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Epidemiology of Human Herpesvirus Type 8 Infection in Cardiopathic Patients

[1]Angela Ingianni, [1]Maria A. Madeddu, [1]Francesca Carta, [2]Anna Reina,
[3]Carlo Lai and [1]Raffaello Pompei
[1]Section of Applied Microbiology, University of Cagliari, Cagliari
[2]Immunohaematology Service, Brotzu Hospital, Cagliari
[3]Division of Cardiology, S. Trinità Hospital, Cagliari

Abstract: Problem statement: The possible contribution of viruses to vascular pathology is still a controversial issue. Human herpesvirus type 8 (HHV-8) has been suggested to participate to the pathogenetic events associated with atherosclerotic lesion establishment and progression. Recently, a high incidence of infection of HHV-8 (11%) has been verified in the island of Sardinia. The aim of this study was to evaluate a possible relationship between the HHV-8 infection and cardiovascular diseases in the South of Sardinia. **Approach:** The presence of HHV-8 genome was detected in DNA extracted from peripheral leucocytes, by nested-PCR and Southern blotting, in either acute or chronic cardiopathic patients (n = 180); healthy blood donors were examined as controls (n = 108). **Results:** The results demonstrated a significant increase (p = 0.035) in HHV-8 DNA isolation from cardiopathic patients (22.8%) in comparison to healthy controls (12.0%). **Conclusion:** HHV8 infection can be considered, among others, as an additional risk factor for cardiovascular disease development, although it was not necessarily the starting cause. More extensive studies were needed to define the exact role of HHV-8 infection in cardiopathic patients.

Key words: Human herpesvirus type 8, epidemiology, ORF26, cardiovascular disease

INTRODUCTION

The human herpesvirus 8 (HHV-8) is known as the Kaposi Sarcoma Associated Virus (KSAV). It is also responsible for multicentric Castleman disease, for Primary Effusion Lymphomas (PEL) and for serum Cavity Primary Lymphomas (BCBL)[1-3]. HHV-8 has a specific tropism for lymphocytes and endothelial cells and is highly carcinogenic in both immunocompromised adults and in AIDS patients[4]. To date, no full elucidation exists of the properties of virus transmission and the mechanism of viral switching from the latent to the lytic phase, causing the relevant clinical symptoms[5,6].

Recently, an increase in HHV-8 infection has been detected in the general population of some regions of Italy, namely Sicily, Sardinia and the Po valley. Some authors have claimed that the presence of endemic diseases, such as malaria, G6PD defects and thalassemia can at least partially be responsible for the selection of highly sensitive subjects to the HHV-8 infection[7,8].

Some herpesviruses, along with other viruses and bacteria, have been considered as playing a role in the formation of atheromatous plaques[9,10]. This hypothesis has been emphasized by the finding that HHV-8 is able to replicate in and damage endothelial cells. Moreover, some genes (ORF) homologous to the human genes involved in cell proliferation and angiogenesis have been described[3] in the HHV-8 genome.

This research has been designed to verify whether the presence of HHV-8 DNA in cardiovascular patients can be considered an additional risk factor for atherosclerosis and heart pathologies.

MATERIALS AND METHODS

The HHV-8 DNA was searched for in 180 patients (127 males and 53 females) from the UTIC Cardiology Division of the SS Trinità Hospital, Cagliari (Sardinia, Italy). These patients were divided into 4 groups based on the different cardiovascular diseases: 77 patients had an Acute Coronary Syndrome (ACS), 5 a non-acute coronary syndrome (NACS), 45 had an Acute Non-

Corresponding Author: Angela Ingianni, Department of Science and Biomedical Technology, Section of Applied Microbiology, University of Cagliari Via Porcell, n°4 09124 Cagliari, Sardegna, Italy
Tel: +39.070.6758487 Fax: +39.070.6758482

Coronary Syndrome (ANCS) and 53 a Non-Acute Non-Coronary Syndrome (NANCS). One hundred eight subjects (83 males and 25 females) from the Immunohaematology Service at the Brotzu Hospital of Cagliari were selected as healthy controls. Both patients and controls were divided by age and sex.

The presence of HHV-8 DNA was detected by nested PCR technique using two sets of primers (outer primers: ORF-fw 5' AGCTAGCAGTGCTACCCCCA 3' and ORF-rev 5' ATCGTCAAGCACTCGCAGGG 3', inner primers: ORF26-fw 5' AGCCGAAAGGATTCCACCA 3' and ORF26-rev 5' TCCGTGTTGTCTACGTCCAG 3'; corresponding to position 47261-47531 and 47287-47500 of the published sequence on the Gene Bank Accession N° U75698[11]), which were specific for the highly conserved gene for the minor capsid protein (open reading frame ***orf26***). The nested PCR was realized by a starting denaturation at 95°C for 2 min followed by 35 cycles of 94°C for 1 min, 52°C for 1 min and 72°C for 1 min, followed by a final extension of 5 min at 72°C. The inner PCR was run for a further 25 cycles with the following thermal profile: 94°C for 20 sec, 58°C for 20 sec and 72°C for 20 sec. Each PCR mixture, that contained 25 pmol of each primer, 200 µM of deoxynucleotide triphosphates (Invitrogen, Carlsbad, California), 1.5 µM of MgCl2, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 U Taq polymerase (Invitrogen, Carlsbad, California) in a final volume of 50 µL, was processed in a GeneAmp PCR system (model Mastercycler Personal 5332, Eppendorf, Hamburg, Germany). The expected amplified segments of 290 bp were detected with an electrophoretic assay on 2% agarose gel; these segments were then stained with ethidium bromide and read with a UV-transilluminator. The PCR product specificity was confirmed with a Southern blot assay of the amplified segments, which were hybridized with an internal probe for the specific gene labeled with digoxigenin (DIG-DNA Labeling kit, Roche Biochemicals, USA), according to standard protocols. For each experiment, a positive control DNA extracted from human B lymphocytes containing the HHV-8 genome (BC-3 cells, American type culture collection, Manassas, Va.) was employed and a negative control without a DNA template was also performed. The integrity and efficiency of the DNA was confirmed by the amplification of a 268 bp fragment of the gene for the human β-globulin.

The χ^2 test was used for statistically analyzing significant differences in HHV-8 DNA detection between healthy and cardiopathic subjects.

RESULTS

The HHV-8 DNA was detected in the peripheral lymphocytes of 41 patients with a clinically established cardiovascular pathology (23.6 males and 20.7% females), (Table 1). Considering the different pathology groups, HHV-8 was found in 19 patients with ACS (27.2%), 1 with NACS (20.0%), 10 with ANCS (22.2%) and 11 with NANCS (20.3%). No significant differences in HHV-8 frequency were detected either among the various pathogenic groups, or between males and females. Healthy controls showed a percentage of 12.0% positivity for HHV-8 DNA isolation in both males and females. The difference in HHV-8 detection in cardiovascular patients (22.8%) with respect to the healthy subjects (12.0%) was found to be statistically significant (p = 0.035). Patient age was found to have an important influence on HHV-8 detection (Fig. 1).

Table 1: Detection of HHV-8 DNA by means of a nested PCR method in the peripheral lymphocytes of cardiovascular patients and healthy controls

Samples	No. of subjects examined	No. positive for HHV-8	Percentage
Controls:			
Males	83	10	12.0
Females	25	3	12.0
Total	108	13	12.0
Cardiovascular patients:			
Males	127	30	23.6
Females	53	11	20.7
Total	180	41	22.8
ACS	77	19	27.2
NACS	5	1	20.0
ANCS	45	10	22.2
NANCS	53	11	20.3

ACS: Acute Coronary Syndrome; NACS: Non-Acute Coronary Syndrome; ANCS: Acute Non-Coronary Syndrome; NANCS: Non-Acute Non-Coronary Syndrome. Statistical significance between controls and cardiopathic patients: p = 0.035

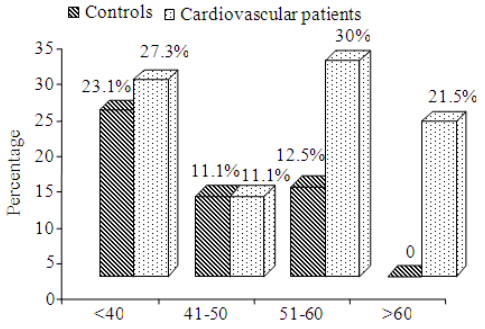

Fig. 1: HHV-8 DNA positivity in patients with cardiovascular diseases and healthy controls divided into <40, 41-50, 51-60, >60 age groups. The difference in HHV-8 DNA detection was statistically significant between patients and controls (p = 0.035)

While in the <40 and 41-50 age groups, positivity for HHV-8 was quite close between patients and controls (27.3% in the cardiopathic patients compared to 23.1% in the healthy population for the <40 group and 11.1 and 11.1% for the 41-50 group, respectively); the older age groups showed extremely divergent values between patients and healthy subjects. In the 51-60 age group, the values were 30.0% positivity for the cardiovascular patients compared to 12.5% for controls, whereas in the >60 group the percentages were 21.5 and 0% respectively.

DISCUSSION

The HHV-8 DNA prevalence found in this study was in accordance with other studies performed in Sardinia or in other parts of southern Italy[12-14]. Considering that there is a high percentage of HHV-8 infection in the general population in Sardinia, it will be worth performing a comparative analysis of HHV-8 infection and vascular diseases in other hospitals of southern Italy and in other regions of Italy and Europe. However, contrasting results have been obtained by different authors as regards the isolation of HHV8 from cardiovascular patients. Whereas some authors found a prevalence of HHV8 in some cardiopathic patients, other authors reported the absence of Herpesvirus genomes in atherosclerosis[10,15]. The discrepancies may be due to different methods of analysis (detection of HHV8 specific antibodies or genomic DNA) or to the various materials tested (peripheral blood cells, bioptic samples, specimens from atheromatous lesions). In this study the nested PCR method employed was shown to be very specific and extremely sensitive for detecting the HHV8 genome. Therefore, the standardization of the methods and materials for searching for HHV8 genes as well as analysis carried out on more patients and subjects from different hospitals, considered together with environmental, genetic, alimentary and immunologic factors, will be necessary for defining the real role of HHV-8 infection as a further threat for the development and evolution of cardiovascular diseases.

CONCLUSION

The results of this study, despite being limited to a relevant number of patients from a single hospital of the Sardinian Region, suggest that in patients with various cardiovascular pathologies, there is a higher frequency of HHV-8 DNA isolation from peripheral blood lymphocytes, than in healthy control subjects of the same age. This difference is statistically significant overall, but the differences are particularly important for the older age groups, where the percentage of positivity can vary by 20 or more points. No relevant differences were found among the various cardiovascular pathologies or between males and females. These findings taken as a whole may suggest that HHV-8 infection can be considered as a further risk for the development of cardiovascular disease, at least in patients aged over 50.

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