Kaposi’s sarcoma-associated herpes virus—a new concern for human reproduction?

Mark R. Howard¹ and Gulam Bahadur²,³

¹Department of Virology, University College London Medical School and ²Department of Obstetrics and Gynecology, Reproductive Medicine Unit, University College London Medical School and University College London Hospitals Trusts, 88–96 Chenes Mews, London WC1E 6HX, UK

The detection of the recently discovered Kaposi’s sarcoma-associated herpes virus (KSHV) in human immunodeficiency virus-uninfected donor semen and in blood from a normal blood donor has led us to review this new area of health concern, with emphasis on a number of studies conducted into the presence of the virus in semen and the possibility of transmission during assisted conception procedures.

Key words: donor insemination/human immunodeficiency virus/Kaposi’s sarcoma-associated herpes virus

Before 1982, the acquired immunodeficiency syndrome (AIDS) pandemic Kaposi’s sarcoma (KS) was largely restricted to specific geographical areas of the world such as sub-Saharan Africa and the south-eastern Mediterranean (Reynolds et al., 1965). However in 1982, cases of this rare tumour were noted in young gay men from New York and San Francisco (Centers for Disease Control, 1981). Not only did this observation herald the arrival of AIDS and HIV-associated disease in Western society but, because of the particular population in which KS was occurring, it served to reinforce the notion of a distinct infectious cause for KS itself. By the beginning of the 1990s epidemiological data showed that among all HIV-infected individuals homosexual men are far more likely to develop KS. In some studies up to 30% of patients with AIDS who were homosexual were suffering from the disease (Fauci et al., 1985; Beral et al., 1990). These data were interpreted as supporting the presence of an infectious agent in the homosexual population which was involved in the pathogenesis of KS (Beral et al., 1992).

It was not, however, until 1994 that a likely candidate agent for the pathogenesis of KS was described (Chang et al., 1994). Researchers at Columbia University published nucleotide sequence data derived from a novel herpes virus with homology to members of the gamma-herpes virus family, which includes the human pathogen Epstein–Barr virus (EBV) and other non-human herpes viruses such as herpes virus saimiri (HVS). The virus has now been shown to have many characteristics of viruses from this group, including a tropism for CD19-positive B-lymphocytes (Ambroziak et al., 1995) and the presence in its genome of a number of genes (such as vbl-2 and v-cyclin) with homology to human cell-cycle regulation genes which may be involved in the transformation of cells in vivo (Li et al., 1997; Hardwick et al., 1997). In general, however, the physical and genomic organisation of the virus is common to all herpes viruses. Visualization by electron microscopy has shown the same membrane-surrounded capsid structure (~150 nm diameter) typical of herpes viruses and the recently completed sequencing of the genome shows familiar organisation of well-defined herpes virus genes such as thymidine kinase, the structural capsid proteins and the DNA polymerase genes into a number of unique and multiply-repeated genome sections (Renne et al., 1996).

Soon after the initial discovery of viral genetic material the same group from Columbia also showed that parts of this sequence could be commonly and exclusively amplified by the polymerase chain reaction (PCR) from KS tissue (Chang et al., 1994). Over the next few months following this report these same sequences were detected in all forms of KS, including those not associated with HIV infection: classical KS affecting predominantly elderly southern Mediterranean males, KS following organ transplantation and African endemic KS (Boshoff et al., 1995; Moore et al., 1995; Schalling et al., 1995). Although this evidence increasingly suggested a link between the virus and the disease, controversy arose over the interpretation of the association (Cohen, 1995). In order to explain the observed patterns of disease any agent responsible for KS development should be largely restricted to the populations at greatest risk, such as homosexual males. The occurrence of KS in AIDS patients had already been noted to differ widely depending on the risk factor that the patient had for HIV infection. In general HIV-infected patients who had not acquired HIV through homosexual contact had an extremely low incidence of KS. Heterosexuals, intravenous drug users and haemophiliacs with HIV infection all had incidences of KS <5% and only African HIV-infected patients had an incidence of KS similar to homosexuals at ~30% (Beral et al., 1990). If KS was caused by this new herpes virus such a pattern of infection was at odds with one of the fundamental characteristics of all known human herpes viruses; their globally high prevalence in human populations.

With this in mind a number of researchers suggested that rather than causing KS, the new virus now termed Kaposi’s sarcoma-associated herpes virus (KSHV), also known as human herpes virus 8 (HHV8), was instead an opportunist, able to preferentially replicate in already established KS tissue. In order to resolve this dilemma it was necessary to determine both a temporal association between the virus and the development of KS disease and define the prevalence of the virus on
a population basis. A collaborative group from University College London and the Institute of Cancer Research, London were able to show using PCR amplification that detection of viral DNA in the peripheral blood of a group of HIV-infected and uninfected men appeared to be restricted to those individuals with, or at greatest risk of, KS (Whitty et al., 1995). Importantly it was also shown that detection of KSHV in the peripheral blood of those patients at risk of, but who had not yet developed, KS was highly predictive of subsequent disease development with such detection possible up to 4 years prior to the onset of KS (Whitty et al., 1995). Although in this study an association between the level of immune suppression, as measured by CD4-positive T-lymphocyte count, was not fully established, recent data (Bigoni et al., 1996) have shown that viral DNA is easier to detect by PCR analysis in individuals with a very low CD4 count. It has also been found that KSHV DNA is relatively difficult to detect in the peripheral blood of patients with KS who are not HIV-infected and have intact immune systems. Both these findings suggest that replication of the virus is, in vivo, under some form of immunological control.

Although detection of viral DNA in the peripheral blood was a useful diagnostic tool, it became apparent that by DNA detection alone it would be difficult to determine the true prevalence of the virus, at least until secure serological assays were available. Of paramount interest was the question as to whether KS was a rare manifestation of a common infection or a common manifestation of a rare infection? Until serological analysis became possible it was also important to determine the likely transmission route of the virus. With the restricted prevalence of KSHV it appeared unlikely that transmission of the virus was by casual contact with infected saliva which has been demonstrated for human herpes virus 6 (HHV6), cytomegalovirus and EBV transmission (Gerber et al., 1972; Harrett et al., 1990). The only other route of transmission which fitted the epidemiological profile of KS was sexual. Sexual transmission of herpes viruses has been previously demonstrated for herpes simplex viruses 1 and 2 (HSV 1 and HSV 2) and also for cytomegalovirus (CMV), especially in homosexual sexual contact (Corey et al., 1983; Collier et al., 1987). As up to 30% of HIV-infected homosexual men developed KS, anal intercourse appeared to be a highly probable transmission event with semen being an immediate candidate for horizontal virus passage.

Notwithstanding the poor sensitivity of DNA detection, a number of important studies have now been conducted regarding the prevalence of KSHV in the semen of men with both low and high risk of developing KS. The findings were in two broad categories; those who found KSHV DNA in a significant percentage of all semen collected from both HIV-infected homosexuals and HIV-uninfected semen donors, and those who found the virus restricted to the semen of those individuals with or at greatest risk of developing KS. In the first of such studies Ambroziak et al. (1995) reported that they were unable to detect KSHV in semen from five HIV-infected men with KS. However, in two subsequent studies the virus was found not only in the semen of a high percentage of HIV-infected KS patients (90%), but also in a significant number of semen from HIV-uninfected semen donors (23%) (Lin et al., 1995; Monini et al., 1996). The claim to find KSHV in donated semen immediately raised serious concern as to the findings of previous studies that had suggested a limited spread of KSHV outside high-risk group individuals, and sparked renewed debate as to the casual or causal association between KSHV and KS. A number of groups have since attempted to repeat and confirm these findings by examining semen and prostate tissue from KS patients and from control patients residing in similar geographical locales as in the previous studies of Monini and Lin. To date three studies have been published. Corbellino et al. (1996) examined semen from HIV-uninfected Italian donors but were unable to detect the virus in any. Tasaka et al. (1996) were similarly unable to detect KSHV in prostate tissue from ten Italian men and ten men from the United States, all of whom were HIV-uninfected. Gupta et al. (1996) did detect KSHV in the semen of a small percentage (14%) of HIV-infected homosexual men with KS, but were unable to detect virus in the semen of a control group of HIV-infected men with no KS from the USA.

The discrepancies between these studies cannot be easily explained by differing viral DNA detection sensitivities or disparate patient and control populations. In order to relate these observations to UK populations our collaborative group initiated a study to determine the prevalence of KSHV in semen from HIV-infected homosexual men with, and without, KS attending a London HIV outpatient clinic, and to compare this with the KSHV prevalence in a large number of semen samples donated and stored at the Middlesex Hospital fertility clinic (Howard et al., 1997). By examining donor semen from the fertility clinic we hoped to determine whether there was a measurable risk of KSHV transmission to women receiving assisted conception techniques using donor semen, which would not have been eliminated by the current donor screening protocols.

In our study we were able to show, using purified DNA from the KSHV-containing cell line, HBL-6, that our absolute PCR detection sensitivity was two copies of KSHV genome, a level at least as sensitive as all previous studies. Quantification of cellular DNA from each sample enabled us to confirm that comparable amounts of DNA from both the HIV-infected and uninfected semen were analysed. We also confirmed that no extractions contained inhibitors which might have affected PCR sensitivity and that DNA extraction from all samples was equally efficient. Semen from 115 donors attending the fertility clinic were analysed and although four contained CMV DNA, we did not detect KSHV DNA in any samples. We were however able to detect KSHV in the semen of 25% of our HIV-infected homosexual men, a frequency similar to that reported by Gupta and colleagues, confirming that KSHV may indeed be found in seminal fluid. There was no significant difference in the prevalence of KSHV in those HIV-infected homosexual men with or without KS, and it remains to be determined whether detection of the virus in semen from HIV-infected individuals without KS is predictive of subsequent KS development in an analogous way to its finding in peripheral blood and bronchoalveolar lavage fluid (Whitty et al., 1995; Howard et al., 1995).
It is also difficult to currently determine whether a co-factor, such as HIV-1, is necessary for KS development in individuals who are infected with KSHV. Although some groups have argued that certain HIV-1 gene products, including the tat protein, may influence the formation of KS, it should be noted that KS may occur in groups of individuals who are well characterised as being HIV-uninfected (Ensoli et al., 1993). However, it does appear that the severity of KS is usually increased if an individual has an HIV infection with the accompanying reduced level of immune system function.

As the above data might suggest that there is currently no consensus view as to the prevalence of KSHV in semen from healthy donors, especially in the USA and Italy. It was initially suggested that the findings of Monini and Lin were due to the specific geographical areas from which their HIV-negative donors were drawn. The donors analysed by Monini were recruited from a region of Italy in which classical, HIV-unassociated KS is 30 times more common than in the UK. However, with a number of recent studies disputing the reported high prevalence of KSHV in semen of healthy semen donors from similar geographical localities, it might be that some form of technical difficulty, possibly in the form of laboratory PCR contamination, may have been responsible for the very high numbers of virus-positive samples found by the two early studies. Unless great care is taken, 'false-positive' PCR results which overestimate the percentage of positive samples in a study may be difficult to avoid. Currently our study remains the only one to investigate semen from UK sperm donors and we are confident that the precautions and controls included in our experimentation ensure the validity of our findings. We remain convinced of the low prevalence of KSHV infection in the UK and this work has led to a recent review which supported the current evidence suggesting a sexual route for KSHV transmission (Blackbourne and Levy, 1997).

In the long term, the most accurate studies of the prevalence of KSHV are likely to come from population-based serological studies. Recently first generation assays have become available in the form of indirect immunofluorescence (IIF) and recombinant antigen assays, both of which have been shown to be between 80 and 90% sensitive in detecting those individuals with KSHV antibodies (Miller et al., 1996; Gao et al., 1996; Simpson et al., 1996). Preliminary serological results in general support the theory of a low prevalence of the virus in the general blood donor population, infection being essentially restricted to the already identified groups at risk of KS. Already two reports exist which are highly suggestive of KSHV transmission between different sex partners; the first involved a heterosexual woman who had a single sexual encounter with an HIV-infected bisexual man (Barry et al., 1991). The man developed KS 3 years after the encounter, the woman developed KS 2 years after her ex-partner. The woman had only one other, HIV-uninfected, sexual partner. It would appear that this one exposure to theoretically KSHV-contaminated semen was sufficient to enable infection of the recipient and subsequent disease development. The second documented transmission involved an HIV-positive heterosexual man with multiple partners, who claimed to have had no homosexual contact (Tirelli et al., 1996). He developed KS of the skin, oesophagus and penis. His wife was subsequently found to be infected with HIV; her only risk factor was sexual contact with her husband. She subsequently developed Castleman's disease, previously shown also to be associated with KSHV (Soulie et al., 1995). KSHV viral DNA was detected in both individuals. Although both these cases of heterosexual transmission involve a donor who was HIV-infected, they serve to underline the possibility of transmission of the virus to both male and female partners of infected individuals. Whether such transmission would result in disease if the individual was not also infected with HIV remains to be determined, as currently the risk of an immunocompetent individual developing KS following KSHV infection is unknown.

The proportion of semen taken from KSHV-infected donors that will be infectious for a recipient also remains unquantified. Nor for that matter is it known whether the detection of KSHV DNA indicates infectivity, although PCR analysis does currently remain the only effective method of identifying viral presence in semen. Interestingly among the 115 donor semen we have tested, donated at a time prior to CMV antibody
screening, only four contained CMV DNA, an incidence similar to that previously described by culture of CMV from the semen of healthy donors (McGowan et al., 1983). It would therefore appear that only a minority of CMV seropositive donors shed CMV in semen and the same may be true for KSHV. Until such time as a reliable assay for anti-KSHV antibodies is readily available and suitable for screening donors, the potential hazard of unknowingly using semen from KSHV-infected males remains. Therefore the possibility of inadvertent KSHV transmission during assisted conception procedures should not be overlooked.

Our study of UK sperm donors is to some extent reassuring for UK assisted conception centres practising donor insemination treatments. However, there is a need for similar studies around the world to establish the incidence of KSHV among their regional sperm donor populations. Assisted conception procedures involving couples should not overlook the potential for KSHV transmission between husband and wife. As HIV-positive patients are not excluded from assisted reproduction procedures (Semprini et al., 1992), serious consideration should be given to providing information on KSHV and encouraging KSHV tests for high-risk patient groups. This additional information could be helpful during the counselling and assessment of the welfare of the child and of the mother. However, further discussion of the significance of the test to both the patients and to their clinical management must now be initiated between the relevant disciplines. The increasing number of reports of supposed KSHV sexual transmission and knowledge of the 2–5% seroprevalence of KSHV in UK blood donors underlines the importance of heightening awareness of this new virus to a wider range of public health care workers. Thus far, we feel that the published epidemiological and serological studies do suggest a potential sexual route for KSHV transmission.

References


Bigoni, B., Dolcetti, R., de Lellis, L. et al. (1996) Human herpes virus 8 is present in the lymphoid system of healthy persons and can reactivate in the course of AIDS. J. Infect. Dis., 173, 542-549.


Received on April 10, 1997; accepted on August 8, 1997