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Seroepidemiology of human herpesvirus 8 (HHV-8) infection in injecting drug users

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SUMMARY

A cross-sectional study was carried out in injecting drug users (IDUs) from Greece to assess the seroprevalence of human herpesvirus 8 (HHV-8) and to identify potentially associated risk factors. A total of 288 IDUs were tested for K8.1 antibodies to HHV-8 lytic antigen. Associations between HHV-8 serostatus and potential risk factors were examined using univariate and multivariate logistic regression analysis. Seroprevalence of HHV-8 was 24·3% (95% CI 19·5–29·7), increasing with age from 19·4% in those aged <30 years to 52·9% in those aged $\geqslant 40$ years (P for trend $=0{\cdot}003$). No statistically significant associations between HHV-8-positive status and gender, educational level, age at first drug injection, needle sharing, number of imprisonments, complications from drug overdose, HIV and HCV were observed. In the multivariate logistic regression analysis, older age ($\geqslant 40$ *vs.* <40 years, OR 3·30, 95% CI 1·14–9·56) and report of septicaemia/abscess (yes *vs.* no, OR 1·80, 95% CI 1·01–3·18) were each independently associated with higher HHV-8 seroprevalence. HHV-8 is highly prevalent in the IDU population in Greece. The independent association between HHV-8 and reported abscess or septicaemia supports the hypothesis that poor hygiene conditions in the setting of drug injection may contribute to HHV-8 transmission.

Key words: HHV-8; injecting drug users (IDUs), risk factors, seroepidemiology.

INTRODUCTION

Human herpesvirus 8 (HHV-8) is an oncogenic virus with a causal role in several diseases, such as Kaposi's sarcoma (KS), primary effusion lymphoma, and multicentric Castelman's disease [1–4]. Although the mode of HHV-8 transmission has been investigated extensively, it is still not understood. Transmission appears to occur by both sexual and non-sexual routes, and epidemiological patterns differ by population and geographical location [5].

The high incidence of AIDS-associated KS in men who have sex with men [6–8] and the high seroprevalence of HHV-8 in this group [9–11] have provided evidence for sexual transmission in

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homosexual men, whereas evidence of heterosexual spread of the virus is inconsistent [9, 12–15]. Other possible transmission routes, such as blood-borne exposures, are being investigated [16]. In this regard, studies on HHV-8 infection and injecting drug use have yielded conflicting results. Some studies have found an association with frequency of drug injection [14, 17, 18], whereas an investigation of HHV-8 in Dutch injecting drug users (IDUs) found little evidence of viral transmission through drug injection [19]. Furthermore, results of another survey involving IDUs from San Francisco, after controlling for sexual behaviour, suggested that HHV-8 seroprevalence increased with duration of drug use [20]. An additional investigation conducted in HIV-infected pregnant women found that HHV-8 was associated with injecting drug use [21].

Very few studies of HHV-8 seroprevalence have been conducted in Greece [22, 23], which along with other Mediterranean areas is known to have a high incidence of KS [24, 25]. In addition, data are lacking for rates of HHV-8 infection in the IDU population in this area. The objective of the current study was to assess the seroprevalence of HHV-8 infection in a group of IDUs from Greece. To elucidate modes of HHV-8 transmission in this population in Greece, we also attempted to identify the associations between HHV-8 seropositivity and potential risk factors, including injecting drug use.

METHODS

Subjects – study design

This cross-sectional seroprevalence study involved a sample of 288 heroin IDUs from Greece participating in a drug-treatment programme at the Eginitio Psychiatric Hospital. All participants were asked to provide a blood sample. Trained staff obtained informed consent and interviewed participants and data were collected anonymously. The Ethics Committee of the Athens University Medical School approved the study. Participants were asked about demographic characteristics and their injecting drug history, including age at first injection, duration of injecting drug use, and use of shared needles. Additional information included the number of imprisonments since their first injection and complications associated with drug-use overdose. In addition, participants were asked whether they had ever suffered abscess/septicaemia and whether they had hepatitis. Serum samples were stored at $-80\,^{\circ}C$ until required.

Laboratory methods

All sera were analysed for antibodies against HHV-8 as previously described [23, 26]. Specifically, a second-generation ELISA was used to detect antibodies directed against the lytic phase glycoprotein K8.1. Briefly, *Escherichia coli*-expressed K8.1 recombinant protein was diluted 1:5000 in 0·05 M carbonate/bicarbonate buffer at pH 10·0. Serum samples were diluted 1:20. A sample was considered to be seropositive if the optical density (OD) was $\geqslant 0{\cdot}174$. In a previous evaluation of the performance characteristics of the present assay in the Greek population, the sensitivity and specificity of the test for this specific cut-off value were 94% and 100%, respectively [23].

Hepatitis B core antibody (anti-HBc) was tested by Axsym-MEIA (Abbott Diagnostics Division, USA). Participants were also tested for HCV antibodies (anti-HCV) using a third-generation MEIA (Abbott Laboratories, USA); antibody testing for HIV (anti-HIV) was performed using Axsym-MEIA (Abbott Diagnostics Division) with confirmation by Western blot (GeneLabs Diagnostics, 2.2 assay).

Statistical analysis

The prevalence of antibodies to HHV-8 was calculated and expressed as a percentage together with the 95% confidence interval (CI). Furthermore, age-specific prevalence rates (along with 95% CI) for the Greek IDUs were calculated and compared to the corresponding prevalences of 955 healthy urban workers from Greece; this group of healthy urban workers was previously tested for the presence of anti-HHV-8 by the same laboratory using the same immunoenzyme assay [23].

Univariate analysis using the χ^2 test and t test, as appropriate, was performed to examine the presence of associations between HHV-8 serostatus and all questionnaire variables. A multivariate logistic regression model was used to identify risk factors independently associated with the presence of HHV-8 antibodies. Specifically, a model containing all variables whose univariate test had a P value $<0{\cdot}25$ was considered. Then, variables that did not contribute to the model, based on their Wald statistic, were eliminated and the new model was compared to

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the old through the likelihood ratio statistic. With the exception of the duration of injecting drug use, variables whose exclusion gave a non-significant likelihood ratio statistic ($P<0{\cdot}05$) were omitted from the model.

RESULTS

Characteristics of the study population and HHV-8 seroprevalence

Of the 288 IDUs included in the study, 230 were males (79·9%) and 58 (20·1%) were females. The age of the study population ranged from 17 to 58 years with a mean value (S.D.) of 29·3 (6·2) years. A total of 15·6% of the study population had an education level above high school. Most participants reported a relatively long history of injecting drug use. In this regard, the mean (S.D.) duration of injecting drug use was 8·2 (5·9) years, whereas the mean age at first drug injection was 21·0 (5·0) years. The majority of the study population (52·8%) reported that they had never injected with a used syringe, and 35 (12·1%) reported having been in prison more than 10 times since their first drug injection. A total of 34·1% reported a history of abscess/septicaemia. Anti-HBc was detected in 53·0% of the study population, and 20·0% reported having suffered from hepatitis. The majority of the study population (90·5%) was HCV positive, while the HIV prevalence rate was extremely low with only one patient being HIV positive (0·35%); as a result of this low frequency, this variable was not considered for further analyses.

Of the 288 participants, 70 were reactive for anti-HHV-8 antibodies, indicating an overall seroprevalence of HHV-8 in IDUs from Greece of 24·3% (95% CI 19·5–29·7). The age-specific prevalence of HHV-8 in IDUs compared to healthy workers was 19·4% *vs.* 6·5% (<30 years old); 27·7% *vs.* 5·7% (30–39 years old); and 52·9% *vs.* 9·1% (⩾40 years old), with no overlap in the corresponding 95% CI [23].

Univariate analysis

Table 1 illustrates the univariate associations between HHV-8 serostatus and participant characteristics. In the univariate analysis, few variables were associated with positive HHV-8 serology. HHV-8 infection was associated with advancing age (P for trend $=0{\cdot}003$). More specifically, HHV-8 seroprevalence increased with age from 19·4% in participants aged <30 years to 52·9% in those aged ⩾40 years. Furthermore, seroprevalence was higher in IDUs with a history of abscess/septicaemia (32·6% *vs.* 20·1%, $P=0{\cdot}019$). An association of borderline significance was detected between HHV-8 seropositivity and duration of injecting drug use (P for trend $=0{\cdot}073$).

No significant associations were observed between HHV-8 infection and gender, educational level, age at first drug injection, needle sharing, number of imprisonments since first drug injection, or complications from drug overdose. Moreover, self-reported hepatitis, anti-HBc, anti-HCV, and anti-HIV were not significantly associated with HHV-8 status.

Independent risk factors for HHV-8 infection

A multiple logistic regression model was used to identify independent risk factors for HHV-8 infection (Table 2). In this model, HHV-8 was significantly more prevalent in individuals aged ⩾40 years compared to those aged <40 years (OR 3·30, 95% CI 1·14–9·56, $P=0{\cdot}03$). Moreover, a history of septicaemia or abscess (OR 1·80, 95% CI 1·01–3·18, $P=0{\cdot}05$) was independently associated with higher HHV-8 seroprevalence. There was no association between duration of drug injection and HHV-8 infection in the multiple logistic regression model.

DISCUSSION

Non-AIDS-related KS is more frequent in Greece [24, 25] than in northern Europe or the USA. In the current cross-sectional study, we observed a high HHV-8 seroprevalence in Greek IDUs (24·3%), a much higher rate than that reported in healthy urban workers from Greece. The higher prevalence persisted even after we controlled for age. Moreover, HHV-8 prevalence reported for Greek IDUs was considerably higher than the rate observed in the majority of studies performed with IDUs [17, 20, 21, 27–31]. However, these differences could be in part attributed to the fact that these studies employ different serological assays – with different sensitivity and specificity – to detect HHV-8 antibodies; research indicates that HHV-8 seroprevalence estimates, measured in absolute terms, depend strongly on test performance [26].

The seroprevalence of HHV-8 was independently associated with advancing age, a pattern consistent with results from previous age-specific prevalence studies [32–34]. We also identified an independent association between HHV-8 serostatus and

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Table 1. *Univariate analysis of HHV-8 seroprevalence according to demographic variables, reported behaviour and status of HIV, HCV and anti-HBc*

Characteristic/risk factor	No. of IDUs	HHV-8 seropositivity *n* (%)	*P* value
Gender			0·973
Male	230	56 (24·3)	
Female	58	14 (24·1)	
Age (years)			0·003†
<30	170	33 (19·4)	
30–39	101	28 (27·7)	
⩾40	17	9 (52·9)	
Education (years)			0·124
⩽12	243	55 (22·6)	
>12	45	15 (33·4)	
Age at first injection (years)			0·342
⩽20	150	33 (22·0)	
>20	138	37 (26·8)	
Duration of injecting drug use (years)			0·073†
⩽2	40	7 (17·5)	
3–10	168	38 (22·6)	
>10	80	25 (31·2)	
Ever injected with a used syringe			0·771
No	152	38 (25·0)	
Yes	136	32 (23·5)	
No. of imprisonments since first injection			0·854
0	60	14 (23·3)	
1	63	14 (22·2)	
2–5	99	23 (23·2)	
5–10	31	10 (32·2)	
>10	35	9 (25·7)	
Complications from drug overdose*			0·758
No	143	36 (25·2)	
Yes	144	34 (23·6)	
Abscess/septicemia*			0·019
Yes	98	32 (32·6)	
No	189	38 (20·1)	
Self-reported hepatitis*			0·491
Yes	57	16 (28·1)	
No	228	54 (23·7)	
Anti-HBc*			0·206
Positive	140	35 (25·0)	
Negative	124	23 (18·5)	
Anti-HCV*			0·293
Positive	248	61 (24·6)	
Negative	26	4 (15·4)	
Anti-HIV*			0·072
Positive	1	1 (100)	
Negative	286	67 (23·5)	

* Missing values because of lack of response from some participants.
† The *P* value is for a test for linear trend.

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Table 2. *Multiple logistic regression: determinants of HHV-8 seropositivity*

Variables	OR	95% CI	*P*
Age (years)			
<40	1·00		
⩾40	3·30	1·14–9·56	0·03
Duration of injecting drug use (years)			
⩽2	1·00		
3–10	1·30	0·53–3·18	0·57
>10	1·45	0·54–3·94	0·46
Abscess/septicaemia			
No	1·00		
Yes	1·80	1·01–3·18	0·05

OR, Odds ratio; CI, confidence interval.

self-reported abscess/septicaemia. Skin abscess and septicaemia are frequent medical complications of intravenous drug use [35, 36]. Potential sources of infection include the skin, dirty syringes, contaminated drugs, and solutions used to clean equipment and dissolve drugs [35, 36]. In the present study, IDUs who self-reported abscess/septicaemia had an increased risk of being HHV-8 infected compared to those reporting no evidence of these complications. One possibility is that the study population used injection practices without following fundamental hygiene conditions (e.g. having unsterile skin before injection or using dirty syringes). Such conditions might contribute to transmission of HHV-8 infection.

The prevalence of HCV infection was high in this group (90·5%) and this high rate is in accord with findings from other studies conducted in Greek incarcerated IDUs [37]. Because HCV was highly prevalent in this study population, a possible association between HCV and HHV-8 infection could not be assessed, but this association between the two infections has already been suggested in previous studies [38, 39]. Earlier authors have suggested that transmission of HHV-8 might occur through needle sharing [14], but we did not identify sharing as a risk factor for transmission in the current study. However, in view of the high prevalence of anti-HCV in our study group, the validity of this negative finding should be viewed with caution. Although we found an independent association between self-reported abscess and HHV-8 infection, injection with a used syringe did not emerge as being associated with HHV-8-positive status.

In conclusion, we identified a high seroprevalence of HHV-8 infection in IDUs from Greece. Evidence suggests that inappropriate hygiene conditions in the setting of drug injection may contribute to HHV-8 transmission. Understanding the epidemiology of HHV-8 is critical for designing interventions to decrease the transmission of this virus.

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DECLARATION OF INTEREST

None.

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