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# Occurrence and Risk Factors of Human Herpes Virus-6 among Renal Transplant Recipients: A Single-Center Study

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**Abstract**

**Background:** Human herpesvirus-6 (HHV-6) is one of herpesviruses family known to reactivate after kidney transplantation and associated with several clinical manifestations. However, risk factors for active viremia remain unclear.

**Subjects and Methods**: Blood samples collected from 49 renal transplants during the first post-transplantation year for three successive months, and from 49 age and sex-matched normal donors as controls, HHV-6 viremia detected by real time PCR for HHV-6-*pol*-gene.

**Results**: Actively increasing viral load was detected in 8/49 (16.3%) of renal transplants, all of them were symptomatic (p=0.002), and six of these eight (75%) had renal allograft rejection. Only recipients who had received allograft from living-related donor was recognized as a risk factor for active HHV-6 infection (P=0.018), and all the controls were negative for the virus.

**Conclusion**: HHV-6 should be considered an emerging pathogen that might be associated with some post-transplants diseases, which also could include renal allograft rejection.

**Key words:** HHV-6, Renal transplantation, real-time PCR

**List of Abbreviations**

HHV-6: Human Herpes Virus-6
RTR: Renal transplantation Recipients
PTP: Post-transplant period
qRT-PCR: quantitative Real time polymerase chain reaction

## INTRODUCTION

Viral infections are among the major causes of morbidity and mortality after tissues and organ transplantation. Because transplants imply the use of immunosuppression drugs to avoid graft rejection, one of the most studied families of viruses in organ transplantation is the Herpesviridae, which encompasses eight human different viruses, the majority of them is highly prevalent in the general population, and shows immuno-modulatory effects. (1-3)

Human Herpes Virus-6 (HHV-6), the etiologic agent of exanthema subitum, like other herpesviruses, can remain latent in the host's cells, and can reactivate as soon as immunosuppression starts. The common sites for latency include salivary glands, mononuclear cells, lymph nodes, and liver and renal parenchyma. Clinically, HHV-6 causes a mononucleosis-like syndrome, lymphadenopathy, pneumonitis, hepatitis, bone marrow suppression, and encephalitis, after liver transplantation. (1-5)

The clinical role and the epidemiology of the latent and early-active HHV-6 infection after kidney transplantation are not well defined clear (1,6,7). In addition, the diagnosis of active HHV-6 infection is complex for many reasons include, latency, chromosomal integration, and episodic short replication cycles without clear clinical association (8,9).

Several diagnostic methods have been used, among them; the detection and/or quantification of viral DNA by means of PCR in blood or plasma samples is the method of choice, although there are still no well-established viral load thresholds for the levels of viral replication. (2,10-13)

In Iraq, kidney transplantation program was started successfully in 1973, and since then, renal transplantation is being done in some centers (14-16). Few Studies were recently conducted on detecting viral infections or reactivations in Iraqi renal transplant recipients (RTRs) (17,18). However, to the best of our knowledge, there is no previous study on HHV-6 in RTRs in Iraq, therefore, this study aimed to prospectively investigate the prevalence of HHV-6 in Iraqi RTRs using quantitative real time PCR (qRT-PCR).

## MATERIALS AND METHODS

### Patients

This prospective study conducted from January to June 2015, 49 RTR including 36 males and 13 females, their ages ranged from 18-55 years, from the Center of Kidney Diseases and Transplantation in the Medical City of Baghdad, were enrolled in the study, and 49 age and sex-matched normal donors were enrolled as controls. The study approved by the ethical committees of the Ministry of Health and the College of Medicine-Al-Nahrain University /Baghdad/Iraq.

Patient's inclusion criteria were age more than 18 years, post-transplant period (PTP) ranging between 1-12 months and

obtainment of informed consent. Patients follow-up consisted of clinical assessment (clinical symptoms and serum creatinine) and 3ml whole blood samples were collected for three successive months.

Clinical parameters (immunosuppressive regimens, acute rejection episodes, transplant function and late complications) obtained from patient's medical records. Two main Standard immunosuppressive regimens were mainly followed in RTRs; either the cyclosporine A (CSA), mycophenolate (MMF), and prednisolone, or the regimen that included tacrolimus (TAC) instead of CSA, in addition to MMF and prednisolone. And induction with monoclonal anti-CD25 antibodies (Basilixibam/Daclizumab)

Treatment with oral CSA was started before surgery (10 mg/kg/d) to obtain therapeutic CSA blood levels, and then was adjusted, based on a target level of 150–250 ng/ml in the first four weeks, and then 150–200 ng/ml thereafter.

The maintenance dose of MMF was 1.0–2.0 g/d. Methylprednisolone 5.0 mg/kg/d was administered on three consecutive days from the day of RT. While oral prednisolone was started on the first day after operation at 0.5 mg/kg/d and reduced gradually till 5.0–10 mg/d. For those patients who were on TAC regimen; the starting dose was 0.05 mg/kg at induction, then 0.05-0.15mg/kg according to the blood level which should be 6-12 ng/mlin the first 3 months and then 4-8 ng/ml maintenance immunosuppression.

Diagnosis of acute rejection (AR) episodes confirmed by renal biopsy. The histological features graded according to the Banff 2005. AR episodes treated with intravenous methylprednisolone 500 mg/d for three consecutive days, while steroid resistant cases treated with anti-thymocyte globulin (ATG) at 4 mg/kg for 7–10 days.

**Viral Detection and Monitoring**

**First: Viral DNA Extraction:** Viral DNA was extracted from 100µl of blood using (DNA-sorb-B-Sacace/Italy) Kit, according to the manufacturer's protocol using DNA lysis and sorbent solutions, and then DNA was eluted in 50µL of DNA-eluent.

**Second: Viral DNA Quantification:** Quantification of HHV-6 viral load was done using (HHV6 Real-TM Quant- Sacace/Italy), which is an *in vitro* Real Time amplification test for quantitative detection of HHV-6-*pol*-gene in the biological materials. Internal Control (IC) Test contains an IC (*b*-globine gene) which serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

Amplification results of HHV6 DNA are detected on the Joe/HEX/Yellow and b-globine gene used as Internal Control is detected on the Fam/Green channel. The kit contains quantitative standards for quantitation of HHV-6 DNA in samples, and Human DNA.

For real-time PCR the following amplification protocol was used: 1 cycle at 95$^{o}$C for 15 min followed by 5 cycles consisting of 5 s at 95 $^{o}$C, 20 s at 60 $^{o}$C, and 15 s at 72 $^{o}$C, and then 40 cycles consisting of 5 s at 95 $^{o}$C, 30 s at 60 $^{o}$C, and 15 s at 72 $^{o}$C. The detection threshold was 400 copies /mL.

**Risk factor analysis:** Patients with increasing viral load for the consecutive three months were compared with patients with either single episodic DNA detection or no detection at all, in all the clinical and lab parameters obtained for each RTRs.

**Statistical analysis** was performed with the SPSS version 21.0, categorical data formulated as count and percentage. Chi-square or Fisher exact test was used to describe the association of these data. Numerical data were described as median, 25-75 percentile, Mann-whitney U test was used for comparison between groups. The lowest level of accepted statistical significant difference is bellow or equal to 0.05.

## RESULTS

All the 49 RTRs completed the full 3-month follow-up, and no patient excluded during the study. The general characteristics of the patients wre shown in Table 1.

Out of 49 RTR involved in this study; 27 (55.1%) were on TAC regimen and the remaining 22 (44.9%) were on CSA regimen, and during their follow up; 6 out of these 22 patients were shifted from CSA to TAC, table 1.

Active HHV-6 infection was observed in 8 of 49 (16.3%) RTRs; their mean PTP was 6.4±3.5 months. Six out of these eight patients (75%) had biopsy-proven rejection during the follow up period (P<0.001). And all of them 8/8 (100%) were symptomatic (p=0.002), with (4 out of 8 [50%]) had fever, 2 of 8 (25%) patients with skin rash, and another 2 of 8 (25%) patients had upper respiratory tract infection.

**Table 1: General characteristics of the 49 RTRs**

| Characteristic | | Count | % |
|---|---|---|---|
| Age/years (Mean 33.49±11) | <40 years | 31 | 63.27% |
| | ≥40 years | 18 | 36.73% |
| Gender | Female | 13 | 26.53% |
| | Male | 36 | 73.47% |
| PTP/months Mean 5.92±3.4 | < 6 months | 25 | 51.02% |
| | ≥ 6 months | 24 | 48.98% |
| Serum Creatinine/ mg/dl Mean 1.28±0.44 | > 1.2 | 30 | 61.22% |
| | ≤ 1.2 | 19 | 38.78% |
| Diabetes mellitus | | 39 | 79.59% |
| Hypertension | | 24 | 48.98% |
| Rejection | | 10 | 20.40% |
| Immunosuppressive drugs | CSA | 22 | 44.90% |
| | TAC | 27 | 55.10% |
| Shift from CSA to TAC | | 6 | 12.24% |
| Ganciclovir treatment | | 18 | 36.73% |
| Donor | Living Related | 31 | 63.27% |
| | Living Unrelated | 18 | 36.73% |
| Clinical presentation | Cough | 8 | |
| | Fever | 4 | |
| | skin rash | 5 | |

**Table 2: Comparison between the 8 RTRs, who had active HHV-6 infection and the remaining patients.**

| | | Active infection | | No active infection | | Total | p value |
|---|---|---|---|---|---|---|---|
| | | Count | % | Count | % | | |
| **Gender type** | **Male** | 5 | 13.89% | 31 | 86.11% | 36 | 0.442 |
| | **Female** | 3 | 23.08% | 10 | 76.92% | 13 | |
| **Age groups** | **≥ 40 years** | 3 | 16.67% | 15 | 83.33% | 18 | 0.961 |
| | **< 40 years** | 5 | 16.13% | 26 | 83.87% | 31 | |
| **PTP/months** | **≥ 6 months** | 6 | 25.00% | 18 | 75.00% | 24 | 0.108 |
| | **< 6 months** | 2 | 8.00% | 23 | 92.00% | 25 | |
| **Diabetes mellitus** | **Yes** | 2 | 20.00% | 8 | 80.00% | 10 | 0.725 |
| | **No** | 6 | 15.38% | 33 | 84.62% | 39 | |
| **Hypertension** | **Yes** | 4 | 16.00% | 21 | 84.00% | 25 | 0.950 |
| | **No** | 4 | 16.67% | 20 | 83.33% | 24 | |
| **Transplantation** | **First** | 8 | 17.39% | 38 | 82.61% | 46 | 0.430 |
| | **Second** | 0 | 0.00% | 3 | 100.00% | 3 | |
| **Rejection** | **Positive** | 6 | 60.00% | 4 | 40.00% | 10 | **<0.001** |
| | **Negative** | 2 | 5.13% | 37 | 94.87% | 39 | |
| **Serum creatinine** | **> 1.2** | 6 | 20.00% | 24 | 80.00% | 30 | 0.382 |
| | **≤ 1.2** | 2 | 10.53% | 17 | 89.47% | 19 | |
| **IS drugs** | **CSA** | 4 | 18.18% | 18 | 81.82% | 22 | 0.751 |
| | **TAC** | 4 | 14.81% | 23 | 85.19% | 27 | |
| **Shift of IS drugs** | **CSA to TAC** | 2 | 33.33% | 4 | 66.67% | 6 | 0.229 |
| | **Non** | 6 | 13.95% | 37 | 86.05% | 43 | |
| **Ganciclovir** | **Not used** | 7 | 21.88% | 25 | 78.13% | 32 | 0.149 |
| | **Used** | 1 | 5.88% | 16 | 94.12% | 17 | |
| **Donor** | **Living-unrelated** | 0 | 0.00% | 18 | 100.00% | 18 | **0.018** |
| | **Living-related** | 8 | 25.81% | 23 | 74.19% | 31 | |
| **Complaint** | **Yes** | 8 | 36.36% | 14 | 63.64% | 22 | **0.002** |
| | **No** | 0 | 0.00% | 27 | 100.00% | 27 | |

**PTP: post-transplantation period, IS: Immunosuppressive, TAC: tacrolimus, CSA: cyclosporine**

**Patient's Data Sheet**

| **Patient Code No.:** | **Date:** | **Age:** | **Gender:** | **PTP/months:** | **Diabetes** |
|---|---|---|---|---|---|
| Fever | Skin Rash | Cough | Anemia | Jaundice | Hypertension |
| Cause of renal failure | First Trans-plantation or 2nd | History of rejection | Donor Related or not | Other associated diseases | |
| **Investigations:** | ***1*-*CMV (D/R) sero-state:*** | *IgM (D/R) serostate* | *IgG (D/R) serostate* | | |
| ***2*-**Serum Creatinine during follow up | | ***3*-**Renal Biopsy Results | | ***4*-**Ultrasound Results | |
| **Immunosuppressive regimen:** | Types | Shift to another drug | | Use of ATG | |

Single episodic viremia was observed in 37 of 49 (75.5%) of the patients, more frequently than sustained viral replication. In these patients, the mean PTP was 5.2±3.8 months. The majority of cases were asymptomatic (28 of 37 [75.7%]), with 6/37 (16.2%) patients having upper respiratory tract infection and 3/37 (8.1%) patient presented with skin rash, with no alteration in mental status. The remaining 4/49 (8.2%) did not developed HHV-6 viremia during the follow up period.

Viral loads were higher (median, $1.9x10^5$ copies/mL blood) in patients with actively increasing viremia, compared with patients with single episodic viremia (median, $4.5x10^4$ copies/mL blood), ($p<0.001$).

Analysis for risk factors associated with active HHV-6 infection; only RTRs who were receiving an organ from a living-related donor (P=0.018) was recognized as a risk factor for HHV-6 infection or reactivation. Table 2.

## DISCUSSION

The chronic use of immunosuppressive drugs in RTRs to decrease the rejection rate has led to emergence and reactivation of many opportunistic pathogens among which latent viruses like HHV-6 has been accused as a cause of morbidity in RTRs (1,5,6,19). To the best of our knowledge only one study was conducted in Iraq on HHV-6, which was on the association of this virus with certain hematological malignancies (20)

Most infections after transplantation are thought to result from the reactivation of endogenous latent virus (21,22), in this study active HHV-6 was detected in (16.3%) 8 out of 49 RTR, in whom the viral load was increasing over the successive three months. In RTRs several studies reported conflicting results about reactivation of HHV-6 both in pediatric and adult patients (19,23). The percentage of the presence of HHV-6 genome ranges from 0 to 80%, and these differences are strongly influenced by the techniques employed for the DNA detection (7,23). Additionally, HHV6B reactivates more often than HHV6A, but the replication of HHV6A is more virulent (24) and can also be fatal (21,25,26).

Although active HHV-6 infections in solid organ transplant recipients is usually asymptomatic (7,23). This study showed that all of the 8 patients who had active replication were symptomatic, 4/8 had fever, and 2/ had skin rash and respiratory infections, in addition, 75% of them had graft rejection. These symptoms may or may not be caused by HHV-6 itself, because other pathogens should be excluded (21), though, all of these 49 RTRs in this

study were CMV IgM negative during the three months follow up of the study (unpublished data).

These results can be supported by other studies which showed that HHV6 replication in solid organ transplant recipients can be associated with respiratory infections, encephalitis, fever, skin rash, and transplant rejection (19,22,23,27,28).

In liver transplants, HHV-6 also may cause graft dysfunction and may be associated with rejection (29). Locally in the hepatocytes, HHV-6 infection of the allograft, is associated with increased expression of vascular endothelial adhesion molecules and infiltration of leukocytes, this could lead to local inflammation and damage to the graft leading to dysfunction and rejection (30).

When evaluating single episodic HHV-6 viremia, studies on RTR with different diagnostic methods demonstrated incidence rates ranging from 38% to 68% (27,31-34). In the current study, single episodic viremia was detected in (75.5%) of RTRs, with the majority being asymptomatic.

Generally, there are few studies regarding the risk factors for HHV-6 reactivation in solid organ transplant recipients (35,36). Luiz *et al* (2013) (13), showed that patients who received transplants from living donors also had a greater risk of active viral replication. However in the present study all of the patients had living donor, but all of those who had active viral replication received their allograft from living-related donors. Actually, even recent studies could not find a plausible explanation for this (13,23).

Studies found that in a minority of the cases, the virus is able to integrate its genome in the human chromosomes in a condition known as “chromosomally integrated HHV6 (ciHHV-6), and it is very important since in these cases, HHV6 infection may be inherited (9,37,38), this may partially explain the higher viral replication rate in those who had living-related donors.

Due to high HHV-6 sero-prevalence rate in adults, serology is of limited benefit for the diagnosis of active infection in RTR. Also viral culture of HHV-6 is both time- and resource-intensive, and is not routinely used (7,39). HHV-6 antigenemia assays could detect HHV-6 viral antigens in peripheral blood mononuclear cells using monoclonal antibodies (39,40). However, the cut-off level to determine clinically significant active infection is unknown (41). Quantitative real-time PCR assays are often used for the diagnosis of active HHV-6 infection (42-44); and for quantification of viral DNA in whole blood or plasma. (7,39,44)

In conclusion, HHV6 now is regarded an emerging pathogen that may be associated with some post-transplants disorders, similarly to those caused by CMV. However, the scenario still presents some unsolved issues; in particular, the ubiquitous nature of the virus, chronicity of infection, and the latency of the virus (2,23).

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