

## Original Article

# The Frequency of HHV-8 Infection in Otherwise Healthy Blood Donors as Well as Renal Allograft Recipients Living in Iran

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## Abstract

**Background:** Different reports from Middle East countries demonstrated Kaposi's sarcoma (KS) in transplant population. This vascular malignancy occurs mostly among immunocompromised individuals. Human herpesvirus 8 (HHV-8) appears to be the causative factor for the development of this neoplasm. Transplant programs are concerned about the frequencies of HHV-8 infection either in general population or transplant patients.

**Methods:** The current study was conducted in two phases. Firstly, we detected antibodies against HHV-8 in 790 otherwise healthy blood donors. Secondly, a total of 125 kidney allograft recipients evaluated as being seropositive for HHV-8. We utilized enzyme immunoassay (EIA) for serologic studies.

**Results:** Among blood donors, the male to female ratio was 1.05 (405 vs. 385) while the mean age was  $38.9 \pm 11.7$  years. The serostatus of none of these blood donors were positive for HHV-8. Among kidney recipients, the male to female ratio was 1.9 (82 vs. 43). The mean age was  $39.01 \pm 14.77$  years. Two (1.6%) patients were seropositive for HHV-8.

**Conclusion:** The prevalence of HHV-8 infection among Iranians is likely to be low. Yet, owing to the evidence of this infection among kidney allograft recipients and its probable role in developing post-transplantation KS (PT-KS), further studies appear to be required to keep the various aspects of this infection under close surveillance.

**Keywords:** Human herpesvirus 8, kidney transplantation

**Cite this article as:** Hosseini-Moghaddam SM, Keyvani H, Soleimanirahbar A, Karimi G, Daneshvar S, Basiri A. The frequency of HHV-8 infection in otherwise healthy blood donors as well as renal allograft recipients living in Iran. *Arch Iran Med.* 2013; **16(7)**: 376 – 379.

## Introduction

Human herpesvirus 8 (HHV-8) appears to be the causative factor for the development of Kaposi's sarcoma (KS).<sup>1-3</sup> In 1994, this virus was isolated from KS lesions of a patient who suffered from acquired immune deficiency syndrome (AIDS). Considering the genomic structure and life cycle of this agent, it was named as human herpesvirus 8.<sup>4</sup> Based on the data extracted from International Committee on Taxonomy of Viruses (ICTV), HHV-8 is a Rhadino virus of the subfamily Gammaherpesvirinae, of the family Herpesviridae. Owing to its role in the development of KS, it is also called KS-associated herpesvirus (KSHV). HHV-8 is a DNA virus and its DNA core is covered by the protein and lipid coatings. It has been reported to be likely that some genetic sequences identified in HHV-8 genome may be from host cells in origin.

In spite of the association between HHV-8 and AIDS-related KS that has been clearly identified, the link between PT-KS and

HHV-8 infection has not been completely demonstrated. Many aspects of PT-KS and HHV-8 infection still remain unclear. Epidemiologic data seem to be quite limited and the frequency of HHV-8 infection and its probable consequences possesses a scattered global epidemiologic pattern. For instance, although there are several reports of PT-KS in Saudi Arabia, other reports from this region did not demonstrate a considerable prevalence rate of HHV-8 infection.<sup>5,6</sup>

Considering the limited number of reports on the frequency of HHV-8 infection from our region and considerable numbers of reports related to KS following transplantation from this region, we designed an integrative study to demonstrate the followings: firstly, the seroprevalence of HHV-8 infection in general population; secondly, the frequency of HHV-8 infection in organ transplant recipients; and thirdly, the evidence of previous HHV-8 infection in cases suffering from different types of PT-KS. In this study, we focused on the seroprevalence of HHV-8 infection in the two settings: general population and renal transplant patients.

## Materials and Methods

We designed a two-phase, cross-sectional study. In the first phase of the study, we specifically determined the seroprevalence of HHV-8 infection among general population living in Tehran. In the next phase, we focused on the post-renal transplantation seroprevalence of HHV-8 infection. For both phases, our data collection method was based on a questionnaire including the back-

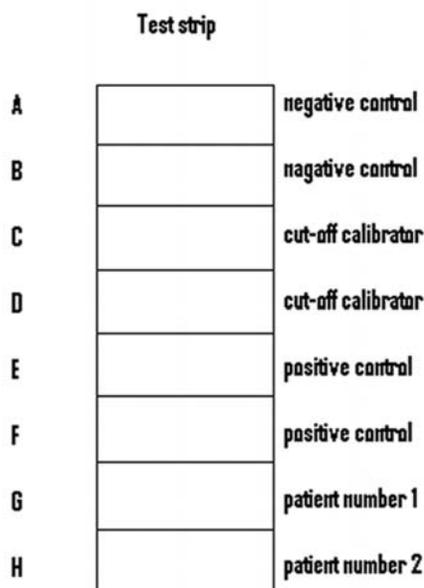
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Accepted for publication: 28 May 2013

**Table 1.** The frequency and the percentage of immunosuppressives use in renal allograft recipients

Period of time	Used drug				
	Prednisolone	Cyclosporine	Mycophenolate mofetil	Azathioprine	Sirolimus
6 months	29 (100%)*	29 (100%)	16 (55.1%)	16 (55.1%)	0 (0%)
1 year	33 (97%)	34 (100%)	13 (38.2%)	18 (52.9%)	11(32.3%)
3 years	30 (100%)	30 (100%)	25 (83.3%)	6 (20%)	0 (0%)
5 years	32 (100%)	32 (100%)	29 (90.6%)	9 (28.1%)	0 (0%)
total	124 (99.2%)	125 (100%)	83 (66.4%)	49 (39.2%)	11 (8.8%)

\*The relative frequencies are given in each group.



**Figure 1.** This figure shows a schematic shape of a test strip and its different wells for the placement of the required materials and specimens.

ground variables as well as risk factors for HHV-8 infection. We also took blood samples from each individual enrolled in this survey after receiving a signed informed consent.

In the first phase, we signed a mutual contract with Iranian Blood Transfusion Organization (IBTO). Subsequently, the research team was allowed to test the anonymous samples of blood donors to determine HHV-8 infection. Human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infections are routinely studied in blood transfusion centers. Determining serostatus of HHV-8 infection is not a routine in Iran; hence, the research team was requested to submit final findings to IBTO after completion of the study. Therefore, the research team focused on five different blood transfusion centers located in the northern, southern, eastern, western, and central districts of Tehran between fall 2006 and spring 2009. A written consent was obtained from each individual before the interview. Blood samples were transported to a laboratory under standard conditions. The serum specimens were maintained at  $-20^{\circ}$  centigrade. Finally, a total of 790 blood donors were enrolled in this phase.

In the second phase, a total of 125 kidney allograft recipients were enrolled. Using a systematic approach, patients were randomly allocated. All enrolled subjects were recipients who received their renal allografts in Shahid Labbafinejad Medical Center, a tertiary referral hospital that provides transplantation services. Renal transplants were regularly referred from differ-

ent provinces of Iran. We excluded subjects with more than one episode of renal transplantation, and cases who received the kidney allografts in other countries. One of the main objectives of this phase was to determine the seroprevalence considering the interval following transplantation. We included renal transplant patients who received allograft within 6 months to 5 years prior to enrollment.

We utilized EIA method (Biotrin Co) to detect anti-HHV-8 antibodies. The diagnostic test included a direct EIA to identify the binding of HHV-8-specific immunoglobulin G (IgG) to lytic peptide antigens (Ag) paired with microtiter test strips (Figure 1). The kit contained 96 wells coated with HHV-8 antigens. The serum samples were placed in the wells and if HHV-8 IgG was present in the specimens, it would bind with the HHV-8 antigens during the incubation step. The sensitivity and specificity of this test have been reported as 90.4% and 93%, respectively.

## Results

In the first phase, we studied a total of 790 blood samples from five different clusters of Tehran. In this phase, male to female ratio was 1.05; 405 (51.3%) to 385 (48.7%). The mean age was  $38.9 \pm 11.7$  years. Individuals in this group were at different educational levels. Considering the marital status, 143 (18.1%) individuals were single, 637 (80.6%) married, and six (0.8%) divorced. In

terms of regional place of living, 162 (20.5%), 160 (20.3%), 172 (21.8%), 147 (18.6%), and 149 (18.9%) individuals were living in Tajrish (north), Azadi (west), Vesal (center), Resalat (east), and Naziabad (south) districts of Tehran, respectively. The history of blood transfusion and drug abuse were positive among 40 (5.1%) and nine (1.1%) individuals, respectively. A total of 153 (19.4%) individuals were cigarette smokers. Totally, 410 (51.9%) individuals had a history of hospitalization  $2.2 \pm 1.6$  times, in average. Additionally, three (0.4%) and 15 (1.9%) subjects were hepatitis B surface antigen (HBsAg) and HCV-Ab positive, respectively. Furthermore, two (0.3%) cases were identified as HIV-Ab positive. In this phase of the study, none of the blood donors were seropositive for HHV-8 infection.

In the second phase, we studied 125 renal transplant recipients who received kidney allografts from living donors. Twenty-nine, 34, 30, and 32 subjects received renal transplants six months, one year, three years, and five years before patient recruitment. The male to female ratio in this group was 1.9; 82 (65.6%) to 43 (34.4%). The mean age was  $39.01 \pm 14.7$  years. With the exception of one subject (0.8%), all recipients received their allografts from non-related donors. Twenty-three (18.4%) and 7 (5.7%) patients had a history of one and 2 episodes of rejection, respectively. None of these patients were seropositive for HBsAg, HCV, cytomegalovirus, or HIV. Notably, two (1.6%) individuals had a history of PT-KS. Surprisingly, the result of HHV-8 antibody assay was negative for these two subjects.

In this phase, HHV-8 antibody assay was positive in two (1.6%) renal transplant recipients. These two patients had no evidence of KS lesions. They received renal allografts five years before taking the blood sample. The immunosuppressive regimen received by the two HHV-8- seropositive cases as well as the two subjects with PT-KS included prednisolone, cyclosporine, and mycophenolate mofetil.

Table 1 demonstrates the frequencies of the different immunosuppressives received by kidney allograft recipients. The patients have been stratified in four distinct groups, based on the interval since the time of transplantation. For each group, the frequencies of immunosuppressive medications are given.

## Discussion

In this study, we focused on an important issue related to the seroprevalence of HHV-8 infection in general population and renal allograft recipients. Our findings appear to be applicable for both epidemiologic and etiologic aspects of HHV-8 infection, considering previously published reports.

There are frequent reports from the Middle East demonstrating a considerable rate of PT-KS in renal transplant recipients. Almuneef, et al. demonstrated that PT-KS is the most common cancer in renal transplants in Saudi Arabia. They reported the incidence rate to be 10 times higher than the United States and the Western Europe.<sup>5</sup> In a retrospective study carried out on 273 renal transplants in Iraq, Altaee, et al. showed that within a 10- year interval, eight patients developed PT-KS. Similarly, they found PT-KS as the most common malignancy in renal transplants.<sup>7</sup> To the best of our knowledge, the registry system for documenting the malignancies and neoplasms do not exist in the majority of the Middle East countries. Thus, one may cast doubt on recognizing PT-KS as the most prevalent neoplastic disease after renal transplantation. Considering different reports on PT-KS from

our region, its frequency does not seem to be negligible. Unfortunately, there are no significant data from this region to show the frequency of HHV-8 infection either in general population or transplant patients.

One may assume that the rate of seropositivity for HHV-8 infection after transplantation is a consequence of infection prior to receiving renal allograft. In the study of Almuneef, et al. HHV-8 antibodies were detectable in 14 out of 201 (6.97%) end- stage renal disease patients prior to the transplantation and 10 out of 258 (3.88%) otherwise healthy control group.<sup>5</sup> This study did not focus on the seroprevalence of HHV-8 infection after transplantation, and the effect of immunosuppression on the frequency of HHV-8 infection remained unclear. In addition, their control group was not representative of general population.

In a study from Saudi Arabia conducted by Alzahrani, et al. it has been demonstrated that 27 of 150 (18%) renal transplant recipients were serologically positive for HHV-8 infection.<sup>8</sup> Considering the findings of this report, the frequency of HHV-8 infection does not seem to be low in solid organ transplant individuals living in Saudi Arabia. In addition, they showed that an increase in the seroprevalence of HHV-8 infection after transplantation appears to be likely. A lack of evidence still seems to exist to compare the seroprevalence of HHV-8 infection, before and after transplantation, in a well-defined population from Middle East countries. Comparing the seroprevalence between the general population and the allograft recipients seems to be another shortcoming.

In our study, only two (1.6%) recipients with HHV-8 infection were identified. Additionally, no HHV-8- positive individual was identified in our population sample from blood donors. Since this clustered sample included blood donors living in different districts of Tehran in a certain period of time, the frequency seems to represent the seroprevalence in the general population. Considering our findings, HHV-8 infection does not seem to be frequent in our population. Further studies focusing on different regions of Iran appear to be required to have a more accurate estimation. Considering previous reports from Saudi Arabia, Iran, and Iraq focusing on either PT-KS or HHV-8 infection, it is still unclear that which areas in our region have a higher rate of HHV-8 seroprevalence. In addition, owing to the huge number of travelers between the neighboring countries, especially trips for religious purposes to Saudi Arabia and Iraq, spreading of HHV-8 infection appears to be very likely.

Consequently, we feel that epidemiologic aspects of HHV-8 infection in this geographic region require more studies focusing on seroprevalence before and after transplantation. The frequency of HHV-8 infection in other immunocompromised patients, such as HIV- infected patients, has not been studied in this region. We suggest that physicians are better to bear in mind the possibility of HHV-8 infection in immunocompromised patients living in the Middle East countries regardless of their national seroprevalence.

In our study, two PT-KS patients were seronegative for HHV-8, whereas, two recipients with no PT-KS were HHV-8 seropositive. As it was explained before, the association between HHV-8 infection and PT-KS, in spite of other types of KS, has not been clearly determined. Immunosuppression after transplantation may affect humoral immunity and antibody production. For some viral infections such as cytomegalovirus infection the role of serologic tests for the diagnosis of infection after transplantation is under debate. It is not clear that how immunosuppressive regimen may affect the accuracy of the serologic tests for HHV-8 infection. The role of HHV-8 infection in the development of PT-KS seems to be still

under debate. Biologic models appear to be required to show the association between HHV-8 and KS in non-HIV immunocompromised patients such as transplant patients.

Prolongation of immunosuppression after transplantation may increase the probability of HHV-8 infection. Zamanian, et al. from Iran have demonstrated that there was a higher probability for the development of PT-KS among those renal transplant recipients who received immunosuppressive regimens for over five years.<sup>9</sup> Although the incidence of PT-KS may be associated with the prolongation of immunosuppression, the frequency of HHV-8 infection in different intervals following the transplantation has not been clearly determined. The same finding can be extrapolated from our results demonstrating that both cases with PT-KS received allografts 5 years prior to enrolment. Thus, the frequency of PT-KS does not appear to be noticeable in early post-transplantation period.

The immunosuppressives, particularly calcineurin inhibitors such as cyclosporine, have been noticed to play a significant role in the development of PT-KS.<sup>2</sup> In our study, both PT-KS cases and those two HHV-8-positive kidney recipients were receiving the conventional immunosuppressive regimen consisted of calcineurin inhibitors (cyclosporine). Farge, et al. have demonstrated that immunosuppressive drugs are the major risk factors for PT-KS.<sup>10</sup> Ecdar, et al. as well as Firoozan and colleagues in two different reports demonstrated that the most effective method for the treatment of PT-KS is the cessation of calcineurin inhibitor immunosuppressives such as cyclosporine.<sup>6,11</sup> All the renal transplant recipients included in our study were on cyclosporine therapy. Consequently, we cannot compare the effects of different immunosuppressives in the development of PT-KS.

Considering growing number of reports on PT-KS from Iran and other countries from this geographic region, transplant physicians are concerned about seroprevalence of HHV-8 infection in general population and transplant patients. Our findings showed that the seroprevalence in general population is negligible. We did not find HHV-8-positive serologic test in our two PT-KS patients. This latter finding might be affected by the effect of immunosuppressive regimen on humoral immunity following transplantation. Considering previous reports, the seroprevalence of HHV-8 infection may not be negligible in our region. High traveling rate and dynamicity of population in this region may make our transplant population at risk. Nevertheless, this study was the first cross-sectional

survey on HHV-8 infection seroprevalence in both general population and transplant patients. The frequency of this infection was not considerable in both settings.

Since different provinces of Iran provide services for organ transplantation, further studies appear to be required to determine the seroprevalence in different regions of Iran.

**Note:** This study was approved by UNRC research council. All expenses were compensated by grants given by UNRC.

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