Demographic Indicators and Risk of Infection with Human Herpesvirus Type 8 in Central Italy

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Abstract

Background: A cross-sectional study was conducted in Latium and Abruzzo Regions (Central Italy) to estimate the prevalence of infection with human herpesvirus type 8 (HHV-8) and the association between demographic indicators and risk of HHV-8 infection.

Patients and Methods: Sera from 416 healthy individuals (≥45 years of age), originally recruited in a multicentric case-control study on classic Kaposi's sarcoma (KS), were tested for antibodies against HHV-8. The association between demographic indicators (i.e., urban/rural residence, occupation) and HHV-8 seropositivity was assessed by means of multiple logistic regression (MLR) odds ratios (OR) and 95% confidence intervals (CI), adjusted for age and occupation.

Results: Overall, 20.4% of the study participants had antibodies against HHV-8, 23.2% of the men and 17.0% of the women (p = 0.15). HHV-8 seropositivity rates significantly increased with age (p = 0.01), from 10.0% in those under 65 years of age to 24.9% in 75 years or older (MLR-OR = 2.4). By multivariate analysis, a significantly 2-fold higher risk of HHV-8 was found in individuals living in rural areas, as compared to those living in metropolitan/urban areas (MLR-OR = 2.0, 95% CI: 1.1-3.5), and in farmers, as compared to white collars (MLR-OR = 2.1, 95% CI: 1.1–4.1).

Conclusions: The study findings suggest that demographic factors such as age, urban/rural residence, and occupation are associated with HHV-8 seropositivity among adult individuals living in central Italy.

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Introduction

Infection with Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human Herpesvirus 8 (HHV 8), is necessary to develop Kaposi's sarcoma (KS) [1]. The distribution of HHV-8 varies across geographical areas, showing the highest prevalence in the sub-Saharan Africa and the lowest in USA and Northern Europe, with intermediate rates in the Mediterranean area [2]. In Italy, HHV-8 seroprevalence among blood donors shows an increasing North-South gradient, with peak rates in Sicily and Sardinia, mirroring incidence rates for classic KS [3].

The increase in both incidence rates of KS and of HHV-8 seropositivity found among homosexual men infected with the human immunodeficiency virus (HIV) put in evidence the etiological role of some cofactor(s) associated with sexual life style [4, 5]. Transmission through saliva may also favour HHV-8 spread [2], and the high prevalence of HHV-8 infection among children in sub-Saharan Africa and, to a lesser extent, in other areas at high or intermediate risk for KS, indicated the existence of non-sexual modalities of HHV-8 transmission [6, 7].

As seen for the Epstein-Barr virus (EBV), socio-economic factors may also explain part of the observed geographical variations in HHV-8 seropositivity rates. The aim of this study was to estimate HHV-8 prevalence and to identify demographic factors associated with HHV-8 infection in the adult population of Central Italy, a Mediterranean area with intermediate KS incidence rates [8].

Methods

The present study group was constituted by healthy subjects recruited in a multicentric case-control study on risk factors for classical KS, carried on in various parts of Italy by the National Cancer Institute and the Italian National Institute of Health (ISS) (for details, see [9]).

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Since classical KS is, in general, a disease of older age, the individuals (i.e., the controls) included in this investigation were aged 45 years or older.

As shown in figure 1, the study participants were recruited by general practitioners working in metropolitan areas (i.e., Rome), urban areas (e.g., Civitavecchia) or rural areas (e.g., Bagnoregio) of Latium and Abruzzo regions, Central Italy. Serum samples were obtained from 416 individuals, 45 to 90 years of age, randomly chosen from the list of the general practitioners (for details, see [9]). From each participant, information was elicited on area of birth, area of residence, occupation, and years of education.

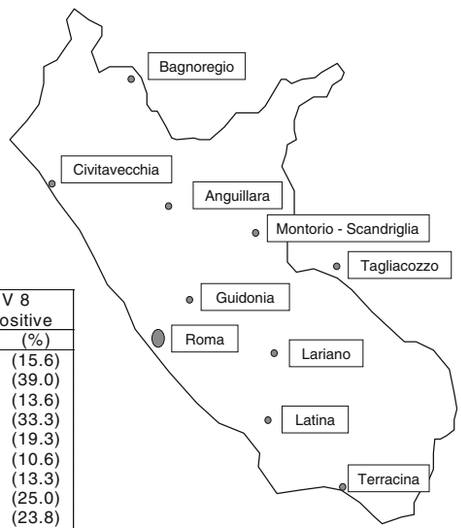
Anti-HHV-8 immunofluorescence assay was performed at the IRCCS San Gallicano, Rome, according to a standard procedure [10]. HHV-8 infected body-cavity-based B-cell lymphoma-1 (BCBL-1), derived from PEL, was cultivated in RPMI 1640, supplemented with 10% Foetal Calf Serum (FCS) and 50 μM Beta-mercaptoetanol. Esponentially growing cells (3×10^5cells/mL) were treated for 72 hrs with 20 ng/mL of phorbol-12-myristate-13- acetate (TPA, Sigma, St Louis, MO). Ten μL of treated cell suspension and 10 μL of untreated cells were dropped on the consecutive wells of multiwell slides, rapidly air dried and fixed in cold acetone for 20 minutes. Fixed cells, treated and untreated, were incubated, in two steps of 30 min at room temperature, in a humid chamber, with two serial dilutions of each serum sample and with FITC-labelled goat F(ab)2-immunoglobulins anti-human IgG (Southern Biotechnology Associates, Inc., Birmingham, USA). The microscopic examination of TPA-treated and untreated cells allowed to establish specific antibody reactivity both to nuclear and cytoplasmic antigens. A titre higher than 1:20 was considered positive.

The association between HHV-8 seropositivity and demographic factors was assessed by means of the chi-square test or, when appropriate, the chi-square test for trend [11]. To take into account the potential role of confounding factors, multiple logistic regression (MLR) equations were fitted and MLR odds ratios (OR) and 95% confidence intervals (CI) — adjusted for age and occupation – were computed [12].

Results

Among the 416 tested individuals, 85 (20.4%; 95% CI: 16.6–24.3) were seropositive for anti-HHV-8 antibodies. Table 1 reports the distribution of study subjects according to selected characteristics and HHV-8 seropositivity, and the association between demographic indicators and HHV-8 infection. More men (23.2%) than women (17.0%) were HHV-8 seropositive, though the difference was not statistically significant (p = 0.15). HHV-8 prevalence significantly increased with increasing age (test for trend, p = 0.01), from 10.0% among persons aged ≤64 years to 24.9% in those aged 75 or older (MLR-OR = 2.4, 95% CI: 1.0–6.2) (Table 1).

HHV-8-seropositivity was significantly higher in persons living in rural areas (24.2%), as compared to those living in metropolitan or urban areas (13.3%, respectively) (p = 0.01). Such a difference in seropositivity rates was associated – at multiple variate analysis – with a 2-fold significantly increased risk for HHV-8 infection (95% CI: 1.1–3.5) (Table 1). With regard to occupation, farmers showed the highest prevalence of HHV-8 infection (34.5%): in comparison with white collars, they showed a 2-fold higher risk of infection (MLR-OR = 2.1, 95% CI: 1.1–4.1). No statistically significant associations emerged between area of birth, education and HHV-8 seropositivity (Table 1).



	Tested	HHV 8 seropositive	
	No	No	(%)
Bagnoregio	32	5	(15.6)
Civitavecchia	23	9	(39.0)
Anguillara	22	3	(13.6)
Montorio - Scandriglia	54	18	(33.3)
Tagliacozzo	31	6	(19.3)
Guidonia	47	5	(10.6)
Roma	143	19	(13.3)
Lariano	24	6	(25.0)
Latina	21	5	(23.8)
Terracina	19	9	(47.4)

Figure 1. Study sites, Central Italy 2000–2003.

Discussion

About 20% of the adult individuals from Central Italy included in this seroepidemiological study was seropositive for HHV-8 antibodies. This prevalence of HHV-8 infection was consistent with the geographic distribution of KS in the Mediterranean area [13, 14], but higher than that previously reported in a population-based study in the Latina province, which found rates of about 9% among men and 6% among women [8]. At least part of such elevated prevalence of HHV-8 infection (as compared to previous studies) may be attributable to the advanced age of our study population (i.e., the median age was 73 years). In agreement with other investigations, HHV-8 infection occurred more frequently in older individuals [1, 2], and persons aged 75 years or older had a 2.4-fold higher risk of having acquired HHV-8 infection than those aged 64 or less. The design of this study (i.e., a cross-sectional investigation) did not allow us to investigate age at acquiring HHV-8 infection, and we can not speculate on potential factors that may increase the risk of infection at older ages. On the other hand, such risk patterns may reflect the cumulative risk of acquiring infection with ageing.

As already reported from other investigations, no significant difference was found between males and females.

Table 1
Multiple logistic regression (MLR) odds ratio (OR) for HHV-8 seropositivity. Central Italy, 2000–2003.

Characteristics	Tested No.	HHV-8 seropositive No. (%)	MLR-OR (95% CI)
Sex:			
Men	228	53 (23.2)	1
Women	188	32 (17.0)	0.8 (0.4–1.6)
Age:			
< = 64	60	6 (10.0)	1
65–74	171	33 (19.3)	1.9 (0.7–4.8)
> = 75	185	46 (24.9)	2.4 (1.0–6.2)
Area of birth:			
Northern Italy	28	5 (17.9)	1
Central Italy	247	54 (21.9)	1.3 (0.4–3.7)
Southern Italy	114	25 (21.9)	1.8 (0.6–5.8)
Not determined	27	1 (3.7)	0.2 (0.0–2.2)
Residence:			
Metropolitan/ Urban	143	19 (13.3)	1
Rural	273	66 (24.2)	2.0 (1.1–3.5)
Profession:			
White collar workers	127	23 (18.1)	1
Blue collar workers	76	18 (23.7)	1.4 (0.7–2.7)
Farmers	84	29 (34.5)	2.1 (1.1–4.1)
Housewife	112	13 (11.6)	0.6 (0.3–1.2)
Education (years):			
0–5	250	58 (23.2)	1
6–8	40	11 (27.5)	1.9 (0.8–4.3)
> = 9	54	10 (18.5)	1.3 (0.5–3.4)

HHV-8: Human Herpesvirus type 8; CI: Confidence interval

This finding strengthens the observation that there are other factors determining a higher risk of KS in HHV-8 positive men compared to women [3, 6, 8, 14].

Individuals living in rural areas and farmers had a 2-fold increased risk for HHV-8 infection. This observation represents an original finding from our investigation, and it has not been clearly noted in other studies. A recent analysis of correlates of HHV-8 infection from the overall multicentric investigation, seems to indicate that socio-economic variables (e.g., childhood crowding) are likely to play a role in increasing the probability of acquiring HHV-8 infection [15]. In this analysis, we were not able to control for all potential confounders of the relationship between HHV-8 seropositivity and socio-demographic indicators (e.g., number of siblings and housing). We controlled for age, which is an important determinant of infection, and found that it did not influence the association with occupation or residence: thus, this finding was not attributable to older age of farmers or of residents in rural areas. Moreover, although farmers had a low degree of education (i.e., 94% of them had less than 6 years of education) education "per se" was not associated with HHV8 infection, suggesting that some peculiar factor related to such occupation might have elevated the risk of infection.

We are aware that our study had some drawbacks, and that, accordingly, the findings should be interpreted with caution. In particular, it is worth stressing the limited number of variables investigated and the fact that the prevalence rate of HHV8 is influenced by the type of assay used for serological diagnosis [16, 17]. In this investigation, the sensitivity and specificity of the assay was about 90% and 95%, respectively [17].

Nonetheless, our findings indicate that there are demographic factors associated with an increased risk of acquiring HHV-8 infection that may explain the high prevalence of HHV-8 infection among older adults living in the Mediterranean area.

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