

Prevalence of antibodies to HHV-8 in the general population and in individuals at risk for sexually transmitted and blood-borne infections in Catania, Eastern Sicily

Prevalenza degli anticorpi anti-HHV-8 in soggetti a rischio di contrarre infezioni a trasmissione sessuale ed ematica e nella popolazione generale della provincia di Catania

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INTRODUCTION

Human herpesvirus-8 (HHV-8) is now widely believed to be a necessary cause of Kaposi's sarcoma (KS) and may be pathogenically related to primary effusion lymphoma (PEL or body cavity-based lymphoma) and multicentric Castlemann's disease, two lymphoproliferative disorders with multifactorial pathogenesis which are also characterized by the presence of HHV-8 [1, 2].

Since its discovery, HHV-8 has been widely investigated not only in terms of pathogenesis, but also in terms of distribution and transmission routes. The distribution of HHV-8 appears quite wide. Researchers agree that antibodies to the virus are present in almost all Kaposi's sarcoma patients and are more common in individuals at risk for this disease as well as in STD clinic attenders than in the general population [3-6]. Besides, studies conducted in specific geographical areas have shown discrepant data on the prevalence of the virus in the normal adult population vary-

ing from 4% to 25% [4, 6]. On the other hand, in Sub-Saharan Africa HHV-8 is very common in the general population, ranging from 49 to 87% [7].

Interpreting these controversial data can be difficult, for example because of the imperfect sensitivity of the methods themselves, which render such assays susceptible of artefacts and contamination. Two major questions fascinate researchers. Firstly, whether HHV-8 exists in healthy individuals at high frequency, and secondly, the existence of one or more transmission routes of the virus.

In this regard, a number of reports have shown sexual routes are the most prevalent transmission path of the virus [8, 9], although in areas where quite a high HHV-8 prevalence in the general population exists, non-sexual routes have been supposed [10-12].

Few studies of HHV-8 seroprevalence have been conducted in Italy, particularly in Sicily which, as well as other southern European countries, has shown significantly high values of KS incidence rates especially in the pre-

AIDS era. Indeed, Sicily showed significantly high rates of KS that were more than 50 times higher than those in Northern Europe [13]. This study represents one of the first surveys of HHV-8 in Sicily on the prevalence of antibodies to HHV-8 in both low and high-risk subjects. This project describes a study whose aims are to evaluate: a) the prevalence of HHV-8 in anti-HIV positive individuals with different risk factors and anti-HIV negative intravenous drug users (IDUs); b) the potential association among HHV-8 infection and other factors, such as behavioural risk factors, human immunodeficiency virus (HIV).

■ SUBJECTS AND METHODS

Study population and survey procedures

Three treatment facilities were the sites for this retrospective analysis of several groups of people: the Outpatients Clinic of Infectious Diseases Unit of Catania University, the Public Outpatients Clinic for drug users in the "Garibaldi" Hospital of Catania, and the "Vittorio Emanuele" Hospital Blood Bank of Catania. Consecutive patients were recruited as study subjects at the 3 chosen sites from January 2001 to April 2002.

Anti-HIV positive patients, both with AIDS and not, were recruited from the Infectious Diseases Clinic. Anti-HIV negative IDUs were recruited from the drug treatment clinic. Blood donors, as a reference group close to the normal population, were recruited as a control group from the Blood Bank. Blood donors were consecutively selected among male and female without any risk factors for sexually or parenterally transmitted infections. They were neither paid nor self-attended.

The study was approved by the ethics committee of the University of Catania. The intent of the study was explained to every respondent along with the principles of confidentiality and anonymity. Every person who attended the outpatient clinics from January 2001 to April 2002 was asked to volunteer for the study. Very few patients (11 anti-HIV positives and 19 IDUs) refused to participate in the study. Those who were under 18 years were not enrolled. The logs of personal names and identification numbers were securely kept for all patients to ensure that confidentiality was not breached. Informed consent was obtained from all the study participants.

A blood sample was also obtained for HHV-8 serology and other tests.

Demographic characteristics and behavioural information about the potential risk for contracting STDs and blood-borne diseases were retrospectively collected from clinical records.

Serological techniques

Serum was separated from clotted whole blood by centrifugation and frozen at -20°C. A total of 424 specimens were obtained and tested by IFA for antibody anti-HHV-8. Testing for the HHV-8 antibody was performed by HHV-8 IFA (Viramed Biotech AG, Planegg/Steinkirchen - Germany) for the latent nuclear antigen (LANA or LNA-1).

For qualitative IgG antibody detection, patient serum, following 1:64 dilution, was applied to cultured cells containing inactivated viral antigens placed on paint delineated wells on glass microscope slides. During a 30 minute incubation period, the antibody specific for HHV-8 antigens formed an antigen-antibody complex. In the subsequent washing step, non-specific antibody and other unreacted serum proteins were eliminated. Fluorescein-conjugated goat anti-human IgG was then applied to the wells on the glass slide. During a further 30 minute incubation period, this conjugate was able to combine with human IgG, if present. The non-reacted conjugate was then eliminated by a second washing step. In conclusion, the slides were viewed by fluorescence microscopy. A positive result was denoted by bright green fluorescence at the antigen sites. Absence of specific fluorescence staining of the infected cells denoted a negative reaction for HHV-8 IgG antibody.

HIV infection was proven by detection of HIV-1 antibodies using a commercial enzyme immunoassay (ELISA) and Western Blot, according to the Centre for Diseases Control criteria. Serology was consistently performed by the same technician and examined by the same clinician.

Variables

The variables used and measured at enrolment were age, sex, HIV status, previous KS and exposure factors to sexually or parenterally transmitted diseases.

Statistical analysis

Association between HHV-8 seroprevalence and other variables was evaluated by calculat-

ing the χ^2 tests with Yates's correction or Fisher's exact test where appropriate.

RESULTS

We retrospectively investigated the prevalence of antibodies to HHV-8 in 424 patients from three different outpatient clinics in Catania, western Sicily. Of the 424 participants recruited, 119 (28.1%) were female and 305 (71.9%) were male. The median age was 37 years.

Study participants were: 196 (46.2%) HIV-positive subjects (group 1); 102 (24.1%) IDUs (HIV-negative) (group 2); and 126 (29.7%) blood donors (non-IDUs, heterosexuals, HIV-negative) (group 3). In the first group, 62 (31.6%) were female and 134 (68.4%) were male. In the second group, 12 (11.8%) were female and 90 (88.2%) were male. In the third group 36 (28.6%) were female and 90 (71.4%) were male. In the context of the first group,

people were present with different behavioural risks, such as homosexual men, heterosexuals with multiple partners, IDUs and blood transfused individuals. The sample is described in detail in Table 1.

The frequency of HHV-8 positive samples was determined (Tables 2 and 3). Of the 424 subjects tested, 98 had antibodies against HHV-8, giving a crude HHV-8 seroprevalence in the study population of 23.1%. Only 12 female were positive for antibodies to HHV-8 in the anti-HIV positive group (group 1). No female was positive in the other groups. In the HIV positive group (group 1), 16 patients with a previous history of KS were present. Among them, 2 were female (12.5%). Fourteen (87.5%), all male, were positive for anti-HHV-8 antibodies.

All patients were stratified by sex and clinic of provenance (groups 1, 2 and 3). When we stratified the study population by sex, no significant association was found ($p=0.4$) with HHV-8 infection. When we stratified the

Table 1 - Characteristics of the study population.

Group	Female (n)	Male (n)	Total (n)
(1) HIV-positives	62	134	196
Homosexuals	(-)	(54)	(54)
Heterosexuals	(54)	(41)	(95)
IDUs	(6)	(26)	(32)
Transfused	(3)	(12)	(15)
(2) IDUs	12	90	102
(3) Control Group	36	90	126
Total	110	314	424

Table 2 - Prevalence of anti-HHV8 antibodies in the study population.

Group	HHV-8 positives n (%)	HHV-8 negatives n (%)	Total n
(1) HIV-positives	89 (45.4) (95 C.I. 38.4-52.4)	107 (54.6)	196
(2) IDUs	6 (5.9) (95 C.I. 1.2-16.2)	96 (94.1)	102
(3) Control Group	3 (3.6) (95 C.I. 0.7-10.1)	81 (96.4)	126
Total	98 (23.1) (95 C.I. 23.8-33.6)	236 (76.9)	424

Table 3 - Prevalence of anti-HHV8 antibodies in the anti-HIV positive group.

(1) HIV positives	HHV8 positives n (%)	HHV8 negatives n (%)	Total n
Homosexuals	42 (77.8) (95 C.I. 64.8-88)	12 (22.2)	54
Heterosexuals	30 (31.6) (95 C.I. 22.4-41.9)	65 (68.4)	95
IDUs	8 (25.0) (C.I. 11.5-43.4)	24 (75.0)	32
Transfused	9 (60.0) (95 C.I. 32.3-83.7)	6 (40.0)	15
Total	89 (45.4) (95 C.I. 38.4-52.4)	107 (54.6)	196

study population by groups, a statistically significant association was found between HIV and HHV-8 infection ($p < 0.001$). When we stratified the HIV-positive group by risk factors, a statistically significant association was found between HHV-8 infection and sexual orientation, both homosexual men ($p < 0.001$) and heterosexual ($p < 0.05$). When we stratified the anti-HIV positive group by the presence of KS and HHV-8 positivity, a strongly significant association was found between HHV-8 infection and KS ($p < 0.001$). In addition, in the HIV positive/KS patients stratified by risk factor, a statistically significant association was found between HHV-8 infection and homosexuals ($p = 0.03$).

DISCUSSION

Anti-HHV-8 prevalence in the general population has shown great differences among different countries; studies confirm a lower prevalence in Northern Europe as well as in North America compared with Southern Europe and Africa [4, 6, 11]. In addition, it has been pointed out that Mediterranean regions show quite high incidence rates of classic KS. Vitale *et al.* have detected high HHV-8 prevalence rates, especially in Sardinia (25%), Sicily (20.3%) and Malta (8.5%) [14].

In this study, the prevalence of anti-HHV-8 antibodies in the control group (group 3), which we assumed to reflect the general population, was 3.6%. The literature on the seroprevalence of HHV-8 in Sicily reports a higher prevalence of infection [11, 15]. Cattani *et al.*

reported a survey conducted among 100 HIV seronegative patients either with (50%) or without (50%) KS showing that all KS patients and 64% (95 C.I. 44-77) of KS-free subjects had HHV-8 antibodies [11].

According to their investigations, the HHV-8 infection among healthy people in Sicily would be widespread. On the other hand, Gao *et al.* have observed an HHV-8 prevalence of 4% (4/107) among northern Italian blood donors, whereas none (0/122) of the North American blood donors was anti-HHV-8 positive [4]. Besides, our data are similar to those of Kedes *et al.* and Simpson *et al.*, who found that HHV-8 infection is uncommon in the general population of the UK and US, having a range of infection under 5%, although this contrasts with Lennette *et al.* who found an unusual 25% of anti-HHV-8 prevalence in the American general population [5, 6, 18]. We hypothesize that blood donors in Sicily are not representative of the general population: even though they were consecutively selected and they came from the same area, such discrepancies could be due to multiple factors. Firstly, the small sample size itself may make it non-representative of the population as a whole and the estimate imprecise. A further factor could be the incomplete sensitivity of the anti-LANA IFA assay. In this regard, Rezza *et al.*, testing an adult population for anti-HHV-8 antibodies by IFA, found that 28% were positive for anti-lytic antibodies and 2.1% were positive for anti-latent, thus suggesting a discrepancy on sensitivity among the two IFA tests [15]. Another possible explanation is that several seroepidemiolo-

logical studies conducted in Sicily focussed on the elderly and children rather than the adult population [14, 16-18].

Our data showed no statistically significant association with sex ($p = 0.4$). This confirms the findings of Vitale *et al.* that HHV-8 infection is present in Sicily with quite a high prevalence in both sexes (20.4% male; 20% female) [14]. On the other hand, it is contrary to the incidence rate of KS, which is much more common in males rather than females all over the world, and in Sicily as well.

In an American study, evidence was found of consistently higher anti-HHV-8 antibodies in anti-HIV positive gay men compared with anti-HIV positive women, thus resembling the lower KS prevalence among women [20].

As regards the anti-HIV positive group (group 1), a high prevalence of the virus (45.4%) was found. Studying group 1 thoroughly subdivided by risk factors, the trend that emerges is that homosexual men showed the highest anti-HHV-8 prevalence in comparison with the other exposure categories ($p < 0.001$). These data seem to parallel those suggested by Lennette *et al.* and Gao *et al.* who, respectively, reported 90% and 35% of HHV-8 seropositivity among anti-HIV positive gay men compared with lower rates among other groups [4, 6]. Concordance also exists with Rezza *et al.* who reported higher HHV-8 infection rates in anti-HIV positive homosexual men (from 38.9% to 83.3%) than anti-HIV positive IDUs (from 6.4% to 55.3%) or anti-HIV positive heterosexuals (from 0% to 25%) [15]. Rezza *et al.* suggested a strong association with sexual activity, as Kedes did in 1996 [5, 15]. Therefore, anti-HIV positives, both homosexuals and heterosexuals, appear to be at higher risk of contracting HHV-8 infection than anti-HIV negatives. Many factors could contribute to these high rates, including the number of sexual partners and type of sexual practices.

Regarding IDUs, serological evidence of infection with HHV-8 was detected in a relatively small number (6/102, 5.9%). This resembles that reported by Simpson *et al.* who found HHV-8 antibodies in 2/63 IDUs using ELISA assay and no positive reaction with IFA test for anti-latent antibodies [19]. Although these are preliminary data, they seem to confirm the much less efficient parenteral transmission of HHV-8 compared with the sexual route as above hypothesized.

The study also focussed on KS. Although our sample of participants with KS was very small we confirmed the findings of others by demonstrating that antibodies to HHV-8 were found in almost all KS patients (87.5%). Ser-raino *et al.* carried out a study focussing on 175 HIV negative individuals affected by different types of cancer and showing a high HHV-8 infection prevalence ranging from 16% (IFA) and 38% (ELISA) [16].

Presuming that KS develops only in HHV-8 infected immunocompromised people, some considerations come from this result. First of all, there may be a causal relationship between HHV-8 infection and the development of KS. In this regard, Whitby *et al.* have suggested that HHV-8 might predispose to the development of the tumour overall in areas considered at risk [17]. Secondly, in our study a large sample of anti-HHV-8 positive individuals was KS-free (82%). If we assume that HHV-8 infection promotes KS progression, high prevalence of anti-HHV-8 antibodies would be consistent with excess risk of development of KS, particularly if those concerned subsequently become immunocompromised.

In the KS group 12 of 14 anti-HHV-8 positives were gay men, the two seronegatives were women. The higher rate of KS among HIV-infected homosexual men may be explained by a higher HHV-8 infection rate among these persons possibly related to specific sexual practices, such as anal sex, as Martin *et al.* have already documented [21]. However, we need to have a more complete picture, for example by evaluating the self-administered questionnaire before making any decision regarding sexual practices involved. Besides, a possible systematic error could arise from the small sample.

The two HHV-8 seronegative women were both 52 years old and partners of anti-HIV positives. Based on strictly generation-gap and cultural diversities, in Sicily there is an ingrained stigmatization of sexual practices such as anal intercourse and they would be unlikely to admit to such practices.

In conclusion, our data showed the following: a low anti-HHV-8 prevalence among the general population; a high anti-HHV-8 prevalence among anti-HIV positive people; a low anti-HHV-8 prevalence among IDUs; support for the sexual transmission of HHV-8; and a causal relationship between HHV-8 infection and KS.

SUMMARY

Background: Human herpes virus 8 (HHV-8) is the putative infectious agent of multifactorial diseases, such as Kaposi's sarcoma (KS), primary effusion lymphoma and multicentric Castleman's disease. However, its exact mode of action as well as its transmission is still under investigation. Besides, little is known about its seroprevalence in the population.

HHV-8 epidemiology has been widely studied all over the world, demonstrating significant differences in distribution among various geographical areas and various population communities. Very few studies of HHV-8 seroprevalence have been conducted in Italy, particularly in Sicily which, along with other Mediterranean areas, is known to have high rates of KS incidence.

Between January 2001 and April 2002, 424 patients were consecutively recruited from three treatment

facilities. An Infectious Diseases Clinic provided 196 anti-HIV positive patients, both affected by AIDS and not. A further 122 anti-HIV negative intravenous drug users were recruited from drug treatment clinics, while as a control group from the Blood Bank 126 blood donors were recruited. Baseline serum samples were assayed for antibodies to HHV-8 latency-associated nuclear antigen (anti-LANA) by IFA (Viramed Biotech AG, Planegg/Steinkirken - Germany).

Anti-HHV-8 antibodies were found in 98 individuals (23.1%). HHV-8 reactivity was more common among anti-HIV positive patients (89/196, 45.4%, 95 C.I. 38.4-52.4) than in IDUs (6/102, 5.9%, 95 C.I. 1.2-16.2) and the control group (3/126, 2.4%, 95 C.I. 0.7-10.1). Overall, anti-HHV-8 antibodies were found in all three groups with large differences between groups.

RIASSUNTO

L'Human Herpes virus 8 (HHV-8) è stato individuato quale agente eziologico di alcuni tumori quali il sarcoma di Kaposi, i linfomi delle cavità basali del corpo e la malattia multicentrica di Castleman. Tuttavia le modalità di azione e di trasmissione del virus sono ancora da studiare; inoltre, anche la prevalenza del virus nella popolazione resta controversa.

L'epidemiologia dell'HHV-8 nel mondo è stata ampiamente studiata ed ha mostrato significative differenze nella sua distribuzione nelle diverse aree geografiche ed in differenti gruppi di popolazione.

Pochi sono stati gli studi di sieroprevalenza effettuati in Italia e particolarmente in Sicilia, dove, così come in altri paesi del Mediterraneo, vi è un'alta incidenza del sarcoma di Kaposi.

Nel periodo compreso tra Gennaio 2001 ed Aprile 2002, sono stati arruolati consecutivamente quattrocentoventiquattro soggetti in tre differenti ambulatori. Di questi, 196 anti-HIV positivi, affetti o no da AIDS, sono stati

arruolati presso un ambulatorio di Malattie Infettive; 110, anti-HIV negativi (soggetti che facevano uso di droghe per via endovenosa), presso un servizio per le tossicodipendenze; 126 donatori di sangue (gruppo di controllo) sono stati arruolati presso un Centro Trasfusionale.

I sieri di questi soggetti sono stati testati per anticorpi anti-latency-associated nuclear antigen (anti-LANA) tramite metodica IFA (Viramed Biotech AG, Planegg/Steinkirken - Germany).

Complessivamente, sono risultati anti-HHV-8 98 soggetti (23.1%); la positività all'HHV-8 è stata del 45.4% fra i soggetti anti-HIV positivi (89/196, 95 C.I. 38.4-52.4), del 5.9% fra quelli che facevano uso di sostanze per via endovenosa (6/102 95 C.I. 1.2-16.2) e del 2.4% nel gruppo di controllo (3/126, 95 C.I. 0.7-10.1).

Gli anticorpi anti-HHV-8, pur riscontrati in tutti i gruppi studiati, hanno mostrato una ampia variabilità di prevalenza.

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