

Prevalence of Herpesvirus 8 Infection in Type 2 Diabetes Mellitus Patients

¹A. Ingianni, ¹F. Carta, ²A. Reina, ³M. Manai, ^{1,4}A. Desogus, ^{1,4}R. Pompei

¹Department of Biomedical Sciences and Technology, Sezione di Microbiologia Applicata e Tecnologie Biomediche, Università degli studi di Cagliari

²Servizio di Immunoematologia, Ospedale Brotzu di Cagliari

³Servizio di Diabetologia e Malattie metaboliche, Ospedale S.Giovanni di Dio, Cagliari

⁴Biotechne, Cagliari

Abstract: In this study a possible relationship between the presence of type 2 diabetes (DM2) and the Human Herpesvirus 8 infection (HHV-8) has been evaluated. The presence of HHV-8 DNA was monitored by nested PCR and Southern blotting in peripheral blood leucocytes of DM2 patients admitted to the Diabetology and Metabolic Disease Service of the "S. Giovanni di Dio" Hospital (Cagliari, Italy); healthy blood donor subjects were examined as controls. The results suggest that the frequency of HHV-8 DNA detection is more elevated in DM2 patients (23.7%) than in healthy control subjects (12%, $p < 0.05$). The association of HHV-8 infection with some other frequent complicating pathologies of DM2 patients was also evaluated. The possible role of HHV-8 in DM2 disease and related intercurrent pathologies is critically discussed.

Key words: HHV-8; type 2 Diabetes mellitus; Molecular epidemiology; Diabetic complicating diseases.

INTRODUCTION

The Human Herpesvirus 8 (HHV-8) is involved in most cases of classic Kaposi sarcoma, and in other rare pathologies, such as multicentric Castleman's disease, pleural effusion lymphoma (PEL) and body cavity-based lymphoma (BCBL) [1-3]. HHV-8 has a specific tropism to lymphocytes and endothelial cells and is highly tumorigenic in immunosuppressed adults and HIV infected patients [4]. To date, the method of virus transmission and the mechanism of viral switching from the latent to the lytic phase, leading to severe clinical symptoms, has not been completely clarified [5, 6]. Recent studies have reported an increasing presence of HHV-8 infection in the general population and in particular in some countries, such as Southern Italy, Sicily, Sardinia, and the Po river valley; genetic pressure, caused by endemic diseases (namely malaria, thalassemia, G6PD deficiency), has been claimed by some authors as being the possible cause of the selection of a population with an increased sensitivity to HHV-8 infection [7, 8].

Although the number of subjects infected by HHV-8 is relatively high in the general population, only a small number of individuals develop the Kaposi sarcoma; other risk factors are probably involved in

tumor presence and evolution: the Kaposi sarcoma has been detected with a high frequency in type 2 diabetes mellitus patients. This disease leads to a decrease in the number of T lymphocytes, a depression of killer cell activity and to a decrease in human resistance to Herpesvirus infections [9]. A diminished resistance to Herpesvirus infections was also observed in an experimental model of diabetes in mice [10].

Herpesviruses have also been involved in other important pathologies: HHV6 was claimed to have some role in the development of drug hypersensitivity [11, 12]; Cytomegalovirus and HHV-8 were described as having a possible relationship with the formation of atheromatous plaques [13-15]; this last hypothesis is also sustained by the property of HHV-8 to multiply in and to damage endothelial cells [16]. Furthermore, the HHV8 genome contains several open reading frames (orf) which are homologous to human genes that inhibit the immune response and modulate cell replication and angiogenesis [3]. The present study has the aim of verifying whether the HHV-8 infection is more frequent in DM2 patients in the southern part of our country and whether it can be considered an additional risk factor for general medical complicating pathologies of this disease.

Corresponding Author: Dr. Angela Ingianni, Dipartimento di Scienze e Tecnologie Biomediche, Sezione di Microbiologia Applicata, Via Porcell. n°4, 09124 Cagliari, Italy, Tel 070.6758487; Fax 070.6758482

MATERIALS AND METHODS

Patients: The search for HHV-8 DNA was performed on 114 patients (53 males and 61 females) with type 2 diabetes mellitus from the Diabetology and Metabolic Disease Service of the “S.Giovanni di Dio” Hospital (Cagliari, South Italy) and on 108 healthy subjects (83 males and 23 females), who were blood donors in the Immunohematology Service of the Cagliari “Brotzu” Hospital. In the diabetic patients, all the other related complicating pathologies were scored, namely, atherosclerosis, hypertensive cardiopathy, ischemic cardiopathy, cardiac conduction block, heart infarct, hypertensive retinopathy, peripheral vasculopathy, diabetic foot, various tumors, hepatopathies, neuropathies, hypercholesterolemia and diabetic retinopathy. Sex and age were also recorded and the patients were divided into 4 groups, i.e. <40, 41-50, 51-60, >60 years old.

HHV-8 DNA detection: Lymphocytes (about 4×10^6 /ml) were isolated from 3 ml of heparinized blood by the Lymphoprep technique (Nycomed Pharma AS, Oslo, Norway); the DNA was extracted (Easy-DNA, Invitrogen, San Diego, Ca.) and suspended in 20 μ l of TE (10 mM Tris-HCl-1 mM EDTA).

The presence of HHV-8 DNA was detected by nested PCR technique using two sets of primers (outer primers: ORF-fw 5' AGCTAGCAGTGCTACCCCCA 3' ORF-rev 5' ATCGTCAAGCACTCGCAGGG, inner primers: ORF26-fw 5'-AGCCGAAAGGATTCCACCA-3', ORF26-rev 5'-TCCGTGTTGTCTACGTCCAG-3'; corresponding to position 47261 to 47531 and 47287 to 47500 of the published sequence on the Gene Bank Accession N^o. U75698), which were specific for the highly conserved gene for the minor capsid protein (open reading frame, ORF 26). According to Chang's et al. modified protocol [17], a 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 s, 58°C for 20 s and 72°C for 20 s. Each PCR mixture, that contained 25 pmol of each primer, 200 μ M of deoxynucleotide triphosphates (Invitrogen, Carlsbad, California), 1.5 μ M 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 μ l, 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.

Statistical analysis: Differences in the frequencies of results for the study groups were analyzed by the chi-square test; p values of <0.005 were considered statistically significant.

RESULTS

HHV-8 infection of DM2 patients: Among 114 samples from DM2 patients, 27 (23.7%) resulted positive for HHV-8 DNA; 10 out of 83 (22.6%) were males and 15 out of 61 (24.6%) females (Figure 1); the difference between males and females was not significant. Among the healthy controls, 13 patients were found positive for HHV-8 out of 108 (12.0%; 12.9% male and 12.0% female). The difference observed between DM2 patients and controls for HHV-8 infection was found to be statistically significant (p = 0.037)

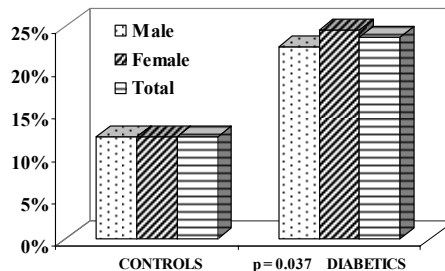


Fig. 1: Prevalence of HHV-8 infection in type 2 diabetes mellitus patients and healthy controls. The data are reported as a percentage of positivity for HHV-8 infection in controls and DM2 patients. The difference of positivity between DM2 patients and controls was statistically significant in the χ^2 test (p<0.05). The percentage of positivity for HHV-8 males and females is also shown, but in this case no significance was detected.

HHV-8 infection in DM2 patients related to age: In Figure 2, the rate of positivity for HHV-8 is also

reported for the different age groups; as expected, most DM2 patients were in the >60 year-old group, where a positivity of 26.7% for HHV-8 was found; very few patients (a total of 28 subjects with a positivity of 14.2%) were included in the age-groups <40, 41-50, 51-60. Among the controls, most subjects fell into the 51-60 age-group, where a percentage of 12.5 positivity to HHV-8 was detected, but the peak for HHV8 was found to be in the youngest group (<40), with a positivity of 23.1%. No significant differences among the age-groups were detected in both the controls and DM2 patients.

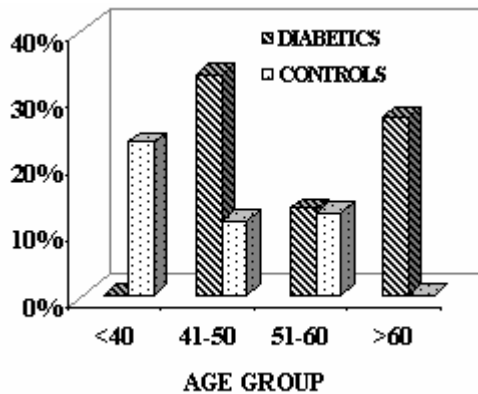


Fig. 2: Distribution of HHV-8 infected and uninfected DM2 patients for age-groups. Most diabetic patients fell into the >60 group (26.7% of positivity to HHV-8), whilst in the other groups only a minority of patients were included. In the controls, the peak of positivity to HHV-8 was observed in the youngest group (<40, 23.1%). No significant differences were detected between the various age groups.

Relationship of HHV-8 infection and medical complicating diseases in DM2 patients: The DM2 patients were monitored for the most common complications that are the consequence of this type of disease (Figure 3). Although some important differences were observed between HHV-8 positive and negative patients, none resulted statistically significant. In particular, atherosclerosis (22.2% versus 13.8%), cardiac ischemia (18.5% versus 9.1%), hypertensive cardiopathy (18.5% versus 14.9%), and diabetic retinopathy (14.8% versus 10.3%), appeared more represented in HHV-8 positive than in HHV-8 negative patients; however, the absolute numbers were too low

to find any kind of significance among the various groups.

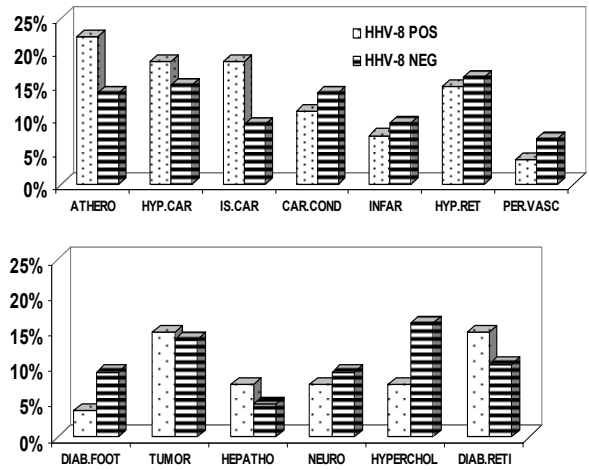


Fig. 3: Frequency of complicating diseases in HHV-8 infected and uninfected type 2 diabetes mellitus patients. In Fig. 3A some frequent intercurrent cardiovascular diseases are described; in Fig. 3B other various pathologies that often complicate diabetes are reported. Abbreviations: atherosclerosis (ATHERO), hypertensive cardiopathy (HYP CARD), ischemic cardiopathy (IS CAR), cardiac conduction block (CAR COND), heart infarct (INFAR), hypertensive retinopathy (HYP RET), peripheral vasculopathy (PER VASC), diabetic foot (DIAB FOOT), various tumors (TUMOR), hepatothopathies (HEPATHO), neuropathies (NEURO), hypercholesterolemia (HYPERCHO), diabetic retinopathy (DIAB RETI). No significant differences were detected in the frequency of any complicating diseases between HHV-8 positive and negative DM2 patients.

DISCUSSION

Since diabetes is known to induce a state of immunosuppression, an increase of the infection by HHV-8 was expected in the DM2 patients enrolled in this study, as compared to a healthy population of blood donors. Actually, a considerable increase in HHV-8 DNA detection was observed in DM2 patients with respect to controls, but it was less than might have been hypothesized, considering the state of the patients' immunosuppression, their age and the reports found in the literature [3, 5, 6, 14, 16]. The frequency of HHV-8 infection in DM2 patients was a little more than double that of healthy subjects. The difference was found to be statistically significant, but DM2 can be considered as only a small additional risk factor for HHV-8 infection

and evolution. It must be also considered that all the patients included in this study were undergoing careful treatment in a specialized center for diabetes and thus the disease was well compensated. Furthermore, their general conditions, as well as their immune systems, were fairly good.

As regards age and sex, no relevant differences were found between males and females, with a low prevalence of the HHV-8 infection in females with respect to males. On the contrary the age of the patients was important, since the highest number of HHV-8 positive DM2 patients were included in the >60 age-group. This fact is understandable, since type 2 diabetes is typically a disease of the elderly.

Is there a relationship between HHV-8 in DM2 patients and some intercurrent medical diseases? In the light of our findings this conclusion cannot be accepted. It is true that some common cardiovascular complications, typical of DM2 patients, were found more frequently in HHV-8 positive than in negative patients; this is the case for heart ischemia (twice as frequent in HHV-8 positive than in negative patients), atherosclerosis and diabetic retinopathy. However, in no case did these differences have a definite significance; this fact could also be due to the low number of patients included in each group of complicating diseases, which was generally not statistically relevant. It would be worth enrolling more patients so as to verify a possible influence of HHV-8 infection in the presence and evolution of DM2 medical complicating pathologies. Diabetes is reported as opening the door to many infections, especially in patients who have had the disease for many years and are not well compensated, but this observation is valid for many infectious agents and especially for all the Herpesviruses, without a specific preference for HHV-8 or other viruses [4, 11-13, 15].

In conclusion, almost 80% of DM2 patients showed no signs of HHV-8 DNA in peripheral blood lymphocytes and consequently, the most important risk factors for typical diabetic complicating diseases still remain those generally accepted by most clinicians, namely genetics, life style, smoking and immunosuppression [2, 14, 15]. An additional study with more patients from other different hospitals in the Sardinian Region will be necessary to understand the real role of HHV-8 infection in the establishment and

progression of intercurrent medical diseases in DM2 patients.

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