Case report
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Human herpesvirus 8 DNA sequences in classic Kaposi's sarcoma

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Recent evidence about human herpesvirus 8 DNA sequences in Kaposi's sarcoma patients with or without HIV infection indicates the possible impact of this virus in etiopathogenesis of this disease. To confirm this hypothesis, two samples of cutaneous lesions and one extracutaneous sample from patient with classic Kaposi's sarcoma have been analyzed for the presence of human herpesvirus 8 DNA sequences. Identification of viral DNA sequences was performed with nested PCR method. In all three samples presence of identical DNA sequences of human herpesvirus 8 have been documented. In consistence with previous research obtained results support the hypothesis of possible role of human herpesvirus 8 in pathogenesis of classic Kaposi's sarcoma.

KEY WORDS: *Sarcoma, Kaposi; Skin Neoplasms; Herpesvirus, Kaposi Sarcoma-Associated*

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INTRODUCTION

Kaposi's sarcoma (KS) is a multicentric malignancy that, apart from skin, can affect various internal organs. It became significant medical and dermatovenereological problem after 1981 when its association with AIDS was observed. Moritz Kaposi for the first time described it in 1872 and named it "idiopathic, multiple, pigmented sarcoma" (1). KS is characterized by proliferation of vascular endothelial and lymphoreticular cells, which is rather reactive than neoplastic. The disease is important due to possible involvement of internal organs, what is a cause of death in most cases. On the basis of clinic and epidemiological features, four types of KS can be recognized: classic, endemic, iatrogenic and epidemic. Classic KS with the greatest frequency occurs in Europe and North America, among the elderly male of Mediterranean or Eastern European descent, while endemic form prevails in sub-Saharan region of Africa among the young and adult black male and prepubertal children. Iatrogenic KS can be seen in immunosupressed organ transplantation recipients, and epidemic form is AIDS related. Considering KS as the most common malignancy in AIDS, Center for Disease Control and Prevention in USA recommended in 1993 KS to be the one of criteria for diagnosis of AIDS disease (2).

Etiology and pathogenesis of KS are not clear yet. Occurrence of the disease in specific population or geographical region indicates the possibility that multiple factors such as genetic, environmental and/or ineffective, alone or in combination, may promote the disease appearance. In etiology of KS by sexually transmissible agent, the possibility that TAT-protein of HIV virus, cytomegalovirus and human papilloma virus may have the importance in disease development has been attractive for long time (3-5). Other pathogens, such as human herpesvirus 6, Hepatitis B virus, Mycobacterium avium intracellulare or Mycobacterium penetrans were also considered to be possible causes of KS (6). Recent findings of HHV-8 DNA sequences in tumorous lesions and peripheral blood mononuclear cells of patients with all forms of KS today offer a different picture about the possible etiopathogenetic mechanism of the disease (7, 8).

The objective of this article was to prove the presence of HHV-8 in cutaneous and extracutaneous tumorous lesions of classic KS and in this way to contribute to the actual hypothesis about the importance of of HHV-8 in KS.

MATERIALS AND METHODS

Diagnostic process was performed in three different institutions: Clinic for Dermatovenereology of Clinical Centre Novi Sad, Institute for Histology and Embryology in Modena, and Institute for Virusology in Modena.

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The analysis was performed on deparaffinised biopsy specimens of skin (2 tumorous skin lesions) and extracutaneous site (subcutaneous tumorous infiltrate in left retroauricular region). All 3 lesions were pathohisthologically verified KS. HIV serology was negative. Deparaffinisation of tissues was done in xylene, and for DNA extraction proteinase K and chloroform/phenol were used. Extracts were dissolved in 60 μl of de-ionized water, and approximately 100 ng of extracted DNA from each sample were used for PCR.

PCR analysis

DNA sequences of HHV-8 were detected by nested PCR method. The amplification of HHV-8 DNA from deparaffinised samples was performed in 25 successive cycles during the first phase of reaction . Pairs of specific primers were used: KS1-5'-AGC CGA AAG GAT TCC ACC AT-3'; KS-2-5'-TCC GTG TTG TCT ACG TCC AG-3', obtained from $KS330_{233}$ sequence of HHV-8, in order to increase the fragment of 233 pairs of bases (pb) (8). Second round of amplification was done in 30 successive cycles in order to increase the 160 pb fragments within K1/K2 products. 5 μl of PCR products obtained with K1 and K2 primers were added to the PCR mixture, which consisted of primers NS1 and NS2 (NS 1-5'-ACG GAT TTG ACC CCG TGT TC-3'; NS 2-5'- AAT GAC ACA TTG GTG GTA TA-3') (9). To avoid false positive or false negative results every PCR series contained negative control (DNA extracted from negative sample) and positive control (DNA with KS330233 sequence). After electrophoresis, samples were analyzed in 2% agarose gel with ethidium-bromide. Ultraviolet light was used to visualize products of HHV-8.

RESULTS

HHV-8 DNA sequences were confirmed in all 3 specimens (Figure 1). Sequences corresponded to the 160 pb amplicone within KSS 330 region of viral DNA.

DISCUSSION

Using PCR method Chang et al. in 1994 for the first time confirmed the presence of HHV-8 DNA sequences in tissue samples of HIV infected KS patient (8). Detected sequences of 631 and 330 nucleotides were characterized as herpesvirus-like sequences (HVSS) or KS-related herpesvirus (KSHV). One of these sequences, named KS330 showed high level of similarity with capsid-coding genes of Herpesvirus saimiri and Epstein-Barr virus (8). Two years afterwards, by sequencing the complete HHV-8 genome, these sequences confirmed to be the constitutive parts of this virus genome (7,10). Later studies confirmed Chang's work, and detected HHV-8 DNA sequences in classic, endemic and iatrogenic KS also (11-14).

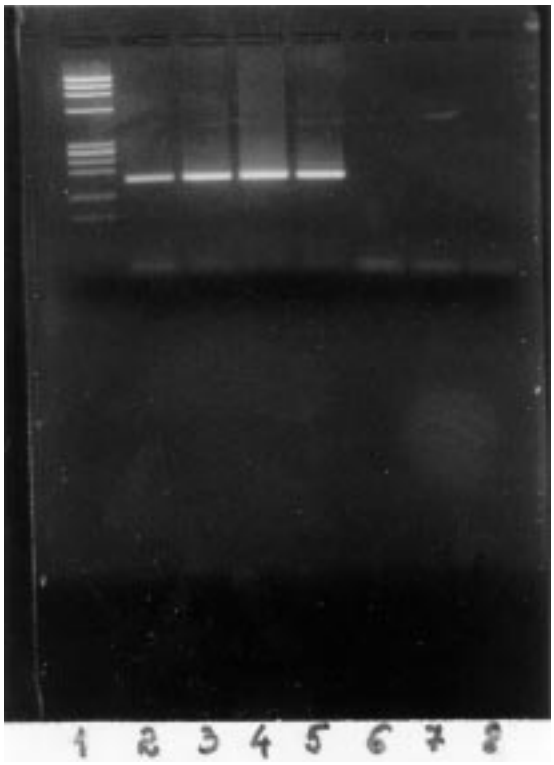

Figure 1. Nested PCR analysis of HHV-8 DNA in tissue specimens of classic KS Legend: Column 1: DNA marker Φ 174; Column 2 and 3: specimen of tumorous skin lesion; Column 4: positive control (DNA extracted from positive specimen); Column 5: specimen from extracutaneous lesion; Column 6: HHV-8 negative control (DNA extracted from negative sample); Column 7 and 8: empty laboratory dish

Due to similarity with Epstein-Barr virus and Herpesvirus saimiri (squirrel monkey virus), HHV-8 is included in Gamma-herpesvirus family. Previous investigations confirmed that virus has affinity for B-lymphocytes, vascular endothelial cells and spindle cells within KS tumors, causing cytopathic effects. Nowadays, it is believed that endothelial and spindle cells of KS lesions are the site of virus latency and active replication. Presence of virus in peripheral blood mononuclear cells of HIV positive KS patients was proved in 50% of cases, and level of virus detection was in correlation with the decrease of CD4+ cells number and clinical stadium of KS. It has been proved that the infection of peripheral blood mononuclear cells with HHV-8 precede and predict KS appearance in the majority of patients with any of HIV related tumors (15). Increased seroprevalence of HHV-8 in HIV infected patients and proved time relationship (3-10 years) from the infection with HHV-8 to the appearance of KS, together with findings of HHV-8 DNA sequences in KS patients, indicate the etiopathogenetic connection of KS and HHV-8. Findings of herpesvirus-like sequences mRNA in 100% of samples of HIV related KS, and 72% of KS without HIV infection, support the possible etiologic role of this new herpesvirus in the pathogenesis of this tumor (13).

Results of Moore et al. who detected HHV-8 DNA sequences not only in tissue samples of HIV related KS but also in samples of classic KS and KS of HIV-negative homosexuals, support the possibility that HHV-8 is the cause of KS with or without accompanied HIV infection (7). How exactly this virus leads to KS development has not been understood yet. Is it directly involved in carcinogenesis, or is it acting together with other pathogens and genetic factors are the questions that have still to be answered. The possibility that virus is responsible for tumor appearance indicates the fact that viral genome in host cells encodes numerous viral homologues in cellular genes, oncogenesis promoters, such as cellular proliferation inducers (υ cyclin), proinflammatory cytokines (υ -MIP-I, υ -MIP-II), or inhibitors of apoptosis (υ -Bcl-2, υ -IL-6, υ -FLIP). Hypothesis about HHV-8 as a cause of KS has also been confirmed by the presence of virus in lymphoproliferative disorders, such as lymphomas of body cavities or Castelmann disease, which are often but not always HIV related (10). In consistence with the results of other authors, our results confirmed presence of DNA sequence of HHV-8 in changes of clasical KS. The identified sequence is one of many sequences of viral DNA that was previously found in different lymphproliferative diseases with or without HIV infection, KS and lymphatic system of healty persons (9,10). This sequence if a fragment of HHV-8 genome (KS330) which has high level of homology with ORF 26 fragment of herpesvirus saimiri and BDLFI ORF fragment of Epstein-Barr virus and can codify numerous proteins (16). This sequence of 160 bp is a fragment of capsid proteine gene of 233 pb that is responsible for synthesis of capsid protein VP23 (16).

The exact mode of viral transmission has not been resolved yet. Most of evidence goes in favour of sexual transmission (17-19). Seropositivity in children and detection of virus in nasal secretion, saliva and sperm of healthy immunocompetent persons indicate the possibility for another mode of transmission (20).

Exact data about the total HHV-8 seroprevalence have not been obtained yet. Previous results were based on serology with antilytic or anti-latent antinuclear antibodies. Lennett´s research from 1996 indicated about 25% seroprevalence in general population, including volunteer blood donors (21). Antilytic and latent antibodies to HHV-8 antigens were detected in 50% of African population (32% in Zimbabwe, 56% in Nigeria, 80% in Uganda, Zaire, Zambia, and 100% in Ivory Coast). In Great Britain, North America and Italy prevalence was less than 5%. Different seroprevalence in different geographic regions were not in correlation with KS development. Non-specific reactivity of lytic immunofluorescent assay, and lower sensitivity of latent immunofluorescent assay might have been the reason for inaccurate seroprevalence data.

CONCLUSION

The finding of HHV-8 DNA sequences in cutaneous and extracutaneous lesions of classic KS is one more confirmation to the hypothesis of possible etiopathogenetic role of HHV-8 in Kaposi's sarcoma development, independent from the HIV infection.

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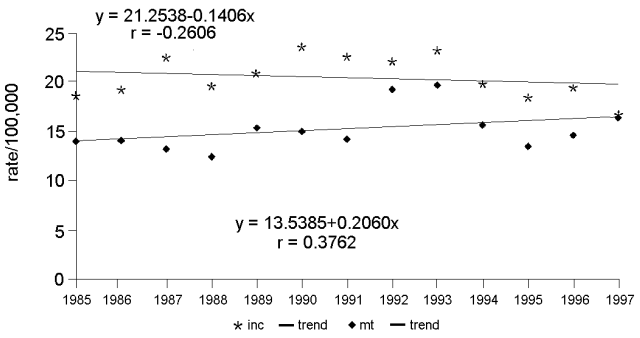
INCIDENCE AND MORTALITY OF PROSTATE CANCER



Prostate cancer accounted 4.2% of all male cancer cases and 5.3% of all cancer deaths in 1997 in Vojvodina. Incidence rates have been increasing until 1993 and decreased afterwards without clear reason. This may be partly due to decreasing histological examination as an important source of information for prostate cancer. Mortality has been increasing until 1993 than it was decreasing from 93-95 and started increasing again from 1995.

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