 31-35 | 0 | 23.9 | 56.5 | 19.6 |
| 36-40 | 0 | 15.6 | 37.8 | 46.7 |
| 41-60 | 0 | 20.9 | 14.0 | 61.1 |
| 61 and above | 0 | 2.8 | 44.4 | 52.8 |

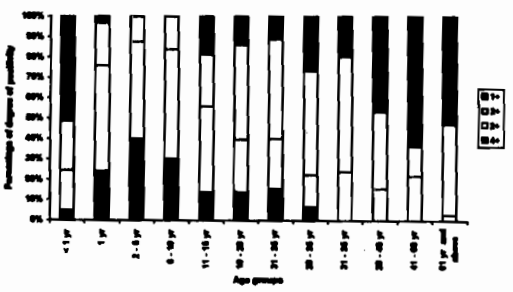

Fig 3–Percentage of the degree of seropositivity against HHV-6 by age group.

transplacental transfer of antibody against HHV-6 and that primary infection occurs early in life. This is in agreement to what was found in other epidemiological reports (Briggs *et al*, 1988; Knowles and Gardner, 1988; Levy *et al*, 1990b, Yanagi *et al*, 1990). By six to ten years of age, 86% of the children has already been exposed to the virus. With the increasing of age, especially after 30 years of age, the antibody titer against HHV-6 starts to decline. This may account for the low seroprevalence rate of the older age group (61 years old and above) as by that time, the antibody titer has declined to the level beyond the detectable limit (1 :

20 dilution) of the assay used in this study.

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