ORIGINAL ARTICLE

Age-specific seroprevalence of Human Herpesvirus 8 in Mediterranean regions

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Objective Human herpesvirus 8 (HHV8) is believed to be transmitted mainly by sexual contact; epidemiological data from Africa show, however, that non-sexual transmission routes may also play an important role. To evaluate better the distribution of HHV8 infection in the Mediterranean area, we performed an age-specific seroprevalence study.

Methods Sera were collected from subjects from different geographical areas. The sera were analyzed by immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). A total of 1083 patients were studied, 667 patients from various regions of Italy and 416 from Albania. The patients were stratified into six age groups. Multivariate logistic regression was used to evaluate associations between HHV8 and demographic data.

Results An overall seropositivity rate of 17.6% was observed. The highest rate was observed in Sardinia (25.0%) and the lowest was found in Albania (13.9%). The prevalence rate increased linearly with age, from 9.7% in patients belonging to the 0–14 years age group to 26.3% for patients more than 59 years old. Seropositivity for HHV8 was significantly associated with membership of the 59 years-plus age group. Rates of seropositivity were significantly higher in patients from central southern Italy (OR = 1.7) and Sardinia (OR = 1.8) than in patients from Albania.

Conclusions The data suggest that HHV8 is widespread in the Mediterranean area, including regions like Albania that have not been previously investigated. The statistically significant association between HHV8 seropositivitity and increasing age suggests that non-sexual transmission routes may be involved in the spread of the virus.

Keywords Human herpesvirus 8, Kaposi's sarcoma-associated herpesvirus, seroprevalence, anti-HHV8 antibodies

Since its initial discovery in 1994 [1] Human Herpesvirus 8 (HHV8), otherwise known as Kaposi's sarcoma-associated Herpesvirus (KSHV), has

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been closely linked with all four clinical forms of Kaposi's sarcoma (KS). HHV8 DNA sequences have been detected in virtually all KS lesions, regardless of clinical form and histological stage [2,3]. This suggests that HHV8 infection may play a causal role in the pathogenesis of KS [4–8].

Recent epidemiological studies have suggested that HHV8 could be widespread in the general population, at least in geographic areas where

KS is prevalent [3,9–12]. HHV8 seroprevalence rates are reported to be higher in Africa and in Italy than in other geographical regions where KS is less prevalent [13–19]. This observation supports the hypothesis that the prevalence of HHV8 infection is causally related to the incidence of KS.

Olsen et al. have observed that, in several regions of Africa, HHV8 infection is widespread from adolescence onwards [18]. HHV8 was found in body sites and fluids that may be involved in virus spread [9,15,20–23]. These data suggest that, at least in specific geographical areas, horizontal transmission of HHV8 infection, possibly via non-genital fluids, may play an important role in maintaining HHV8 in the population.

Despite numerous studies, the overall epidemiological pattern of HHV8 infection in different countries remains unclear. This is particularly true in areas, such as Italy and Albania, where KS was present in the classic or iatrogenic forms before the current acquired immune-deficiency syndrome (AIDS) epidemic. In this paper we investigate the endemic or epidemic nature of HHV8 infection in Italy and Albania using an age-specific seroprevalence study.

MATERIALS AND METHODS

Population and sampling

A total of 1083 healthy individuals from different areas of the Mediterranean were enrolled for the study. These included 517 patients attending the Outpatients Unit of the Institute of Microbiology, Università Cattolica, Rome, Italy for routine examinations, 416 attending the Poliambulatorio 'Padre L. Monti', Tirana, Albania, and 150 attending the Laboratorio Analisi Chimico-Cliniche e Microbiologiche, Ospedale Santa Maria Annunziata, Sassari, Italy. This last group of subjects from Sassari (Sardinia) was included in the study because classic KS is known to be endemic on the island. The group enrolled by the Institute of Microbiology in Rome was classified by geographical region of origin: 357 subjects were born and currently lived in the Lazio region (central Italy), while 160 came from various regions of southern Italy. Individuals with possible risk factors for HHV8 infection (sexually transmitted disease, human immunodeficiency virus infection, organ transplantation) were excluded from the study groups.

After informed consent had been obtained, serum samples were collected along with demographic data on delivery (Caesarean delivery versus vaginal birth), ancestry, area of birth and residence, travel, housing and working conditions, risk factors for parenterally transmitted and sexually transmitted infection, sexual habits and medical history. At the end of the study, completed questionnaires were available for 788 subjects.

Serological assays

An immunofluorescence assay (IFA) was developed using the BC3, HHV8-positive, Epstein–Barr virus (EBV) -negative cell line (American Type Culture Collection, Rockville, MD, USA), as described elsewhere [15]. The Ramos HHV8 and EBV-negative cell line (American Type Culture Collection) and the Hep2 cell line (Eurobio, Les Ulis, France) were used as controls. BC3 cells (10^6/ml) were induced with 20 ng/ml of the phorbol ester 12-*O*-tetradecanoyl phorbol-13-acetate (TPA, Sigma, St Louis, MO, USA) to activate the lytic replicative cycle of the virus. Uninduced and TPA-induced cells were spotted on slides and incubated with two-fold dilutions of human sera, beginning at 1 : 80. The slides were washed three times and then incubated with a prestandardized dilution of Kallestad™ fluorescein-conjugated goat F(ab')$_2$ fragment anti-human immunoglobulin G (IgG; Sanofi Diagnostics Pasteur, Chaska, MN, USA). After incubation, slides were washed, air-dried and examined under a fluorescence microscope.

As a control, anti-human IgG fluorescein-conjugated antibodies were also tested without human sera. Cells showing specific reactivity at a dilution of 1 : 80 with both uninduced and TPA-induced BC3 cells and no reactivity with Ramos and Hep2 cells were considered positive for HHV8 antibodies.

All sera that tested positive by IFA were retested for the presence of HHV8-specific antibodies at a dilution of 1 : 80 using an HHV8 IgG antibody enzyme-linked immunosorbent assay (ELISA) kit (ABI, Columbia, MD, USA). The assay was conducted in accordance with the manufacturer's protocol.

For the purposes of the present study sera were considered positive if both assays showed specific reactivity.

Statistical analysis

The χ^2 test was used to test for the presence of a statistically significant association between seroprevalence and age. The association between HHV8 seropositivity, age, sex and geographic area of origin was evaluated using logistic regression. For a subgroup of 788 individuals, for whom complete demographic information was available, a second logistic model was used to determine if the association with age varied with geographic area. All statistical analyses were performed using STATA 6.0.

RESULTS

Out of 1083 patients in the study population, 191 (17.6%) were identified as seropositive for HHV8, by IFA and ELISA.

The overall geometric mean titre (GMT) for antibodies to HHV8, detected by IFA, was 240.47 (range 80–2560), with no significant difference among different age groups.

During IFA, reactive sera showed a speckled nuclear fluorescence due to latent viral antigens, in both uninduced and TPA-induced cell preparations, while in a high proportion of cells a homogeneous cytoplasmic fluorescence pattern was observed only after TPA-induced activation of the viral lytic cycle and release of viral particles [17,24,25]. As expected, fewer samples displayed reactivity to latent antigen than to lytic antigen [10,13]; these results generally coincided with ELISA data obtained with viral lysate antigens.

Anti-human IgG fluorescein-conjugated antibodies, tested without human sera, failed to show any background immunofluorescence.

All sera were tested with HHV8-negative cell lines (Ramos and Hep2) to analyse the specificity of the observed pattern of fluorescence. Not surprisingly, a pattern resembling the specific pattern for HHV8 was identified in a number of samples, often from elderly people; in these cases the positive reaction with Ramos and Hep2 cells showed that the observed pattern was the result of an autoimmune response due to the presence of antinuclear antibodies. Samples which reacted in this way were excluded from the study.

HHV8 seroprevalence was similar in men and women (18.3% and 16.9%). The study did, however, detect significant differences in prevalence

Table 1 Prevalence of anti-HHV8 antibodies in 1083 subjects grouped by age

| Age group (years) | Subjects (*n*) | HHV8 seropositive | |
		(*n*)	(%)
I (0–14)	143	14	9.7
II (15–29)	209	34	16.2
III (30–39)	232	33	14.2
IV (40–49)	176	28	15.9
V (50–59)	141	34	24.1
VI (>60)	182	48	26.3
Total	1083	191	17.6
Male	534	98	18.3
Female	549	93	16.9

between different geographic areas. The highest seroprevalence rate was found in Sardinia (25.0%) and the lowest in Albania (13.9%). Rates for patients from central and southern Italy (18.3%) were intermediate.

To study the effects of age the population was stratified into six age groups. Rates of seroprevalence for each age group are summarized in Table 1. It was observed that seroprevalence increased from 9.7% in the first group (0–14 years) to 26.3% in the last group (>59 years), showing a statistically significant association ($\chi^2 = 8.66$, $P = 0.03$), apparently linear in form. Interestingly, anti-HHV8 antibodies were found in 10.3% of a subgroup of 29 children less than 1 year old (two males aged 4 and 10 months, and one female aged 6 months).

In a subgroup ($n = 788$) of patients for whom complete demographic information was available, we used logistic regression to study the relationship between seroprevalence and a series of demographic variables. In Table 2 we report the demographic characteristics of the subgroup with complete data on sex, age and geographic area as well as the odds ratio (OR) and the related 95% confidence intervals (95% CI) generated by the logistic model.

Logistic regression showed no association between HHV8 seropositivity and sex. It was observed however, that the OR for being HHV8 positive increased with increasing age and was statistically significant for individuals older than 59 years (OR = 3.6, 95% CI = 1.8–7.2). We also observed an association with geographic area: individuals from central-southern Italy and from Sardinia had a higher risk of being HHV8 positive

Table 2 Characteristics of the subgroup ($n = 788$) with complete demographic data and results from multivariate logistic regression

Demographic variable	Prevalence of HHV8			
	n	%	OR	*P*-value
Sex				
Male	378	15.9	1.00	
Female	410	19.8	1.27	0.218
Age (years)				
0–14	139	10.8	1.00	
15–29	164	17.1	1.64	0.166
30–39	193	15.5	1.66	0.156
40–59	192	17.7	1.82	0.084
>59	100	34.0	3.60	<0.001
Geographic area				
Albania	374	12.6	1.00	
South-central Italy	264	21.2	1.70	0.025
Sardinia	150	25.3	1.84	0.018

compared to people from Albania (OR = 1.7, 95% CI = 1.0–2.7; OR = 1.8, 95% CI = 1.1–3.0, respectively).

Stratification by geographic area showed that in patients from Albania there was a significantly increased risk of HHV8 seropositivity in the 30–39 years age group (OR = 5.8, 95% CI = 1.3–25.8); in Italian patients, on the other hand, the analysis showed significantly increased risk only in the >59 years age group. This result confirmed the results for the general population.

DISCUSSION

Seroepidemiological studies have shown that the seroprevalence of HHV8 infection is geographically diversified. In Africa and some Mediterranean regions HHV8 seroprevalence rates are reported to be higher than those for the United States or northern European countries [10,13, 16–18,26–28], The highest rates have been reported in African countries, such as Zambia and Uganda, where endemic KS has been described in HIV-seronegative patients who are younger than patients with classic KS in the Mediterranean area. Differences in HHV8 seroprevalence between northern and southern Italy have also been reported. These correlate with different rates of prevalence for classic KS [28]. In Sardinia, one of the world's highest incidence areas for classic KS, the rate of HHV8 in non-KS individuals is the highest reported for Italy [15,26]. In contrast very

little is known regarding the distributions of KS and of HHV8 infection in Albania [29]. Rates of HHV8 seropositivity increasing with age have been reported in countries with endemic KS, such as Zambia, Cameroon and Egypt, and in individuals at risk for sexually transmitted infections in Italy [13,14,18,30].

The aim of the work reported in this paper was to investigate further the distribution of HHV8 infection in the Mediterranean area and to evaluate whether here, as in Africa, the infection can be acquired early in life and by non-sexual transmission routes. The analysis of our data has produced a number of interesting findings.

Classic KS is three times more frequent in males than in females [31]. The absence of any significant difference between HHV8 seroprevalence in men and women suggests therefore that sex-specific risk factors may be necessary for disease progression.

We found differences in the prevalence of HHV8 among individuals from different regions. In particular, we observed the highest seroprevalence rate in Sardinia and the lowest in Albania. These data are in accordance with results already reported by other authors [13,28].

The seropositivity rate increased with age. Of particular interest was the rate of 9.7% observed in children less than 14 years old, suggesting that routes other than sexual transmission can be involved in the spread of HHV8. While the anti-HHV8 antibodies present in two children aged 4 and 6 months could be of maternal origin, the presence of specific antibodies in a 10-month-old child is strongly suggestive of perinatal infection, which is a common transmission route for other herpesviruses.

The logistic regression model applied to a subgroup of 788 subjects, for whom complete demographic data were available, showed that, although the risk of being HHV8 positive is higher in Sardinia and central-southern Italy, in Albania the association with HHV8 seropositivity is statistically significant in a younger age group. This situation could be due to different socio-economic conditions in the two countries.

These results, together with the finding of a linear relationship between seroprevalence and age, are consistent with the prominent role of non-sexual transmission. Ongoing transmission from a range of sexual and environmental sources would explain higher seropositivity rates in adulthood.

Overall, our analysis supports suggestions in previous studies that a non-sexual mode of transmission may be important for HHV8 diffusion, not only in African countries, but also in Mediterranean areas with a relatively high prevalence of classic KS (0.5–1.5 per 100 000 population) [10,32]. Our results provide additional insight into the epidemiological pattern of HHV8 infection in different Mediterranean regions, suggesting that in some geographic areas HHV8 is likely to have continuous and multiple transmission routes, as is typical of endemic pathogens, and is not restricted to subjects at risk for developing KS.

ACKNOWLEDGMENTS

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