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Prevalence of Human Herpesvirus 8 DNA Sequences in Human Immunodeficiency Virus-negative Individuals without Kaposi's Sarcoma in Greece

GEORGE ZAVOS[1], MARIA GAZOULI[2], IOANNIS PAPACONSTANTINOU[1], JOHN C. LUKAS[3], ANASTASIOS ZOGRAFIDIS[1], ALKIVIADIS KOSTAKIS[1] and GEORGIOS NASIOULAS[4]

[1]Transplantation Unit, Laiko Hospital, Athens; [2]Department of Biology, School of Medicine and [3]Laboratory of Biopharmaceutics and Pharmacokinetics, School of Pharmacy, University of Athens; [4]Molecular Biology Research Center HYGEIA "Antonis Papayiannis", Athens, Greece

Abstract. *Background: It has already been established that the prevalence of human herpes virus 8 (HHV8) in the general population varies among different geographical areas. The objective of this study was to evaluate the prevalence of HHV8 infection in the Greek population. Materials and Methods: Eight hundred blood samples were collected consecutively from human immunodeficiency virus (HIV)-negative individuals without evidence of Kaposi's sarcoma (KS). All individuals were Greeks and were classified according to gender, age and geographic origin. HHV8 DNA sequences were detected by nested-PCR. Results: An overall HHV8 positivity rate of 9.6% was found. Analysis of the KS330 region within ORF-26 revealed that the HHV8 strains were distributed to the C1, C3 or A1 subtypes. Logistic regression showed no association between HHV8 presence and geographic regions in Greece. The results indicate that very few individuals (4.3%) were exposed to HHV8 before 15 years of age. The infection rate peaked (16.4%) between the ages of 31 and 40. Females and males showed similar prevalence of HHV8. Conclusion: The data suggest that HHV8 is spread in Greece, but to a lesser extent than that observed in other Mediterranean countries. The fact that HHV8 was also found in individuals under 15 years of age indicates that a small percent of HHV8 transmission could occur through non-sexual contacts.*

Human herpes virus 8 (HHV8) has been associated with different forms of Kaposi's sarcoma (KS), including the rare cases of HIV-positive body cavity-based lymphoma and the multicentric Castleman disease (1, 2).

HHV8 is not, however, restricted to KS patients. Epidemiological and molecular studies have suggested that HHV8 could be widespread in the general population, especially in geographic areas where KS is prevalent (3-5). However, the mechanism by which HHV8 is spread and the magnitude of this phenomenon within defined populations remains unclear. In developed countries, depending on the study, 1%-25% of the general population is HHV8 sero-positive (3, 6-9). The infection rates are much higher in developing countries. HHV8 DNA has been detected in 22.5% of peripheral blood mononuclear cells of blood donors in central Africa (10), a sero-positive rate of 50% has been reported for children in Uganda (11) and a high sero-positivity of 80%-100% has been observed in blood samples from Gambia and the Ivory Coast (8).

In contrast to the abundance of studies from the United States, Europe, Africa, Asia and Mediterranean areas, little information is available about HHV8 in Greece, an area considered endemic for "classic" KS (12). In order to evaluate the prevalence of HHV8 DNA sequences in the Greek population, a large-scale analysis of sera samples from HIV-negative individuals without KS was performed.

Materials and Methods

Patients. A total of 800 individuals, 405 females and 395 males, aged between 0 and 95 years, from different areas of Greece, were enrolled in the study. Individuals were classified by geographical region of birth and residence, sex and age. Regarding the region of birth and residence, 407 subjects had been born and lived in the central region of Greece, 185 in the Peloponnese, 44 in Epirus, 40 in Thessaly, 39 in the Aegean islands, 36 in Macedonia, 26 in Crete, 17 in the Eptanisa, and 6 in Thrace. Individuals with possible risk factors for HHV8 infection (*i.e.* HIV infection, organ transplantation) were excluded

Correspondence to: George Nasioulas, Ph.D., Molecular Biology Research Center HYGEIA "Antonis Papayiannis", Kifissias Ave. & 4 Erythrou Stavrou St., 15123 Maroussi, Athens, Greece. Tel.: +30-210-686-7932, Fax: +30-210-6867923, e-mail: g.nasioul@hygeia.gr

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Table I. *Prevalence of HHV8 DNA in 800 individuals with complete demographic data and results from multivariable logistic regression analysis.*

		Prevalence of HHV8 DNA sequences			
Demographic variables	Subjects (n)	HHV8-positive (n)	%	OR ; 95% CI	*p*-value
Sex					
Male	395	35	8.9	1.00	
Female	405	42	10.4	1.11; 0.68 – 1.81	0.672
Age (years)					
0-15	46	2	4.3	1.00	
16-30	114	10	8.8	0.99; 0.20 – 4.95	0.999
31-40	128	21	16.4	4.19; 0.89 – 19.62	0.069
41-50	103	8	7.8	0.98; 0.19 – 5.03	0.982
51-60	121	12	9.9	1.27; 0.26 – 6.15	0.769
61-70	180	13	7.2	0.89; 0.18 – 4.29	0.885
>70	108	11	10.2	0.78; 0.16 – 3.84	0.761
Geographic area					
Central Greece	407	45	11	1.00	
Peloponnese	185	20	10.8	0.99; 0.56 – 1.76	0.984
Epirus	44	5	11.4	1.16; 0.42 – 3.17	0.773
Thessaly	40	0	0	-	
Aegean islands	39	2	5.1	0.56; 0.13 – 2.45	0.441
Macedonia	36	4	11.1	0.79; 0.26 – 2.46	0.695
Crete	26	1	3.8	0.37; 0.05 – 2.84	0.339
Eptanisa	17	0	0	-	
Thrace	6	0	0	-	

from the study. Sera were collected by centrifugation of blood at 1500 rpm for 5 min and then stored at –20˚C until tested.

DNA extraction and PCR assay. DNA from blood was extracted using the QIAamp Blood Kit (Qiagen, Hilden, Germany). To confirm the DNA integrity, a 430-bp sequence in the human *GAPDH* (glyceraldehyde-3-phosphatate dehydrogenase) gene was amplified. Nested PCR detection of HHV8 was performed as previously described. A primer set amplifying a 700 bp fragment (sense and antisense, respectively) HHV8AF (5' AGCACTCGCAGGGCA GTACG 3') and HHV8AR (5' GACTCTTCGCTGATGAACTGG 3') was used (13). The amplification of the external region was 2 min at 94˚C for initial denaturation, 35 cycles at 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 45 sec, and 72˚C for 5 min as final extension. The PCR product (2 μl) was added to an inner PCR reaction mixture and amplified for 30 additional cycles using primers amplifying the 233-bp $KS330_{233}$ region of the sequence associated with HHV8: HHV8BF (5' AGCCGAAAGGATTCCACCAT 3') and HHV8BR (5' TCCGTGTTGTCTACGTCCAG 3'). Amplification products were analyzed for the presence or absence of the 233 bp expected band (ORF-26) on a 2% agarose gel containing ethidium bromide. In order to closely monitor the occurrence of PCR false-positive results, negative controls, including reaction mixtures lacking any DNA template or HHV8-negative human DNA, were regularly analyzed in each reaction. Negative controls were also included during both sample preparation and DNA extraction. To prevent carry-over contamination, all pre- and post-PCR reactions were conducted by separate personnel in different laboratories. Positive results were confirmed by DNA sequencing of PCR products (*Taq* DyeDeoxy Terminator Cycle Sequencing kit and ABI 373A DNA Sequencer; Perkin-Elmer, Forest City, CA, USA).

Alignments and phylogenetic analysis. HHV8 subtypes based on ORF-26 were assigned on the basis of previous reports (14, 15). Phylogenetic analysis of the sequences was performed using the PHYLIP package, version 3.5C. A majority-rule consensus tree was computed from 100 bootstrap replications using that package (16).

The DNA sequence data for the prototype *A1(BCBL-R)*, *A4(BCBLB)*, *A5(Ug374)*, *B1(431KAP)*, *B2(Ug81)*, *B2*(UgD1)*, *BCBL-1*, *C1(ASM72)*, *C3(BC2)*, *C3(BC3)*, *D1(TKS10)* and *D2(ZKS3)* subtype genes are available from GenBank (accession no. AF133038 to AF133044, AF130289, AF130291, AF13092, AF170531 and U86667).

Statistical analysis. Differences in the frequencies of results for the study groups were analyzed by the Chi-square test. *P* values of 0.05 or less were considered statistically significant. Inference was performed with SPSS 11.0 (SPSS Inc., Chicago, IL, USA) and GraphPad 3.0 (GraphPad Software,Inc., San Diego, CA, USA).

Results

A total of 800 specimens from HIV-negative individuals with no evidence of KS were obtained and analyzed by PCR for the presence of HHV8 DNA sequences. HHV8 DNA was detected in 77 of these 800 specimens (9.6%). By studying

the KS330 region within ORF-26, the HHV8 strains were found to be distributed to the C1, C3 or A1 subtypes.

HHV8 detection was similar in men and women (8.9% and 10.4%, respectively, $p=0.672$). The highest rates were found in Epirus (11.4%), Macedonia (11.1%), Central Greece (11%), and the Peloponnese (10.8%), and the lowest in the Aegean islands (5.1%) and in Crete (3.8%). The study did not, however, detect significant differences in HHV8 prevalence between these different areas. No HHV8 DNA was found in samples derived from Thessaly, the Eptanisa and Thrace regions. However, from the two latter areas very few samples were available.

In order to study the effects of age, the population was stratified into seven age groups (Table I). HHV8 presence in individuals under 15 years of age was low (4.3%). Specifically, HHV8 DNA was found in a 2-year-old child and an 11-month-old infant. The rate of HHV8 positivity rose in subjects between 16 and 30 years of age (8.8%) and peaked in the 31- to 40-year (16.4%) age group. Thereafter, rates declined to around 7-10%. Nevertheless, logistic regression showed no significant differences between age groups nor association between HHV8 positivity and sex.

Discussion

Although, many attempts have been made, using molecular and serological assays, to define the prevalence and transmission of HHV8, exact prevalence rates are still uncertain. However, there is consensus that the prevalence of HHV8 infection is geographically diversified. In some Mediterranean regions, such as Italy, and in African countries, such as Zambia and Uganda, HHV8 prevalence rates among HIV-negative individuals are reported to be higher that those for the United States or northern European countries (5, 6-8, 17, 18).

Greece has been considered as endemic for "classic" KS (12), but little is known regarding the prevalence of HHV8 infection in the general population. The aim of the present work was to investigate the distribution of HHV8 DNA sequences among healthy individuals in Greece and to evaluate whether the infection can be acquired by non-sexual transmission routes.

In general, a moderate HHV8 positivity rate (9.6%) was found in HIV-negative subjects, without KS, in Greece. To our knowledge, the other only study of HHV8 presence among healthy blood donors in Greece was that of Simpson *et al.* (19), who tested 26 HIV-negative Greek individuals, without KS, 11.5% of whom tested positive for HHV8 (19). Our results are similar, indicating that the prevalence of HHV8 in Greece is, roughly, 10%.

Our results did not reveal any significant difference of HHV8 presence among females and males, although KS has been reported to be more frequent in males than in females (20). It is possible that sex-specific risk factors may be implicated in KS development. Furthermore, although southern Greece has a high incidence of KS (21), we did not observe statistically significant differences in the prevalence of HHV8 among individuals from different regions.

Similar to findings from the United States and in contrast to those from Africa and Italy, this study indicates that, before 15 years of age, HHV8 infection in Greece is rare (4.3%) (3, 4). However, this also suggests that routes other than sexual transmission, such as direct transmission, may be involved in the spread of HHV8. Most of the infection in Greece was noted to occur between 31 and 40 years of age, and the 4-fold increased risk, relative to the 0 to 15 year age group, approached statistical significance. Analysis of the KS330 region within ORF-26, revealed that the HHV8 strains were distributed to the C1, C3 or A1 subtypes. In general, our findings are in agreement with previous studies reporting that the A and C subgroups are common in Europe (22, 23).

Overall, our results indicate that there is considerable presence of HHV8 in the general population in Greece and that non-sexual routes may also contribute to HHV8 transmission. In Greece, the prevalence of classic KS that has been reported is 0.47/1,000,000 population (24). Thus, the relatively significant proportion of HHV8 detection supports the hypothesis that KS occurs more frequently in populations with a higher rate of HHV8 infection.

Finally, our results suggest that HHV8 is not restricted to individuals at risk of developing KS, but may establish infections in the general population which remain latent until various factors contribute to activation of the virus.

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