ORIGINAL ARTICLE

A Retrospective Review of Mucocutaneous Infections by Human Herpesvirus 1 and 2 in an Urban Population in Malaysia

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Summary

This is a 10-year retrospective review of mucocutaneous infection by human herpesvirus 1 (HHV1) and human herpesvirus 2 (HHV2) carried out by the virus diagnostic unit of University Malaya Medical Centre (UMMC). A total of 504 specimens from UMMC and a private clinic in the same city (KLSC) were tested; 198 samples from patients with oral lesions and 306 from patients with genital lesions. HHV1 was found to be responsible for 98.4% of oral lesions whereas HHV2 was the cause of 83.6% of all genital lesions. Detailed analysis showed no statistical difference by age group, race or gender among the patients with oral and genital lesions.

Two laboratory methods were used in this study. Of the total 504 specimens tested, 18.0% specimens were positive by direct immunofluorescence (IF), 55.0% by virus isolation and 56.5% when both methods were used in combination. Although IF can provide a more rapid diagnosis, it is, however, less sensitive and can be attributed partly to inadequate collection of specimens.

Key Words: Human herpesviruses 1 and 2, Infection, Malaysia

Introduction

The placement of viruses within the family *Herpesviridae* is based on the morphology and architecture of the virion[1]. A typical herpes virion consists of a core containing a linear double-stranded DNA, enclosed by a icosadeltahedral capsid approximately 100 - 110nm in diameter containing 162 capsomeres, an amorphous material of viral encoded proteins surrounds the capsid designated as the tegument, and an envelope containing viral glycoprotein spikes on its surface[2]. Since the discovery of human herpesvirus 6 in 1986[3], followed by human herpesvirus 7 in 1990[4], members of the Herpesviridae family have been reclassified by the International Committee on the Taxonomy of Viruses and renamed with a formal binomial nomenclature such that herpesviruses are now designated by serial Arabic number and the family or subfamily by the natural host[5]. Thus, the former Herpes simplex viruses (HSV) were renamed as human herpesvirus 1 (HHV 1, formerly known as

This article was accepted: 15 January 2002
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herpes simplex virus type I) and human herpesvirus 2 (HHV 2, formerly referred to herpes simplex virus type II). Both are members of the family *Herpesviridae*, subfamily *Alphaherpesvirinae* and genus *Simplexvirus*[1]. They are identical morphologically under electron microscope and their genomes show considerable homology but are biologically and antigenically distinct from each other[6-8]. Their antigenic variation was first demonstrated in the early 1960s[9], and subsequently the two distinct serotypes were established by serum neutralization test[10]. Presently, the two serotypes can be easily differentiated by type-specific monoclonal antibodies and by a number of molecular techniques without resorting to the cumbersome neutralization test[11-14]. Both HHV 1 and HHV 2 have a short replicative cycle which is cytolytic and, unlike the other 6 known human herpesviruses, they grow easily in-vitro in a number of mammalian tissue culture cell-lines[2].

HSV infections of humans have been documented since ancient times[8,15]. Records of human HSV infections began with descriptions of cutaneous spreading lesions thought to be herpetic in aetiology, particularly in the writings of Hippocrates, as reviewed by Nahmias and Dowdle[8]. HHV 1 and HHV 2 are among the most common infectious agents of man. Infections with either virus may be clinically inapparent or produce symptoms that range from mild and trivial to those of severe disease[16]. They appear to have different modes of transmission[17,18]. Classically, HHV 1 is transmitted chiefly via a non-genital route, whereas HHV 2 is most often transmitted venereally or from maternal genital infection to the newborn. The mode of spread of each of the two virus types is reflected by its relative prevalence at different ages and by its pattern of clinical distribution within the host. Thus, HHV 1 infections occur most frequently during childhood and usually affect body sites above the waist. HHV 2 infections, on the other hand, occur most often during adolescence and young adulthood and involve body sites below the waist, primarily the genitalia.

Limited study of HSV infection in Malaysia showed that HSV accounts for 20% of genital ulcerative lesions[19]. The objectives of this study are to review retrospectively the distribution of HHV 1 and HHV 2 mucocutaneous infections with respect to the clinical sites and age of patients in Malaysia and to determine the accuracy of the laboratory tests in support of the clinical findings.

Materials and Methods

Population

From January 1990 to December 1999, patients with a provisional clinical diagnosis of mucocutaneous HSV infections of the oral and genital areas seen in University of Malaya Medical Centre (UMMC) and a Kuala Lumpur private clinic for sexually transmitted diseases (KLSC) were included in the review.

Sample collection

A sterile cotton swab was used to collect the fluid from the vesicular lesion after it was aseptically ruptured using a sterile lancet and kept at 4°C in viral transport medium (VTM) containing amphotericin B (15µg/ml), penicillin G (100 units/ml) and streptomycin (50µg/ml). Swab was similarly collected from any ulcerative lesion and kept in VTM. Subsequently, a second sterile cotton swab was used to scrap cells from the base of the vesicular or ulcerative lesion and deposited onto 2 wells of a Teflon coated slide.

Virus detection and isolation

Cells on each well were air-dried and fixed for 10 minutes in cold acetone. The detection of HHV 1 or HHV 2 antigen was performed by direct immunofluorescece (IF) using the Bartels Herpes Simplex Virus Typing DFA Kit (Bartels Inc., USA). Briefly, 20µl of anti-HHV 1 specific fluorescein isothiocynate (FITC) conjugated

Table I
The Number and Percent of Positive Results Obtained by Direct Immunofluorescence (IF), Virus Isolation and a Combination of Both Methods

Year	No. Tested	*No. +ve (%)	IF +ve (%)	Isolation +ve (%)
1990	30	30 (100)	7 (23.3)	30 (100)
1991	36	30 (83.3)	5 (13.9)	29 (80.6)
1992	40	31 (78.3)	11 (27.5)	31 (78.3)
1993	83	40 (48.2)	16 (19.3)	36 (43.3)
1994	100	41 (41.0)	14 (14.0)	41 (41.0)
1995	74	42 (56.8)	17 (23.0)	39 (52.7)
1996	47	23 (48.9)	7 (14.8)	23 (48.9)
1997	47	28 (59.6)	4 (8.5)	28 (59.6)
1998	24	10 (41.7)	4 (16.7)	10 (41.7)
1999	23	10 (43.5)	6 (26.1)	10 (43.5)
Total	504	285 (56.5)	91 (18.0)	277 (55.0)

**A positive result was based on positive IF and/or virus isolation*

mouse monoclonal antibody was added to the first well and a similar volume of anti-HHV 2 specific FITC-conjugated mouse monoclonal antibody was added to the second well. The slide was incubated for 30 minutes at 37°C in a humid chamber, rinsed with sterile phosphate buffered saline (PBS) and washed for another 10 minutes in PBS in a magnetic stirrer. The slide was subsequently air-dried, mounted and examined under the ultra-violet light microscope (Olympus BX50, Japan) for the presence of HHV 1 and HHV 2.

Vero tissue culture cell-line (ATCC, CCL-81) was used for virus isolation. Briefly, 200µl of VTM containing the clinical material was inoculated onto a monolayer of Vero cells and allowed for pre-adsorption for 30 minutes before 1.5ml of Eagle's Minimal Essential Medium containing 2% fetal calf serum was added. The inoculated tube was incubated at 37°C, examined daily for cytopathic effect (CPE), and typed as described.

Table II
The Number Positive Tests Obtained by Direct Immunofluorescence (IF) and Virus Isolation

	IF Positive	IF Negative	Total
Isolation positive	83	194	277
Isolation negative	8	219	227
Total	91	413	504

Statistical analysis

The statistical analysis of any significant association between variables in the data set was based on Chi-square test. The association between parameters was accepted as significant if the p-value was equal to or less than 0.05.

Results

Over the 10-year study period, 504 specimens consisting of 198 oral lesions and 306 genital lesions were tested by both IF and virus isolation. All the oral specimens were from UMMC whereas

Table III
Oral Specimens Tested for HHV1 and HHV2 by Age Group, Race and Gender

Age-group (yr)	No. Tested	No. +ve	HHV1 +ve (%)*	HHV2 +ve (%)*
< 5	72	31	30 (96.8)	1 (3.2)
5 -15	111	25	25 (100.0)	0 (0.0)
16 and above	15	7	7 (100.0)	0 (0.0)
Total	198	63	62 (98.4)	1 (1.6)
Race				
Malays	83	25	25 (100.0)	0 (0.0)
Chinese	85	23	23 (100.0)	0 (0.0)
Indians	24	13	12 (92.3)	1 (7.7)
Others	6	2	2 (100.0)	0 (0.0)
Total	198	63	62 (98.4)	1 (1.6)
Gender				
Male	126	36	35 (97.2)	1 (2.8)
Female	72	27	27 (100.0)	0 (0.0)
Total	198	63(31.8)	62 (98.4)	1 (1.6)

(%) = percent of the total number of positive result for that age group, racial group or gender*

Table IV
Genital Lesions from UMMC and KLSC Tested for HHV1 and HHV2

Source	No. Tested	No. +ve (%)	HHV1 (%)*	HHV2 (%)*
UMMC	102	55 (53.9)	29 (52.7)	26 (47.3)
KLSC	204	165 (80.9)	7 (4.2)	158 (95.8)
Total	306	220 (71.9%)	36 (16.4)	184 (83.6)

(%) = percent of the total number of positive result*

102 of the 306 gentital specimens were from UMMC, the rest were from patients treated in KLSC. Of the 504 specimens, 285 (56.5%) were positive, 91 (18.0%) by IF and 277 (55.0%) by virus isolation (Table I and Table II). The positivity rate by IF was generally lower than by virus isolation for all the years, ranging from 8.5% in 1997 to 27.5% in 1992 whereas the rate by virus isolation was from 41% in 1994 to 100% in 1990 (Table I)

Table III shows the results of oral specimens tested for HHV1 and HHV2 by age, race and gender. Of the 198 specimens, 63 (31.8%) were positive of which 62 (98.4%) was due to HHV1. There was no statistical difference in the distribution HHV1 infection among the different age groups (x^2=0.01, p=0.9958), racial groups (x^2=0.03, p=0.9985) and gender (x^2=0.01, p=0.9183).

Table IV shows the analysis of 306 genital specimens based on 102 patients from UMMC and 204 patients from KLSC. Of the 102 UMMC specimens, 55 (53.9%) were positive, 29 (52.7%) for HHV1 and 26 (47.3%) for HHV2. In contrast, of the 204 genital specimens from KLSC, 165 (80.9%) were positive, 7 (4.2%) for HHV1 and 158 (95.8%) for HHV2. Combining the results from the 2 study sites, of the 220 positive specimens, 83.6% were positive HHV2 as compared to only 16.4% for HHV1.

Table V
The Number of Specimens from Genital Lesions Tested for HHV1 and HHV2 by Age-group

Age-gp	UMMC				KLSC			
(year)	No. tested	No. +ve	HHV1*	HHV2#	No. tested	No. +ve	HHV1	HHV2
< 5	2	0	0	0	0	0	0	0
5 -15	1	0	0	0	0	0	0	0
16 and >	99	55	30 (54.5)	25 (45.5)	204	167	9 (5.4)	158 (94.6)
Total	102	55	30 (54.5)	25 (45.5)	204	167	9 (5.4)	158 (94.6)

HHV1 = The value in bracket is the percent of positive HHV1 of the total positive for that age group*
HHV2# = The value in bracket is the percent of positive HHV2 of the total positive for that age group

Table V shows the analysis of genital specimens tested for HHV1 and HHV2 by age. Only 3 specimens were from patients less than 15 years seen at UMMC and they were negative for HHV1 and HHV2. In patients aged 16 years and above, HHV2 accounted for 45.5% of specimens from UMMC and 94.6% from KLSC.

Table VI shows the analysis of genital results based on race and gender. Among the racial compositions of patients in UMMC and KLSC, Malays (40/42) and Indians (33/36) accounted for the majority of patients treated in UMMC whereas Chinese formed the predominant group (196/222) in KLSC. Female patients (96/133) were in the majority in UMMC whereas male patients (167/173) were mainly from KLSC. Although HHV1 genital lesions were more common among Malay patients (72.7%) than Indians (47.6%) and Chinese (40.0%) in UMMC, it was not statistically significant (x^2=1.14, p=0.5667). Neither was there any significant difference in the prevalence of HHV2 with respect to the different racial groups in KLSC (Fisher exact, p=0.9622). There was also no significant difference in genital infection due to HHV2 with respect to gender in UMMC (Fisher exact, p=1) and KLSC (x^2=0.01, p=0.9528).

Discussion

Since the recognition of two distinct HSV serotypes, many studies have been carried out to elucidate the epidemiology and patterns of clinical diseases due to these viruses. Studies in the early 1970s showed that HHV1 was essentially the main serotype causing oral and above waist lesions whereas HHV2 was the main cause of genital and below waist lesions[17,18,20,21]. The distribution of the lesions indicates the mode of transmission and the pattern of disease acquisition, and indirectly reflects on the age of acquiring the infections. In the late 1980s and 1990s, studies in the United States, Europe, Japan and other developed nations have indicated a substantial increase of HHV1 in genital and below waist lesions[22-30]. The study by *Lafferty et al.* (2000) showed a positive association of HHV1 infection with oral sex rather than with vaginal sex, suggesting that oral sex might be a common source of HHV1 transmission[30]. This was supported by the study of Spruance in 1984 where he documented the shedding of HHV1 from the oropharynx in the absence of lesions[31].

In the present study, HHV1 is still the main serotype causing oral lesions. As for genital lesions, HHV1 was responsible for only 4.2% of patients seen in a private clinic and is compatible with the finding of genital infections in developed countries in the 1970s. On the other hand, HHV1 accounted for 52.7% of genital lesions treated in UMMC, a finding similar to that in developed nations in 1990s. This discordance between the two patient-groups within the same city is rather interesting and may reflect on the different sexual practices of the two groups.

Table V
The Number of Specimens from Genital Lesions Tested for HHV1 and HHV2 by Race and Gender

	UMMC				KLSC				Combined (UMMC + KLSC)			
Race	**No. Tested**	**No. +ve**	**HHV1***	**HHV2#**	**No. Tested**	**No. +ve**	**HHV1**	**HHV2**	**No. Tested**	**No. +ve**	**HHV1**	**HHV2**
Malays	40	22	16 (72.7)	6 (27.3)	2	1	0 (0.0)	1 (100.0)	42	23	16 (69.6)	7 (30.4)
Chinese	26	10	4 (40.0)	6 (60.0)	196	161	8 (5.0)	153 (95.0)	222	171	12 (7.0)	159 (93.0)
Indians	33	21	10 (47.6)	11 (52.4)	3	3	0 (0.0)	3 (100.0)	36	24	10 (41.7)	14 (58.3)
Others	3	2	0 (0.0)	2 (100.0)	3	2	1 (50.0)	1 (50.0)	6	4	1 (25.0)	3 (75.0)
Total	102	55	30 (54.5)	25 (45.5)	204	167	9 (5.4)	158 (94.6)	306	222	39 (17.6)	183 (82.4)
Gender												
Male	6	2	1 (50.0)	1 (50.0)	167	141	7 (5.0)	134 (95.0)	173	143	8 (5.6)	135 (94.4)
Female	96	53	29 (54.7)	24 (45.3)	37	26	2 (7.7)	24 (92.3)	133	79	31 (39.2)	48 (60.8)
Total	102	55	30 (54.5)	25 (45.3)	204	167	9 (5.4)	158 (94.6)	306	222	39 (17.6)	183 (82.4)

HHV1 = The value in bracket is the percent of positive HHV1 of the total positive for that racial group and gender*
HHV2# = The value in bracket is the percent of positive HHV2 of the total positive for that racial group and gender

The isolation rate of HSV from genital lesions was similar to that reported in other countries[28]. However, the isolation rate from oral lesions was lower in comparison with that from genital lesions and from findings in other studies. This low isolation rate from oral specimens may infer that other aetiological agents may be involved.

Although rapid diagnosis of HSV infection by IF can be achieved, the detection rate from clinical samples was low and only improved marginally when virus isolation was employed. The insensitivity of IF was probably due to inadequate method of sample collection by swabbing the base of the lesion with a cotton swab since most of the negative results were found with insufficient or no cells.

Acknowledgments

We would like to thank KLSC private clinic for providing the specimens for virological investigation. The project was partially funded by a research grant (IRPA grant no: 06-02-03-0528) from the Ministry of Science, Technology and Environment, Malaysia.

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