Risk of malignant lymphoma associated with human herpesvirus-8: a case–control study in Spain

Kaposi’s sarcoma (KS)-associated herpesvirus, also known as human herpesvirus-8 (HHV-8), has been shown to be causally associated with KS, primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD) (Chang et al, 1994; Soulier et al, 1995; Cesareman et al, 1996). The three conditions are increased in immunocompromised states, as in human immunodeficiency virus (HIV) infection. The association of HHV-8 with other lymphoproliferative disorders in non-HIV-infected subjects remains controversial (Mikala et al, 1999). In the absence of HIV, HHV-8 DNA has been detected in T-cell PEL cells (Lechapt-Zalcman et al, 2001) and in lymphoma cells with plasmacytic differentiation, but not in cutaneous T- and B-cell lymphoma (Dupin et al, 1997) or in mycosis fungoides (Henghold et al, 1997).

A systematic serological evaluation of HHV-8 in HIV seronegative cancer patients failed to identify a significantly increased prevalence among patients with lymphoid neoplasms (Sitas et al, 1999). A similar study design in Uganda identified a slightly higher HHV-8 prevalence among patients with non-Hodgkin lymphoma (61%) and Hodgkin lymphoma (61%) as compared to the control population (50%). Differences were not statistically significant (Newton et al, 2003).

In this study, in Spain, we evaluated the association between HHV-8 infection and malignant lymphoma.

MATERIALS AND METHODS

The study subjects were recruited at four centres in Spain: Barcelona, two in Tarragona (Tortosa and Reus) and Madrid. Cases were consecutive patients newly diagnosed with a lymphoid malignancy between 1998 and 2002 and categorised according to the WHO Classification for Neoplastic Diseases of the Lymphoid Tissues (Jaffe et al, 2003). Controls were randomly selected from the hospital wards and outpatient clinics daily lists and synchronically identified with the cases. Controls were frequency matched to the cases by age, sex and study centre. Subjects with cancer, organ transplant and/or systemic infection as main diagnosis were not eligible as controls.

All included subjects were interviewed on demographic, medical and family history, and environmental exposures. Cases and controls provided a blood sample. Informed consent was obtained from all subjects prior to enrolment, and the Institutional Review Boards of the participating centres approved the study.

Of 700 eligible cases, 526 (75%) were included in the study, 28 refused to participate, 25 died before the interview, 116 did not provide a blood sample and five cases had no interview. Of 655 eligible controls, 599 (91.6%) were included in the study, 23 refused to participate and 33 did not provide a blood sample. Further details of the study have been described elsewhere (de Sanjose et al, 2004).

HHV-8 antibody and HIV detection

Antibodies against the lytic antigen K8.1 were tested using a enzyme-linked immunoassay (ELISA) as described previously (de Sanjose et al, 2002). Samples with optical densities (OD) below 1 were considered to be negative. Antibodies against the open-reading frame 73 (LANA) were tested by a similar ELISA using full-length baculo expressed LANA as antigen and serum diluted.
1:100. Optical densities values below 0.8 were considered to be negative. All the sera were tested blind to the disease status.

HIV infection status was determined by testing the sera with a licensed commercial ELISA (Abbott Diagnostics, North Chicago, IL, USA). All positive subjects were confirmed with Western blot.

**HHV-8 quantitative real-time PCR**

Peripheral blood mononuclear cells (PBMC) were tested for HHV-8 DNA by quantitative PCR in all subjects considered to be seropositive for either anti-K8.1 or anti-LANA as described previously (de Sanjose et al, 2002). DNA quality and cell quantitation was determined using real-time PCR for endogenous retrovirus 3 (Yuan et al, 2001).

**Statistical analyses**

Unconditional logistic regression was used to estimate the odds ratios (OR) and 95% confidence interval (95% CI) in order to measure the association between specific variables and the risk of lymphoma. Questionnaire variables were explored for their association with HHV-8 or with case–control status at P<0.10 and considered for inclusion in the regression model. The contribution to the models by other potential confounding variables was tested by means of the likelihood ratio test.

**RESULTS**

The study population consisted of 526 lymphoma cases and 599 controls. The average age at entry was 59.7 years among cases and 58.0 years among controls.

No differences were observed in the distribution of cases and controls in relation to age, sex, recruitment area, educational level and history of blood transfusion (Table 1). Of all the items explored, low educational level was significantly associated with higher prevalence of HHV-8 among controls, but the educational level did not modify the overall risk estimates (data not shown).

| Characteristics                | Controls | Lymphoma cases |
|-------------------------------|----------|----------------|
| Age (years)                   | 600 (100)| 529 (100)      |
| <43                           | 130 (21.7)| 95 (18.0)      |
| 43–56                         | 123 (20.5)| 106 (20.0)     |
| 57–67                         | 124 (20.7)| 106 (20.6)     |
| >74                           | 114 (19) | 124 (23.6)     |
| Sex                           |          |                |
| Males                         | 312 (52.1)| 287 (54.6)     |
| Females                       | 287 (47.9)| 239 (45.4)     |
| Recruitment area              |          |                |
| Barcelona                     | 500 (83.3)| 411 (78.1)     |
| Madrid                        | 55 (9.2) | 68 (12.9)      |
| Tarragona                     | 44 (7.3) | 47 (8.9)       |
| Educational level attained    |          |                |
| Primary                       | 237 (39.6)| 212 (40.3)     |
| Secondary                     | 60 (10.0)| 39 (7.4)       |
| Higher school                 | 17 (2.8) | 20 (3.8)       |
| University                    | 41 (6.8) | 44 (8.4)       |
| Other                         | 38 (6.3) | 33 (6.3)       |
| No degree                     | 138 (23.0)| 110 (20.9)     |
| Never school                  | 61 (10.2)| 67 (12.7)      |
| History of blood transfusion  | 160 (26.7)| 129 (24.5)     |

**Table 2** OR for HHV-8 (K8.1 or LANA) detection among lymphoma categories and age–sex matched controls

| Total, N | K8.1, N+ (%) | LANA, N+ (%) | K8.1 or LANA, N+ (%) | ORb (95% CI) |
|----------|--------------|--------------|------------------------|-------------|
| Controls | 598          | 24 (4)       | 17 (2.8)               | 32 (5.4)    | Ref     |
| All lymphoid neoplasm | 501 | 43 (8.6) | 16 (32) | 29 (5.8) | 1.04 (0.62–1.75) |
| B-cell lymphomas | 464 | 22 (4.7) | 15 (32) | 27 (5.8) | 1.03 (0.60–1.76) |
| Chronic lymphocytic leukaemia | 115 | 6 (5.2) | 3 (2.6) | 7 (6.1) | 1.16 (0.48–2.79) |
| Lymphoplasmacytic lymphoma | 19 | 4 (21.1) | 1 (5.3) | 4 (21.1) | 4.47 (3.14–14.85) |
| Marginal zone | 25 | 0 (0) | 0 (0) | 0 (0) | NA         |
| Splenic marginal zone | 26 | 0 (0) | 1 (3.8) | 3 (11.5) | 2.50 (0.68–9.14) |
| Plasma cell myeloma | 70 | 2 (2.9) | 2 (2.9) | 2 (2.9) | 0.53 (0.12–2.30) |
| Follicular lymphoma | 37 | 1 (2.7) | 0 (0) | 1 (2.7) | 0.46 (0.06–3.57) |
| Diffuse large B cell | 82 | 3 (3.7) | 3 (3.7) | 5 (6.1) | 1.08 (0.40–2.89) |
| Low-grade B and lymphoma B nos. | 9 | 2 (22.2) | 2 (2.2) | 2 (22.2) | 5.82 (1.07–31.73) |
| Other B-cell lymphoma* | 25 | 0 (0) | 0 (0) | 0 (0) | NA         |
| Hodgkin lymphoma | 56 | 1 (1.8) | 2 (3.6) | 3 (5.4) | 0.97 (0.27–3.43) |
| T-cell lymphomas | 37 | 1 (2.7) | 1 (2.8) | 2 (5.4) | 1.02 (0.23–4.50) |
| Mycosis fungoides/sezary | 16 | 0 (0) | 1 (6.7) | 1 (6.3) | 1.56 (0.19–12.94) |
| Other T cell* | 21 | 1 (4.8) | 0 (0) | 1 (4.8) | 0.84 (0.11–6.57) |

*Excluded one control and 17 lymphoma cases HIV positive and eight lymphoma cases organ allograft recipients. aOR adjusted age, sex and centre of recruitment. bOther B-cell lymphoma includes: nine, mantle lymphoma; two, hairy cell; one, Burkitt; three, high-grade lymphoma; nine, precursor B lymphoblastic lymphoma/leukaemia; one, high-grade lymphoma. cOther T-cell lymphoma includes: large granular lymphocytic leukaemia; two, peripheral T-cell lymphomas, unspecified; three, angioimmunoblastic T-cell lymphoma; three, angiocentric lymphoma; eight, anaplastic large-cell lymphoma CD30+; one, lymphoma T not otherwise specified, which was the only one HHV-8 positive. OR = odds ratio; HHV-8 = human herpesvirus-8; CI = confidence interval, HIV = human immunodeficiency virus. NA = not applicable.
controls (OR = 1.04, 95% CI = 0.62–1.75). Within B-cell lymphomas, HHV-8 infection was associated with an increased risk of lymphoplasmacytic lymphoma (OR = 4.47, 95% CI = 1.34–14.85) and of low-grade B-cell lymphoma not otherwise specified (NOS) and lymphoma NOS (OR = 5.82, 95% CI = 1.07–31.73).

HHV-8 DNA was identified in nine of 63 (13.0%) HHV-8 seropositive subjects, including those HIV-infected subjects and organ recipients and in none of the 132 seronegative subjects matched by age and sex to positive subjects and randomly selected from the pool of negatives. Two subjects showed a high HHV-8 copy number, one patient with a T-cell lymphoma (copy number per 10⁶ cells = 305 882), who was coinfected with HIV and one subject HIV negative with a B-cell lymphoma NOS, who had been previously diagnosed with MCD (copy number per 10⁶ cells = 24 444).

**DISCUSSION**

In our study, no overall differences in the HHV-8 prevalence could be found between cases and controls. However, HHV-8 was strongly associated with two subgroups, a four-fold increased risk of lymphoplasmacytic lymphoma and a five-fold increased risk of low-grade B-cell lymphoma and B-cell lymphoma NOS. The detection of HHV-8 DNA in PBMC showed that only one in seven seropositive subjects had viral DNA detectable in blood. A possible aetiologic association with HHV-8 was suspected in two subjects with a very high viral DNA copy number in PBMC. One was an HIV-positive subject with a T-cell lymphoma. This observation is in agreement with a recent case report of a PEL of T-cell origin associated with HHV-8 was suspected in an HIV-negative patient (Lechapt-Zalcman et al., 2001). The other subject was a B-cell lymphoma NOS with a previous diagnosis of MCD, suggesting that HHV-8 in this case is likely to play an aetiological role. This observation is in agreement with other reports where MCD has also been associated with other lymphoid neoplasms such as plasmablastic lymphoma and the recently proposed germinotropic lymphoproliferative disorder (Du et al., 2001) that involves a proliferation of plasmablasts.

In our data, we also observed a slight increased risk associated with HHV-8 for splenic marginal zone lymphomas. These neoplasms can also harbour plasma cells with cytoplasmatic proliferation of plasmablasts.

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