

# Seroepidemiology of human herpesvirus 6 infection in normal children and adults in Spain

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## Summary

We have studied the prevalence of anti-human herpesvirus 6 (HHV-6) antibodies in different population groups from Spain. Serum samples from 271 children between 0 and 15 years old, 512 intravenous drug addicts (IVDA) (262 seropositive for human immunodeficiency virus (HIV)) and 254 healthy individuals (100 pregnant women). The indirect immunofluorescence technique was used for antibody investigation from initial dilutions of 1 : 40. Seroprevalence studies showed antibody presence in 37.6%. The highest positivity was found in the group of children (49.4%,  $P < 0.001$ ), followed by the pregnant women (39%), the IVDA (34%) and healthy subjects (28%). When the IVDA group was split into HIV positive and HIV negative, no significant difference was found between them ( $P = 0.45$ ). Antibody titres oscillated between 1 : 40 and 1 : 2560, with the greatest frequency at 1 : 40 in both male and female patients. A statistically significant difference ( $P < 0.05$ ) was only found between sexes in the control group.

Key words: Human herpesvirus 6, antibodies, prevalence, intravenous drug addicts, HIV

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## Introduction

Since the discovery of human herpesvirus 6 (HHV-6) by Salahuddin et al.<sup>1</sup>, many studies have been undertaken to analyse its molecular structure, pathogenesis, epidemiology and diagnosis. Likewise, its associated pathology remained unknown until Yamanashi's studies were published<sup>2</sup>. At the present time it is considered to be the agent responsible for exanthema subitum in newborns, and in its acute stage to be associated with hepatitis and related to chronic asthenia and proliferative syndromes. It has also been related to human immunodeficiency virus (HIV) in that it could shorten the interval time between infection and illness development<sup>3, 8</sup>.

In view of the ubiquitous nature of this virus, we propose four main objectives for this study: to investigate the prevalence of antibodies in different healthy population groups, to study the evolution of antibodies in a

group of children from birth to adolescence, to evaluate the possibility of percutaneous transmission as a mechanism of spread and to investigate whether infection by HIV is associated with a greater incidence of HHV-6.

## Materials and methods

Serum from 1037 individuals belonging to four different population groups was collected and analysed. Group 1 consisted of 271 children between 0 and 15 yr old, in order to determine the period of time in which seroconversion took place and when the primary infection appeared. This group was divided into different age subgroups: 0–3 months, 4–6 months, 7–9 months, 10–12 months, 2–5 yr, 6–10 yr and 11–15 yr. Group 2 consisted of 512 intravenous drug addicts (IVDA) between 17 and 32 yr old. The aim was to compare this group with another group of healthy individuals so as to evaluate the significance of percutaneous transmission. Individuals in group 2 were divided into two subgroups: 250 who were seronegative for HIV and 262 who were seropositive, in order to elucidate whether seropositivity was associated with a greater prevalence of anti-HHV-6 antibodies. Group 3 consisted of 154 healthy individuals between 29 and 50 yr old. Group 4

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was made up of 100 healthy pregnant women between 20 and 35 yr old, so as to compare seropositivity in this group with that shown by children between 0 and 3 months old, and so complete the evolution curve of seropositivity levels in the infant population.

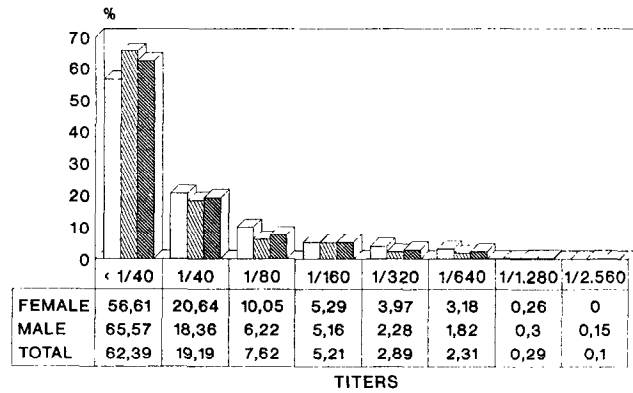
IgG antibody to HHV-6 was determined by an indirect immunofluorescent antibody (IFA) assay described elsewhere, that used twofold dilutions (starting at 1 : 40) of serum and HSB-2 cells infected with an HHV-6 isolate containing both the HHV-6A and HHV-6B genomes<sup>5</sup>.  $\chi^2$  and Mann's U statistical analyses were applied to the results.

**Results**

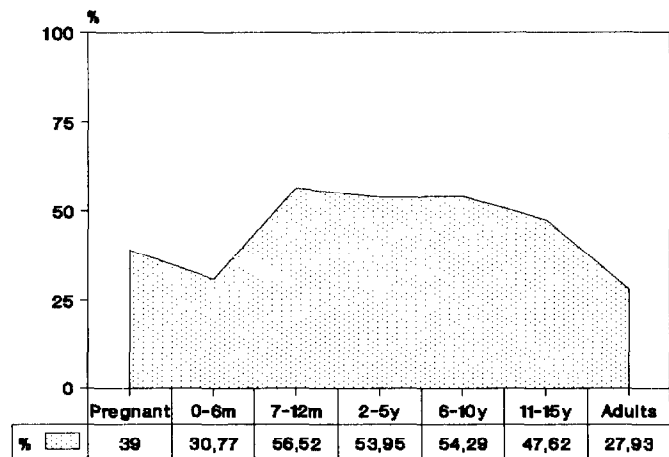
Seroprevalence studies showed anti-HHV-6 antibody presence in 390 individuals (37.6%) (Table 1). The highest positivity was found in the group of children (49.4%,  $P < 0.001$ ), followed by the pregnant women (39%), the IVDA's (34%) and group 3 (28%). When the IVDA group was split into HIV positive and HIV negative no significant difference was found between them ( $P = 0.45$ ).

The general distribution of the titres according to sex is given in Figure 1. Antibody titres oscillated between 1 : 40 and 1 : 2560, with the greatest frequency at 1 : 40 in both male and female patients. No statistically significant difference was found between sexes ( $P = 0.16$ ). Table 2 shows the different percentages of seropositivity according to sex in the children, IVDA's and group 3. A statistically significant difference ( $P < 0.05$ ) was only found between sexes in group 3.

Figure 2 shows the seroprevalence of anti-HHV-6 in children (divided into subgroups according to age), pregnant women and healthy adults. Seropositivity in



**Figure 1.** Distribution of the titres according to sex.  $P = 0.16$ ;  $\chi^2$ ; □, female; ▨, male; ▩, total.



**Figure 2.** Seroprevalence of anti-HHV-6 antibodies according to age. m, Month; y, year.

**Table 1.** Seroprevalence of anti-HHV-6 antibodies according to groups

	Group 1		Group 2		Group 3		Group 4		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Seropositives	134	49.45	174	33.98	43	27.92	39	39	390	37.6
Seronegatives	137	50.55	338	66.02	111	72.08	61	61	647	62.4
Total	271	100	512	100	154	100	100	100	1037	100
Statistics	$P < 0.001$		NS		NS		NS		—	

NS, Not significant.

**Table 2.** Distribution of seropositivity according to sex in groups

Group	Sex	Seropositives No.	Seropositives %	Seronegatives No.	Seronegatives %	$\chi^2$	Total
Group 1	Female	61	48.41	65	51.59	NS	126
	Male	73	50.34				
Group 2	Female	34	45.33	41	54.67	NS	75
	Male	140	32.04				
Group 3	Female	30	38.96	47	61.04	$P < 0.05$	77
	Male	13	16.89				
Group 4	Female	39	39	61	61	—	100
Total		390	37.6	647	62.4	—	1037

Group 1 vs. group 2, group 3 and group 4 :  $P < 0.001$ ;  $\chi^2$ : NS, not significant.

children decreased in time from 31.4% in the first 3 months to 25% in the 4–6 month period. It subsequently increased to 75% between 6 and 9 months to decrease again and level out at about 50%. This value was lower for healthy adults (27.9%), with women showing a significantly higher ( $P < 0.05$ ) seropositivity (38.9%) than men (16.8%). Seropositivity was 39% in the group of pregnant women.

## Discussion

Although indirect enzyme-linked immunosorbent assay (ELISA) methods to determine anti-HHV-6 antibodies exist, they have not become universally established and immunofluorescence continues to be widely used. In this study and previously<sup>9</sup>, sample dilutions were started at 1 : 40, to avoid false positives and to decrease non-specific immunofluorescence.

Different studies from the USA, Japan and Europe reported levels of HHV-6 infection in the whole population of between 60 and 90%<sup>3,4</sup>. However, Civeira et al.<sup>10</sup> and Lozano et al.<sup>11</sup> found only 35% of the population infected, which agrees with our figure of 37.6%. Positivity and antibody titres change with age and socioeconomic conditions of the population<sup>12,13</sup> and with the initial dilutions of the sera used.

In our series 49% of the children reached antibody titres between 1 : 40 and 1 : 640, with a slightly, although not significantly higher prevalence in males. Brown et al.<sup>12</sup> obtained a seroprevalence of 81.6% using similar dilutions. Okuno et al.<sup>14</sup> reported 70% with a dilution of 1 : 40 and Levy et al.<sup>15</sup> found 80.2% positivity with a 1 : 10 dilution, in a similar population group. These prevalences are all higher than that obtained by us for a population with similar characteristics. This could be due to geographical differences, or to the higher dilution used in our study.

The antibody titres observed in the sera from infants were almost uniformly distributed between 1 : 40 and 1 : 640. A greater frequency of the 1 : 40 titre was observed in males. Focusing on the results from infants up to 1 yr, antibodies were detected in 40.3%. A higher incidence was seen between 0 and 3 months, decreasing to a minimum in the group between 4 and 6 months and increasing again in children between 7 and 9 months old. This could be explained by the progressive loss of specific maternal antibodies until 6 months and the beginning of primary infection in the older population subgroup. These specific antibodies seem to follow the classic pattern of IgG evolution. In children between 1 and 15 yr seroprevalence decreased with age, but this difference was only significant when the 1 yr and 15 yr groups were compared. This may be due to a first stage of primary infection, occurring in the later half of the first year, followed by a decrease in antibody level in later age groups with stabilization at a lower antibody level. On comparing these results with others a similar pattern was observed by Knowles et al.<sup>16</sup> and Bagg et al.<sup>17</sup>, whereas in the studies by Fox et al.<sup>18</sup>, Okuno et al.<sup>14</sup> and Levy et al.<sup>15</sup> the evolution curve was

displaced with the primary infection taking place between 5 and 10 yr. The fact that these studies were carried out in different geographical areas (Anglosaxon regions in the former and the USA and Japan in the latter) may account for these divergent observations.

In the epidemiological study of HHV-6, intravenous or sexual transmission has been considered as a possible factor affecting virus distribution<sup>15,18</sup>. However, in the present study, no difference between seroprevalence in the IVDA group and group 3 was found. The HIV positives showed a slightly higher (although this was not statistically significant) positivity than HIV negatives, in agreement with other reports<sup>12</sup>. On the other hand, Lozano et al.<sup>11</sup> and Fox et al.<sup>18</sup> observed a greater number of seropositive individuals in the HIV-positive homosexuals.

No difference in seropositivity between pregnant and group 3 women was observed, although the former presented a higher frequency of the 1 : 80 titre. These results agree with those of another study recently carried out in a close geographical area<sup>11</sup>. A higher seroprevalence in pregnant women was described, but 1 : 20 dilutions were used<sup>19</sup>.

Regarding our control group (group 3), there was a significant difference ( $P < 0.01$ ) between females and males. This higher index of seropositivity in females was also observed by Briggs et al.<sup>13</sup> who found that females older than 15 yr showed a greater frequency of positivity than males of the same age.

It can be concluded that seroprevalence against HHV-6 is not excessively high in our study area, with a higher seroprevalence in female members of the population. A greater, although not significantly different percentage of seroprevalence was found in IVDA's when compared with group 3. Despite the increasing seroprevalence observed in the HIV-positive group, it cannot be stated that infection by HIV increases susceptibility to HHV-6 infection. The seroprevalence in newborn infants was similar to that observed in pregnant women, followed by a decrease in the first few months with an increase between 7 and 9 months, pointing to this date as the moment of primary infection.

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