# DISCUSSION

## 4.1. EPSTEIN-BARR VIRUS

### 4.1a. EBV Seroconversion Profile

From the results obtained from immunofluorescence test for IgG-VCA, 53.3% of the samples in the 0-1 year old had IgG to VCA (Table 4), though many of the positive sera had low antibody titres and GMT for this age group was the lowest among the 12 age groups (Table 5). This study also showed 98% of newborns had IgG-VCA (Table 33) and from the results of a small sample (Table 34) it is likely that transplacental maternal antibodies accounted for the high percentage of IgG-VCA in newborns. In the second half of the first year of life, maternal antibodies waned and children began to get infected and become seroconverted. This caused 95% of children to be seroconverted by 4 years of age. This profile is in agreement with earlier reports on Malaysian Children (Tan & Henle, 1972; Deveraj *et al.*, 1987; Yadav *et al.*, 1990).

IgG titres are high after recent infections and this accounts for the high titres of IgG-VCA in this study. In healthy adults, IgG-VCA is seldom detectable at serum dilution of >1:80.

EBV seroconversion in Malaysia occurs early in life and 100% of children have seroconverted by 9 years of age. This result demonstrates that the apparent improvement of socio-economic conditions in Malaysia has not affected the EBV seroconversion profile and infectious mononucleosis is not and will not be a concern in Malaysia in the near future.

### 4.1b. EBV Seroconversion Patterns in Different Regions

At the beginning of this study, we attempted to divide the sources of serum samples into rural and urban areas, but it was not feasible since sera were collected from general (urban) and district (rural) hospitals in the different states. Instead, the data was divided into 4 regions (Table 8), the seroconversion profiles and the percentages of EBV seropositive sera (Table 9) were found to be similar in the different regions. In conclusion, the seroconversion profiles are similar in different regions of Malaysia.

### 4.1c. EBV Seroconversion Patterns in Males and Females

Results of this study demonstrated that seroprevalence of EBV is independent of sex (Table 11). Moreover, male and female babies and children were equally susceptible to EBV infection (Table 10 & 33).

### 4.1d. EBV Seroconversion Patterns in Different Ethnics Groups

The 3 main races in Malaysia are Malay, Chinese and Indian. There are also indigenous tribes living in Malaysia, mainly in East Malaysia. Though the nation has a cosmopolitan society with shared practices, each ethnic group retains some practices peculiar to that group that may contribute to a different seroconversion profile. In this study, no ethnic variation on EBV seropositive frequency was seen among the races (Table 14).

When GMT of different ethnic groups were compared, the Chinese had statistically higher GMT than other races. However, this current study is unable to give reason(s) for the difference.

### 4.2. ELISA IgG-VCA

ELISA is known to be a very sensitive test, preferred over the IFA if sensitivity, quantitation and speed are the concerns. However, the phenomenon of nonspecific binding resulting in false positives is a big problem. The value of a ELISA kit is highly dependent on the antigens coated onto the ELISA wells.

The ELISA kit (PanBio, Australia) used in this study is not yet commercially available but it has been tested and preliminary result showed it to be comparable to standard IFA. The ELISA appeared to have higher sensitivity in the detection of IgG-VCA (Table 16) but the positive percentages fluctuated among the various age groups and was the lowest in the pre-adolescents, which suggest nonspecific binding and poor repeatability. The result of this study concluded that the traditional IFA remains the more reliable method, since the IFA is scored not only by the quantity of fluorescence but also by location and pattern of fluorescence that is typical of cells containing IgG-VCA.

### 4.3. HUMAN HERPESVIRUS 6

#### 4.3a. HHV-6 Seroconversion Profile

The average seroprevalence in the 900 sera tested in the present study was 90.6%. This high positivity of IgG anti-HHV-6 is in accordance with HHV-6 seroprevalence reported in many countries of the world (Table 35). In contrast, the HHV-6 antibody positivity in this study differ from a few reports. In Thailand, Balachandra *et al.* (1989) obtained a plateau positive rate of 55.6% among the adults and 52.2% positive rate in children and adolescent groups. De Freitas *et al.* (1994) found low seropositivity for IgG anti-HHV-6 among 4 isolated Amazonian Amerindian communities in Brazil, with

seropositivity ranging from 5.4% found among the Oyampi tribe to 14.9% among the Tucano tribe.

Seroprevalence data of HHV-6 in Malaysia has been controversial. Chua *et al.* (1996) screened 600 sera from people aged 1-83 years old and demonstrated HHV-6 seropositive rate was 83.7%. Although the seroprevalence in the present study is slightly higher than that reported in Chua *et al.* (1996), at 90.6% compared to 83.7%, the difference can be attributed to not including sera from adults >20 years of age in the present study. In another study, Yadav *et al.* (1991) reported 58-80% IgG-HHV-6 seropositive sera from 234 healthy sera in Malaysia. It was suggested in the paper that the low prevalence could have been due to low expression of HHV-6 antigens in the infected cells used for the study. They also suggested the low prevalence to be due to HHV-6 with different antigenic properties from the natural circulating HHV-6 being used to infect the cells used for IFA. Another group from USA (Levine *et al.*, 1992), found low seropositivity against HHV-6 in Malaysian Chinese (41.5%) and Malaysian Indians (54.2%) but high seroprevalence in the USA population (96%) and among Ghanaians (100%).

This study has detected much higher serum IgG-HHV-6 prevalence and GMT than both reports of Yadav *et al.* (1991) and Levine *et al.* (1992). The difference could be due to the improved infection efficiency of HHV-6 in the present study. Optimization of HHV-6 infection of HCBMC was carried out by Chua *et al.* (1996) and further improved in the present study. Centrifugation enhancement (Pietroboni *et al.*, 1989) was found to improve infectivity of HCBMC by HHV-6. Additionally, cord blood was obtained from careful withdrawal by using a syringe, and lymphocyte separation was done carefully

resulting in minimal contamination by red blood cell. From observation in the study, infectivity and the number of antigen positive cells are highly dependent on the condition of the isolated lymphocytes. Actively growing and healthy lymphocytes are much more amenable to HHV-6 infection and expression of HHV-6 antigens.

Table 35: Seroprevalence of IgG anti-HHV-6 in The World

| Source | Place/ country | Age range | % positive |
|---|---|---|---|
| Andre & Matz, 1988 | South-west Germany | Whole range | 70% |
| Linde *et al.*, 1988 | Sweden | 0.5 - 70 years | 85% |
| Saxinger *et al.*, 1988 | USA | 6 - 70 years | 80 - 97% |
| Okuno *et al.*, 1989 | Japan | 0 - 59 years | 79% |
| Yoshikawa *et al.*, 1989 | Japan | 2 - 27 years | 69 - 76% |
| Enders *et al.*, 1990 | Germany | 1 - 40 years | 76% |
| Levy *er al.*, 1990 | USA | 1 - 40 years | 91% |
| Yanagi *et al.*, 1990 | Japan | Whole range | 95% |
| Linhares *et al.*, 1991 | North-east Brazil | 0 - 75 years | 76.5 - 77.2% |
| Huang *et al.*, 1992 | Taiwan | Adults | 80% |
| Shanavas *et al.*, 1992 | India | Adults | 76% |
| Ward *et al.*, 1993 | United Kingdom | 60 - 179 weeks | 88% |
| | | 18 - 65 years | 98% |
| De Freitas & Linhares, 1997 | Northern Brazil | 0 - 50 years | 90% |
| Yadav et al., 1991 | | > 13 years | 58 – 80% |
| Chua et al., 1996 | Malaysia | 1 – 83 years | 83.7% |
| Present study | | 0 – 20 years | 90.6% |

**4.3b. HHV-6 Seroprevalence by Age**

From the study, HHV-6 prevalence was the lowest at the first two age groups (0-1 year old and 1-2 years old). Both age groups scored prevalence of 77.3%. In the 0-1 year group, the samples can be divided into 3 serological status groups. First, sera collected from babies up to 2 months of age. Almost all of them have maternal derived IgG against HHV-6 and protected from HHV-6 infection (Table 33). This is in agreement with other reports. For example, 87% of infants at 0-2 months of age in Japan was demonstrated to have maternal IgG (Yoshikawa *et al.*, 1989). The occurrence of maternal antibodies in 100% of infants aged 0-10 weeks was reported in the United Kingdom (Ward *et al.*, 1993).

In the second group of infants aged 3 to 5 months, the levels of maternal antibodies in the body gradually declined. HHV-6 seroprevalence was decreased from 52% to 5% from infants aged 0 to 10 months (Okuno *et al.*, 1989). Another group in Japan (Yoshikawa *et al.*, 1989) also found the lowest HHV-6 seropositive percentage of 6% in infants of 4-5 months. In a study from Taiwan (Huang *et al.*, 1992), all babies in the study lost maternally derived antibodies by 6 months.

Next, with the waning of maternal antibodies, babies aged 6-12 months started to seroconvert against HHV-6. This was shown by detection of serum IgM anti-HHV-6 among infants below 1 year in USA (Saxinger *et al.*, 1988). Farr *et al.* (1990) also detected IgM-HHV-6 mainly at 5-6 months infants.

The evidence for declining maternal antibodies and appearance of seroconversion with respect to HHV-6 has to come from analysis of sera from babies <1 year of age. Such sera are difficult to obtain, and in this study only 60 newborn sera aged 0-1 month

and 9 sera aged 5-11 months from UHKL were able to be collected (Table 32 & 34). IgG-HHV-6 was detected in 95% of newborn sera and this high percentage corresponded with that seen in young adults (Table 33). IgG-HHV-6 was not detected in 4 samples of 5, 6 and 7 months, but was detected at age 8, 10 and 11 months. In spite of the small sample size, the profile of IgG-HHV-6 fits the picture of early protection by maternal IgG till 5 months, and seroconversion begins soon after.

In the second age group (1-2 years old), many young children had seroconverted against HHV-6. This recent HHV-6 infection resulted in many sera having high IgG antibodies titres and the GMT in this age group was the highest compared to other age groups.

Starting from the third age group (2-3 years old), the prevalence of IgG-HHV-6 has reached adult level. This early life seroconversion of HHV-6 is in agreement with Okuno *et al.*, (1989); Levy *et al.*, (1990); Yanagi *et al.*, (1990); Huang *et al.*, (1992) and Ward *et al.*, (1993). This early seroconversion suggests that HHV-6 infection is transmitted horizontally, probably through saliva and oral secretion.

The levels of serum IgG-HHV-6 decrease with time after infection and caused the decrease in GMT when the age increased (Figure 24). This finding is in accordance with Yanagi *et al.*, (1990) and de Freitas & Linhares (1997).

**4.3c. HHV-6 Seroprevalence in 4 Regions of the Country**

There was no statistical difference between the number of HHV-6 seropositive sera in different regions indicating that HHV-6 infection is not influenced by differences in the regions under consideration.

**4.3d. HHV-6 Seroconversion in Male and Female**

Females in this study had higher HHV-6 seropositive percentage (92.5%) compared to males (89.5%). The IgG-HHV-6 titres in both sexes were similar though females had higher GMT compared to the males. However, these differences were not statistically significant. The finding is in agreement with other reports such as the HHV-6 seroepidemiological study in United Kingdom (Clark *et al.*, 1990) where 463 normal sera were tested, Linhares *et al.* (1991) where 434 Brazilians and 250 Japanese immigrants were tested and with Chua *et al.* (1996) where 600 Malaysian samples were tested.

**4.3e. HHV-6 Seroconversion in Different Ethnic Groups**

From the results in this study, variation in culture and way of living in the various ethnic groups do not influence the patterns of HHV-6 seroconversion or the GMT of IgG-HHV-6 significantly (Table 28 & 30). This is in agreement with Linhares *et al.* (1991) and Chua *et al.* (1996) but in contrast with Yadav *et al.* (1991), who found that HHV-6 seroprevalence in Malaysian indigenous tribes (Ibans, Kadazans, Bidayuhs and Orang Asli) to be significantly lower than Malays, Chinese and Indians respectively.

### 4.4. Comparison of EBV and HHV-6 Seroconversion Patterns

Both EBV and HHV-6 seroconversions take place during the first 10 years of life among Malaysians. Most of the young children aged 2 years and more have specific IgG antibodies against the viruses. A noted difference in the seroconversion patterns was the prevalence in the 0-1 year age group of IgG-VCA being significantly lower than IgG to HHV-6 (53.3% compared to 77.3%), indicating that HHV-6 infection occurs earlier than EBV infection. This was also reported by Farr *et al.* (1990) in Western Australia. In the report, HHV-6 seroprevalence reached 65% in babies aged 11-12 months but there were only 20% of EBV seropositive sera in the same group.

The absence of cross-reactivity between EBV and HHV-6 was shown in 123 sera (Table 31), whereby each showed seropositive reaction against only one of the viruses. Absence of serological cross-reactivity between EBV and HHV-6 has been demonstrated in many reports, for example in Andre & Matz (1988), Buchbinder *et al.* (1989) and Farr *et al.* (1990).

There was another difference in EBV and HHV-6 seroconversion profiles. Prevalence of HHV-6 never reached 100% positivity in the age groups studied. In contrast, none of the individuals more than 9 years old was seronegative for IgG-EBV. A few individuals without detectable IgG-HHV-6 could be due to not having been infected by HHV-6 or that the IgG-HHV-6 has waned to levels undetectable by IFA, such individuals are probably susceptible to HHV-6 reinfection and can be considered seronegative.