

## Short communication

# Human herpes virus-6 seroprevalence and leukaemias: a case-control study

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**Summary** The relationships between acute myeloid leukaemia (AML), acute lymphocytic leukaemia (ALL), chronic myeloid leukaemia (CML) and refractory anaemia with excess of blasts (RAEB) and human herpes virus (HHV)-6 antibody level were investigated in a multicentre case-control study. An association between increased HHV-6 seropositivity and geometric mean titre ratio with AML was shown: *P* for trend = 0.022, adjusted odds ratio 1.20, 95% confidence interval 1.07–1.33 respectively. No association was found between HHV-6 and ALL, CML or RAEB.

**Keywords:** HHV-6; leukaemia; viral aetiology

Human herpes virus-6 (HHV-6) was first isolated from peripheral blood leucocytes of patients with lymphoproliferative disorders, including lymphoma and leukaemia (Salahuddin et al, 1986). HHV-6 is predominantly a CD4+ T-lymphotropic virus, although other cell types such as B and CD8+ T-lymphocytes, monocytes/macrophages, megakaryocytes and natural killer (NK) cells can be infected in vitro (Ablashi et al, 1988a; Braun et al, 1997). As for other herpesviruses, primary infection occurs mainly in childhood, being the causative agent of exanthem subitum (Braun et al, 1997). HHV-6 probably remains latent in the host after primary infection since it can be reactivated in immunocompromised patients such as those receiving a bone marrow transplant or those infected with HIV; such patients may present a broad spectrum of clinical disorders in response to infection ranging from asymptomatic infection to illnesses such as interstitial pneumonia or encephalitis (Ragona et al, 1995; Braun et al, 1997).

A role for HHV-6 in haematological malignancies, such as lymphomas has been suggested, though it is primarily a lytic virus; the transforming potential of its DNA has been demonstrated in vitro (Razzaque, 1990). Genomic DNA and several subgenomic clones from HHV-6 variant A can produce malignant transformation of NIH 3T3 cells and human keratinocytes (Razzaque, 1990; Razzaque et al, 1993; Thompson et al, 1994). HHV-6 genome integration has been found in peripheral blood mononuclear cell DNA (Luppi et al, 1993) and HHV-6 DNA sequences have been identified in the pathologic tissue of Hodgkin's disease (Di Luca et al, 1994), non-Hodgkin's lymphoma (Di Luca et al, 1994) and in peripheral blood mononuclear cells from patients with acute lymphoblastic leukaemia (ALL) (Luka et al, 1991) and with T-cell chronic lymphoproliferative disease (Braun et al, 1995).

In addition, HHV-6 seroprevalence was shown to be higher in patients with Hodgkin's disease or acute myeloid leukaemia (AML) as compared with controls (Clark et al, 1990).

In the present study we investigated the relationship between AML, ALL, chronic myeloid leukaemia (CML), refractory anaemia with excess of blasts (RAEB) and HHV-6 antibody level in a multicentre case-control study on risk factors for leukaemias (Mele et al, 1994).

## PATIENTS AND METHODS

This study was conducted in three Italian cities, Roma (central Italy), Bologna and Pavia (northern Italy) between 1 November 1986 and 31 March 1990.

## Recruitment of study subjects

The method of study subject recruitment and data collection is described in detail elsewhere (Mele et al, 1994). Cases were 15 years of age or older with newly diagnosed AML, ALL, CML, or RAEB. Diagnostic criteria were based on the revised French-American-British classification of bone marrow aspirates for acute leukaemias and RAEB; diagnosis for CML was based on typical and cytogenetic laboratory features. Controls were recruited during the study period among outpatients without haematological malignancies and were seen in the same hospital where cases were identified.

The control group was selected by taking the first five outpatients in Rome and the first three in Bologna and Pavia seen on a random day each week. Subjects who had received blood product transfusions were excluded from the study.

A standard precoded questionnaire on medical history, behavioural habits and environmental exposure was administered to both cases and controls. Informed consent was obtained from all study participants.

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**Table 1** Distribution of cases and controls by HHV-6 titre

Titres	Negative at						Total <i>n</i>	Geometric means titres <sup>a</sup>
	1:20 <i>n</i> (%)	1:20 <i>n</i> (%)	1:40 <i>n</i> (%)	1:80 <i>n</i> (%)	1:160 <i>n</i> (%)	1:320 <i>n</i> (%)		
Control	250 (29.3)	88 (10.3)	360 (42.2)	115 (13.5)	35 (4.1)	5 (0.6)	853	29.2
AML	39 (24.5)	15 (9.4)	65 (40.9)	23 (14.5)	12 (7.5)	5 (3.1)	159	35.0
ALL	16 (29.1)	3 (5.5)	25 (45.5)	5 (9.1)	5 (9.1)	1 (1.8)	55	32.3
RAEB	20 (34.5)	1 (1.7)	22 (37.9)	11 (19.0)	4 (6.9)	0 (0.0)	55	30.8
CML	31 (27.2)	18 (15.8)	45 (39.5)	10 (8.8)	8 (7.0)	2 (1.8)	114	29.9

<sup>a</sup>Sera negative at the lowest dilution have been allocated a titre of 1:10. AML, acute myeloid leukaemia; ALL, acute lymphoid leukaemia; RAEB, refractory anaemia with excess of blasts; CML, chronic myeloid leukaemia.

**Table 2** Distribution of cases and controls and geometric mean titres (GMT) by demographic characteristics

	Control			AML			ALL			RAEB			CML		
	<i>n</i>	GMT	<i>P</i>	<i>n</i>	GMT	<i>P</i>	<i>n</i>	GMT	<i>P</i>	<i>n</i>	GMT	<i>P</i> <sup>a</sup>	<i>n</i>	GMT	<i>P</i> <sup>a</sup>
Age															
15–19	41	30.0		8	40.0		12	31.8		0	–		1	10.0	
20–29	194	30.4		19	33.3		14	40.0		1	10.0		7	44.2	
30–39	159	30.8	0.580	20	33.6	0.693	7	32.8	0.876	2	20.0	0.064	20	28.3	0.558
40–49	131	29.6		25	27.9		3	31.8		3	10.0		18	30.5	
≥ 50	328	27.5		87	37.5		19	27.8		52	34.1		68	29.5	
Sex															
Male	313	29.5	0.749	75	31.2	0.212	22	29.2	0.513	36	31.2	0.895	71	28.1	0.355
Female	540	29.0		82	37.4		33	34.5		22	30.1		43	33.0	
Study centre															
North	353	27.1	0.001	71	35.6	0.797	31	26.7	0.181	25	25.2	0.057	37	26.4	0.032
Centre	500	32.5		88	34.2		24	37.4		33	40.0		77	38.5	
Education level															
High <sup>b</sup>	311	31.6	0.045	44	33.6	0.937	17	47.1	0.040	9	31.8	0.988	27	21.1	0.020
Low <sup>c</sup>	534	28.1		108	33.2		37	27.0		46	31.9		82	33.2	
Total	853	29.2		159	35.0		55	32.3		58	30.8		114	29.9	

<sup>a</sup>*P* for trend. <sup>b</sup>≥ 9 years of schooling; <sup>c</sup>≤ 8 years of schooling. Abbreviations as in Table 1.

## Laboratory methods

All sera were stored at –20°C and subsequently tested. Sera were coded prior to laboratory analysis to ensure that investigators were blind to the status of each sample. Coded sera were serially diluted and tested for the presence of antibodies against HHV-6 by indirect immunofluorescence assay (IFA) on HSB-2 cells infected with HHV-6 (GS strain) as viral capsid antigen source (Ragona et al, 1994). Mock infected cells were used to evaluate non-specific background fluorescence. Antibody titres were determined by two independent investigators and expressed as the highest serum dilution yielding detectable immunofluorescence on an appreciable fraction of the antigen-positive cells.

## Statistical analysis

Geometric mean titres (GMT) were computed for each group of cases and compared with those of controls by the ratio and its 95% confidence interval (CI). In calculating GMT, sera were considered negative at the lowest dilution as 1:10. Results were similar when omitting negative sera from analysis. Multiple logistic regression was used to estimate adjusted odds ratios (OR) and 95% CI for each case group as compared with controls. BMDP software was used for all statistical analyses.

## RESULTS

Serum samples from 450 out of 636 recruited cases and from 948 out of 1193 recruited controls were tested. No differences regarding age, sex, education and leukaemia type were observed between tested and untested cases. Eighty-five controls and 74 cases who previously received blood product transfusions (e.g. blood and pooled plasma products) were excluded from the study. Thus, 386 cases enrolled at the time of leukaemia diagnosis, and 853 controls aged 15 years or older were included in this study. Iron deficiency and thalassaemia minor accounted for 43% of control diagnoses; 36% of the control group had no underlying haematological disorder.

In the control group, seropositivity at 1:80 serum dilution was placed at 82 percentile (Table 1); therefore, sera with titres at ≥ 1:80 were considered positive. The seroprevalence population was 18.2%; seroprevalence of the cases were 15.1% (AML), 20.0% (ALL), 25.5% (RAEB) and 17.6% (CML).

Cases and controls from central Italy (Roma) displayed higher seropositivity than cases and controls from northern Italy (Bologna and Pavia). Differences in GMT were found according to education level among controls, ALL and CML cases, whereas no relation with age and sex was found (Table 2).

Multiple logistic regression analysis showed that only AML presented significantly increased HHV-6 seropositivity as

**Table 3** Odds ratios<sup>a</sup> by HHV-6 titre<sup>b</sup>, *P* for trend and GMT ratios in cases and controls

Titres	1:80	1:160	1:320	<i>P</i> for trend	GMT ratios in cases and controls
Control	1.00	1.00	1.00		1.00
AML	1.04 (0.61–1.75)	1.56 (0.74–3.29)	5.58 (1.40–22.3)	0.022	1.20 (1.07–1.33)
ALL	0.61 (0.23–1.61)	2.47 (0.90–6.82)	3.76 (0.41–34.9)	0.121	1.11 (0.99–1.24)
RAEB <sup>c</sup>	1.62 (0.77–3.43)	1.64 (0.52–5.21)	–	–	1.05 (0.94–1.18)
CML	0.67 (0.32–1.38)	1.89 (0.82–4.35)	4.92 (0.85–28.5)	0.102	1.02 (0.92–1.14)

<sup>a</sup>Adjusted for sex, age (five groups), study centre and instruction. <sup>b</sup>Titres  $\leq 40$  have been used as reference group. <sup>c</sup>Four groups of age. Abbreviations as in Table 1.

compared with controls at higher serum dilutions (OR = 5.58, 95% CI 1.40–22.3) (Table 3); similar results were obtained when GMT ratios were analysed. In addition, the OR increased with increasing seroreactivity in the IFA assay (*P* for trend = 0.022).

## DISCUSSION

Our present study, the largest serological case-control investigation conducted on patients with haematologic malignancies who had not yet undergone immunosuppressive chemotherapy, identified a slight significant association between increased HHV-6 seropositivity and GMT ratio with AML (*P* for trend = 0.022, adjusted OR 1.20, 95% CI 1.07–1.33) respectively. The results presented here are in agreement with those obtained in a case-control study in which the prevalence of antibodies to HHV-6 in AML patients was higher than in the control group: GMT = 2.66, 95% CI 1.90–3.74 (Clark et al, 1990). Furthermore, two cases of monoblastic leukaemia were reported in which malignant cells contained HHV-6 DNA, antigens and immature viral particles (Krueger et al, 1994), whereas, in another study, HHV-6 DNA was undetectable by blot hybridization in ten AML patients (Josephs et al, 1988).

The negative results regarding an association between HHV-6 antibody titers and ALL, CML, or RAEB in the present study are also consistent with the literature. Ablashi et al (1988b) found higher levels of HHV-6 antibodies in a small group of children with ALL as compared with normal subjects. However, Levine et al (1992a) found no significant differences in antibody titers between 50 patients with ALL and 50 blood donors. This last finding suggests that the association previously reported for HHV-6 and ALL was a result of the study population age, rather than a true relationship.

Seroepidemiological evidence indicates that HHV-6 infection (IgG antibody titres of  $> 1:20$  by IFA) is common and widespread, with a proportion of subjects ranging from 20% to 80% (Okuno et al, 1989; Levy et al, 1990; Yanagi et al, 1990). In this study, 18.2% of the control population and an average of 22.3% (range 15.1–25.9%) of the cases were seropositive at 1:80 serum dilution by IFA. This positivity rate is actually lower than those previously reported (Okuno et al, 1989; Levy et al, 1990; Yanagi et al, 1990; Torelli et al, 1991). Furthermore, in Italy, Torelli et al (1991) found antibodies against HHV-6 in 54% of 200 blood donors at a 1:40

serum dilution. The differences found in HHV-6 seroprevalence may result from the use of different serological assays and criteria of positivity. The majority of the epidemiological studies were performed with IFA, but the limiting titre for seropositivity varied. The limiting titre adopted in this study was 80, which includes 82% of the control population.

Other factors that may account for differences in HHV-6 seroprevalence include the definition of the control population: there is a possibility that HHV-6 is transmitted by the transfusion of blood or blood components (Wilborn et al, 1994). We excluded those patients who received blood product transfusions from both cases and controls. The possibility that differences in antibody titres could be detected using different isolates remains to be investigated. In a previous study on HHV-6 infection in Italy no variations in seroprevalence or in antibody GMT were observed assaying the sera against the strains G.S., U1102, or Z29 infected cells (Ragona et al, 1994).

Several studies conducted in healthy individuals showed that HHV-6 seroprevalence can be influenced by age, sex and geographic/ethnic origin, titres being higher in children than in adults, in females than in males and Ghanaians compared with Asians and White Americans (Levine et al, 1992b; Ragona et al, 1994; Braun et al, 1997). In our study no differences in seropositivity were observed on the basis of age or sex.

In cancer patients, therapy may play a role in antibody titres, and the high levels of HHV-6 antibody may be a result of therapy rather than relating to the neoplastic disease. The possibility of reactivation of HHV-6 infection by therapy-induced immunosuppression can be excluded because sera were drawn at the time of diagnosis, i.e. well before chemotherapy was administered. In addition, none of the cases studied here had received previous treatments for past malignancies. Furthermore, IFA reactivity was not elevated among cases, as might be expected if there was a general activation of HHV-6 infection. However, because the comparison was performed between values obtained at 1:80 and higher serum dilutions, we conclude that the titre of anti-HHV-6 antibodies and not the frequency of infection is significantly higher in AML individuals than in control subjects. This finding is not clear; however, serology data indicated that immune system of AML patients is activated against HHV-6 antigens. Furthermore, the serological assays currently used for identifying HHV-6 infection do not differentiate between persistent, latent or active

infections. Detection of HHV-6 DNA in serum or plasma has been shown to be a marker of active infection (Secchiero et al, 1995), but does not clarify if HHV-6 has a possible casual role or viral reactivation is triggered by the illness itself. In conclusion, a slight significant association between HHV-6 antibody titres and AML patients not previously treated was observed, while no significant association was found between HHV-6 antibodies and ALL, RAEB, or CML. Further studies should be performed to further explore the relationship between HHV-6 infection and AML leukaemias.

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