Increased seroprevalence of human herpes virus-8 in renal transplant recipients in Saudi Arabia


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Abstract

Background. Human herpes virus-8 (HHV-8) is a herpes virus that is always associated with Kaposi’s sarcoma. Previous studies suggested a high rate of Kaposi’s sarcoma in renal transplant patients in Saudi Arabia. The aim of this study was to investigate the prevalence of HHV-8 in Saudi renal transplant recipients and healthy controls.

Methods. An immunofluorescence technique was used to detect antibodies to the latent nuclear antigen (LANA) of HHV-8 in renal transplant patients, members of a family affected with Kaposi sarcoma, as well as healthy controls.

Results. A significantly higher HHV-8 seroprevalence was detected in renal transplant recipients from Saudi Arabia (27 out of 150; 18%) and in members of a family affected with Kaposi sarcoma (seven out of 10; 70%) relative to the seroprevalence in healthy controls (10 out of 577; 1.7%). Seropositivity for HHV-8 in these transplant patients was not significantly influenced by: the existence of relatives with kidney failure, the donors’ country of origin, the recipients’ home region within Saudi Arabia, the haemodialysis centre, the time that elapsed since the renal transplantation operation and the immunosuppressive regimen used.

Conclusion. The present results provide some explanation for the previously noted high incidence of Kaposi’s sarcoma in Saudi transplant patients.

Keywords: HHV-8 seroprevalence; immunosuppression; renal transplant recipients

Introduction

Human herpes virus-8 or Kaposi’s sarcoma herpesvirus (HHV-8/KSHV), a γ-herpes virus closely related to Epstein–Barr virus (EBV), was first identified about a decade ago [1]. Humans are the only known host for the virus [2]. HHV-8 was shown to infect B cells and endothelial-derived spindle cells of Kaposi’s sarcoma lesion, monocyte–macrophage cells, prostate epithelia and dorsal root sensory ganglion cells [3], where CD19+ B cells represent the main reservoir of asymptomatic infection [2]. HHV-8 is associated with Kaposi sarcoma (hence the name KSHV), primary effusion lymphoma, Castleman’s disease and acute bone marrow failure in renal transplant patients [4]. The known routes of transmission of HHV-8 include sexual transmission, saliva, blood products, allogenic bone marrow transplantation and solid organ transplantation [2]. HHV-8 is considered to be a necessary but not sufficient cause of Kaposi sarcoma [5]. Infection with human immunodeficiency virus (HIV) plays an important role in the development of Kaposi sarcoma, and this factor is believed to be largely responsible for the marked increase in the incidence of the tumour in parts of Sub-Saharan Africa [4,6]. Other forms of immunosuppression, such as found among organ transplant recipients, are also associated with an increased risk of Kaposi sarcoma in HHV-8-infected individuals [7]. The distribution of HHV-8 appears to be related to a combination of geographic, behavioural and genetic risk factors. Outside of Africa, HHV-8 seroprevalence rates roughly match the geographic distribution of Kaposi sarcoma [8]. Serological studies have shown HHV-8 to be comparatively rare in Northern and Western Europe, but more common in countries bordering on to the Mediterranean, in particular
Southern Italy, Greece, the Mediterranean coast of North Africa, and Palestine/Israel, with reported seroprevalence rates in the order of 10–30% using conservative estimates [2]. HHV-8 is more widespread in Sub-Saharan Africa, with a seroprevalence rate range of 20–50% [9]. There has been no report on a difference in HHV-8 infection rates between males and females. There are scant data on the prevalence of HHV-8 in Saudi Arabia. In two hospital-based studies, HHV-8 in the general population of the country was reported to reach seroprevalence rates of approximately 4–7% [3,10].

The previously noted high rate of Kaposi sarcoma in renal transplant patients in Saudi Arabia [10,11] could imply a markedly increased HHV-8 seroprevalence rate in this group of patients. Therefore, the aim of this study was to investigate the seroprevalence of HHV-8 in renal transplant recipients from Saudi Arabia and explore the factors that influence the prevalence of the virus in this group of patients.

Patients and methods

Patients and controls

Participants in this study were 150 kidney recipients who regularly attended the nephrology clinics of King Fahad Military Medical Complex (KFMMC, Dhahran, Saudi Arabia) in the spring of 2004. The main inclusion criterion for participation in this study was that patients should have reached the clinically stable stage following renal transplant surgery, usually 6 months post-operatively. This cohort of patients comprised 76 males and 74 females, of mean age 41 years (range 17–72); they are predominantly Saudi nationals (143), four Yemenis, two Sudanese and an Egyptian. The second group of participants comprised 10 siblings of an extended Saudi family where four had been diagnosed with Kaposi sarcoma. The third group comprised 577 randomly selected healthy Saudi nationals, 464 females and 113 males, of mean age 27 years (range 18–44). The last cohort of individuals were either women attending obstetrics clinics (351) or couples seeking pre-marital genetic counselling (226). Standard clinical care, including medical check ups and tests for renal function, liver function and haematology parameters, was given to the renal transplant recipients in the stable post-operative stage. At this stage, all patients were receiving a standard immunosuppressive regimen consisting of cyclosporin and prednisolone in combination with either azathioprine or mycophenolate mofetil. The administered dosage of each of these drugs was carefully adjusted as necessitated by the clinical status of each patient.

Methods

Serum samples were collected from all study participants, and seropositivity for HHV-8 was determined by testing for antibodies to the latency-associated nuclear antigen (LANA) employing an immunofluorescence assay as described previously [12].

HHV-8 seroprevalence rates in renal transplant recipients were analysed statistically in relation to the following factors: (i) the type and level of immunosuppressive agents administered post-operatively; (ii) the existence of relatives with renal failure; (iii) the country of origin of the kidney donor and whether he/she was alive or dead at the time of donation; (iv) the home region of the recipient within Saudi Arabia; (v) the medical centre where haemodialysis was performed prior to the kidney transplantation; and (vi) the time that had elapsed since the transplantation operation. Statistical analysis of the data was performed using the SPSS statistical package (chi-square or Fisher’s exact tests) to calculate the $P$-values.

Clinical data

In the present study, the kidney donors came from Saudi Arabia (46 patients), the Philippines (22), Pakistan (21), Egypt (19), Bangladesh (10), India (10) and Yemen (6), as well as from six other Asian countries and the USA (16). The number of living kidney donors was 119, and the number of deceased donors was 31. The kidney recipients originated from different regions within Saudi Arabia as follows: northern (74 patients), southern (48), western (19) and eastern (4). For the majority (124 out of 150) of renal transplant recipients, haemodialysis was performed for extended periods prior to renal transplantation in five different medical centres in the eastern region of the country. For the rest of patients, haemodialysis was performed in various hospitals of the other regions of the country. Twenty-one renal transplant recipients, comprising 14% of the total patients, have one or more relatives diagnosed with renal failure. The time that elapsed since the renal transplantation averaged 41.4 months. The primary cause of renal failure in the renal transplant recipients was unknown (63 out of 150; 42%), hypertension (42 out of 150; 28%) and other causes related to 12 different diseases (45 out of 150; 30%), the most prominent of the last category being diabetic nephropathy (18 out of 150; 12%), glomerulonephritis (10 out of 150; 6.7%), chronic pyelonephritis (four out of 150; 2.7%) and systemic lupus erythematosus (three out of 150; 2%).

Results

As measured by immunofluorescence, antibodies to HHV-8-LANA were significantly more frequent in renal transplant recipients (group 1) developed Kaposi’s sarcoma. In siblings of the investigated Saudi family (second group), seropositivity for HHV-8 was remarkably high as antibodies against HHV-8 could be detected in seven out of 10 individuals (70%). Among the seven HHV-8-seropositive individuals of this family there are three persons with renal failure: two sisters (aged 48 and 41 years), and a 46-year-old brother who had undergone kidney transplantation and had been receiving immunosuppressive drugs for 6 years prior to the excision of the transplanted kidney due to the development of Kaposi’s sarcoma. The other four siblings of the family found to be positive for HHV-8 are apparently healthy except for hypertension that affected all of them.
Our data have shown that 52 kidney transplant recipients received azathioprine and 98 patients received mycophenolate mofetil. Both of these two subgroups of patients received comparable low doses of cyclosporin and prednisolone. Relatively more HHV-8 seropositivity was found to be associated with receiving azathioprine (12 out of 52, 23%) as compared with receiving mycophenolate mofetil (15 out of 98; 15%), but this difference was not statistically significant ($P=0.238$). Among both the azathioprine and mycophenolate mofetil subgroups, there were no significant differences between the high daily dose ($\geq 75$ and $\geq 1000$ mg, respectively) and low daily dose ($\leq 50$ and $\leq 500$ mg, respectively) regimens of the two immunosuppressive drugs used with regard to HHV-8 seropositivity.

The present results show that the association of HHV-8 seropositivity in renal transplant recipients was higher (11 out of 42; 26%) in patients with hypertension as the primary cause of renal failure when compared with HHV-8 seropositivity in patients with all known causes of renal failure together (seven out of 45; 15%). However, this difference was not statistically significant ($P=0.221$).

The rate of HHV-8 seropositivity in renal transplant recipients who have relatives with renal failure was slightly higher than that in patients who have no such relatives (five out of 21; 23.8% vs 22 out of 129; 17.1%), but this difference was not statistically significant ($P=0.54$). The HHV-8 seropositivity in renal transplant recipients in relation to the origin of kidney donors, and whether they are alive or deceased, the recipients’ home region within Saudi Arabia, the medical centre where haemodialysis was performed or the time that elapsed since the kidney transplantation operation showed no significant differences between the different groups.

Discussion

The results of the present study confirmed high HHV-8 seroprevalence (18%) in renal transplant recipients relative to its prevalence in the general population (1.7%). We also found a very high HHV-8 seropositivity rate (70%) in an Arab family affected with Kaposi’s sarcoma. Hence, the present results highlight the importance of HHV-8 infection in renal transplant patients.

The observed HHV-8 seropositivity in renal transplant recipients (18%) in this study is lower than that previously reported (28%) in renal transplant recipients from Saudi Arabia [10]. Similarly, it has been found that HHV-8 seropositivity in the healthy control group (1.7%) is much lower than the seropositivity (7%) reported in that study. This difference can most probably be attributed to two factors: first, the LANA immunofluorescence assay for antibodies against HHV-8 used in this study has been reported to have lower sensitivity but higher specificity as compared with the immunoblot assay for ORF65 antigen, as used by the other group. Secondly, in this study, 150 kidney recipients and 577 healthy controls were tested for HHV-8, whereas in the report of Qunibi et al. [10], 32 renal transplant patients and 15 healthy controls were tested.

On the other hand, in a more recent study, Almuneef et al. [3] did not find a significant difference in HHV-8 antibody prevalence between 201 Saudi patients with end-stage renal failure (7.0%) and 258 individuals without renal disease (3.9%). We believe that the difference in seroprevalence between the control groups in their and our study is due to the different antibody assays used. Almuneef et al. [3] measured antibodies to HHV-8 using an indirect immunofluorescence (lytic HHV-8 antigen) technique and confirmation by immunoblots using acetate-induced BC-3 cell extract and the recombinant small viral capsid antigen ORF65. The facts that a significantly higher HHV-8 seroprevalence rate was observed in renal transplant recipients compared with healthy controls in this study, and that Almuneef et al. [3] did not find a significant difference between patients with end-stage renal failure and healthy controls, would suggest that either immunosuppression in the transplant recipients increases the rate of HHV-8 seropositivity or that HHV-8 is frequently transmitted to transplant recipients via the transplanted organ or as a result of more frequent transfusions prior to renal transplantation in these patients. Recently it has been shown that HHV-8 viral load in saliva, or detection of HHV-8 DNA by polymerase chain reaction (PCR), correlates with higher antibody levels to a lytic HHV-8 antigen [13]. Taken together, these observations suggest that increased viral replication during immune suppression can lead to an increased rate of antibody positivity.

In the present study, the mean age in the transplant patient cohort was higher than that of the healthy controls (41 vs 27 years). It has been reported in previous studies that HHV-8 seroprevalence increases with age[14]. However, in our study, the seroprevalence of HHV-8 in the renal transplant recipients is much higher (>10-fold) relative to the healthy controls. Therefore, it is highly unlikely that such an increase could be attributed to the age factor. Nonetheless, the effect of age cannot be completely excluded. In contrast, the effect of induced immune suppression seems to be a more prominent factor.

Infection with HHV-8, and development of Kaposi’s sarcoma, have been observed to be associated with induced immune suppression in many studies [4,5,7]. Hence, immune suppression is hypothesized to exert a significant effect on the seroprevalence of HHV-8. It should be mentioned here that there is an alternative opposite hypothesis that stems from the growing evidence in support of an unanticipated inhibition of viral replication by various immune suppressive drugs including azathioprine and mycophenolate acid. For example, azathioprine has recently been reported to decrease bovine viral diarrhoea virus 10 times more than mycophenolate acid [15].
In this study, there was no notable difference in the duration of follow-up between the azathioprine-treated patients and the mycophenolate mofetil-treated patients. In addition, we have observed a tendency for higher prevalence of HHV-8 in azathioprine-treated patients than in patients treated with mycophenolate mofetil. We have no direct explanation for this difference. However, this finding is apparently in contrast to that of Stangl et al. [15] who reported that azathioprine has a more powerful antiviral effect compared with mycophenolate acid.

As to the possibility of transmission through the transplanted organ or blood transfusions, we did not find any evidence for a link between HHV-8 prevalence rates in Saudi transplant recipients and the provenance of the transplanted organ, the geographic origin of the transplanted patient nor the medical centre where haemodialysis was performed prior to transplantation. Blood transfusion has been linked to HHV-8 transmission in HHV-8 endemic countries [16], but this remains controversial and may not apply to non-endemic countries such as Saudi Arabia. However, several case reports have demonstrated that HHV-8 can be transmitted with the transplanted organ [17], and a recent review of these reports suggests that this may not be an infrequent event [18].

The present study also shows that seropositivity for HHV-8 in renal transplant recipients is not significantly increased in patients with relatives suffering from kidney failure or with time that elapsed since renal transplantation. We therefore did not find any evidence strong enough to support a familial link with the increased seroprevalence in transplant recipients. In contrast, we have observed markedly increased HHV-8 seroprevalence rates in an extended family with a history of Kaposi’s sarcoma. This could be due to intrafamilial spread of HHV-8, which has been shown to be transmitted efficiently from mother to child and from sibling to sibling, but not from father to child [13]. Another possible explanation would be a genetic factor, since an increased rate of classic Kaposi sarcoma in a family has been linked to genetic markers [19], and a genetic factor predisposing to childhood infection has been postulated [20].

Kaposi’s sarcoma has been claimed to be one of the most frequent tumours that develop in organ transplant recipients in Saudi Arabia [7,10]. We have observed that none of the 150 renal transplant recipients (including 27 HHV-8 seropositive patients) in this study developed Kaposi’s sarcoma, although some of them have been exposed to immunosuppressive agents for extended periods of time, exceeding 10 years in some cases. Thus our results reflect an apparent difference from those of Qunibi et al. [10] who reported that 14 (13 of whom are HHV-8 positive) out of 32 renal transplant recipients from Saudi Arabia developed Kaposi’s sarcoma. We have no direct explanation for the fortunate absence of Kaposi’s sarcoma in renal transplant recipients in the present study. This fact underlines the role of other cofactors. However, it should be taken into consideration that the patients in Qunibi’s report were patients referred from different parts of the country to a tertiary medical centre in Riyadh where advanced tumour cases are usually transferred for treatment. Furthermore, the possibility that some patients in that report could have relatives with Kaposi’s sarcoma is not remote, but information on this matter was not given. In comparison, in the present study, Kaposi’s sarcoma was encountered in four individuals who belong to one extended Saudi Arab family. Therefore, we believe that a third factor, possibly genetic make up, other than infection with HHV-8 and exposure to immunosuppressive agents could be involved in the development of Kaposi’s sarcoma.

In conclusion, our results indicate that HHV-8 antibody prevalence, using the LANA immunofluorescence assay technique, is low in the general population of Saudi Arabia, and of a similar range as the rates found in Northern and Central Europe. However, familial clustering of HHV-8 can occur in Saudi Arabia. In contrast, HHV-8 antibody prevalence is increased in renal transplant recipients. If immune suppression increases the rate of HHV-8 antibody positivity as a result of increased viral replication in infected individuals, it may be possible that current serological assays underestimate the true prevalence of HHV-8 in the non-immunocompromised general population.

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References


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