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Research Paper

Serodiagnosis of Human Herpesvirus-8 in Kidney Transplant Recipients: A Single- Centre Study from Iraq

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ABSTRACT:

BACKGROUND:

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus, has garnered significant attention due to its link with the development of various clinical and epidemiological forms of Kaposi's sarcoma(KS). Several studies have indicated that solid organ transplantation plays a significant role in the transmission of HHV-8, and the immune status is also a crucial factor in the development of KS. Therefore, kidney transplantation is an important area for investigating HHV-8 infection.

OBJECTIVE:

This study aimed to investigate the presence of IgG and IgM antibodies against HHV-8 in patients who had undergone kidney transplantation.

MATERIALS AND METHODS:

A case-control study involving 80 participants, with 40 patients who have undergone kidney transplantation within 6 months at Ghazi AL-Hariri Hospital for Surgical Specialties, was compared to a control group of 40 healthy individuals. The detection of HHV-8 antibodies was conducted using ELISA on sera that had been previously stored at Baghdad Medical Laboratories.

RESULTS:

A significant correlation has been established between kidney transplantation and the presence of IgG antibodies against HHV-8, with a calculated P-value of 0.023. Furthermore, a statistically significant association has been identified between HHV-8 IgG positivity and IgM status (p = 0.03). Among the cases positive for HHV-8 IgM, 75% exhibited concurrent HHV-8 IgG positivity. Notably, a predominance of male patients was observed in both IgM and IgG-positive cohorts undergoing renal transplantation.

CONCLUSION:

Patients who have undergone kidney transplantation are susceptible to HHV-8 infection, which poses a significant risk of post-transplant complications.

KEYWORDS: Kaposi sarcoma, Human herpes virus-8, Immunoglobulin G, immunoglobulin M, Enzyme linked immunosorbent assay.

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INTRODUCTION:

Human herpesvirus 8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus, is an oncogenic virus belonging to the γ-herpesvirinae subfamily of the herpesviridae family[(1)]. In immunocompetent individuals, HHV8 infection is associated with mild nonspecific symptoms such as fatigue, diarrhea, rash, and lymphadenopathy. However, in immunocompromised patients, HHV-8 often leads to the development of neoplastic diseases including Kaposi's sarcoma (KS), multicentric Castleman's disease, plasmablastic lymphoma, and primary effusion lymphoma[(2)]. Currently. There are no standardized serological tests with sufficient sensitivity and specificity for the diagnosis of HHV8 infection due to limited access to laboratory techniques that confirm this infection. HHV8 is difficult to isolate on cell cultures, and molecular diagnostic methods for viral DNA detection (PCR) are costly and have limited utility. This limitation is because the virus remains dormant for long periods, with occasional reactivations, resulting in only 10% of asymptomatic patients infected with KSHV having a positive PCR in the peripheral blood[(3)]. Human herpesvirus 8 (HHV-8) infection can occur in both pediatric and adult populations and is transmissible through sexual and non-sexual means, including saliva, blood transfusion, solid organ transplantation, and sexual contact [(4)].

In cases of classic Kaposi's sarcoma unrelated to HIV infection, initial therapeutic modalities may encompass chemotherapy using cytotoxic agents such as liposomal anthracyclines, paclitaxel, vinca alkaloids, and bleomycin, as well as radiation therapy and surgical resection. Contrastingly, AIDS-associated Kaposi sarcoma commonly involves the use of radiation and chemotherapy, with highly active antiretroviral therapy (HAART) indirectly contributing to efficacy in managing Kaposi's sarcoma[5]. Viral infections are prevalent among renal transplant recipients, posing deleterious implications for life expectancy and allograft outcomes.

Besides causing life-threatening infections, viral infections can lead to irreversible renal graft injury. The prevalence of Kaposi's sarcoma in organ transplant recipients ranges from 0.5% to 5%, with a higher incidence of 23% observed in HHV8-positive individuals prior to transplantation. Consequently, Kaposi's sarcoma may arise in transplant recipients due to pre-existing infection in seropositive patients or as a primary infection in individuals lacking HHV8-specific antibodies. In the latter scenario, primary infection can potentially be transmitted through the graft from the donor. In addition, the choice of immunosuppressive therapy can significantly impact the risk of post-transplantation Kaposi's sarcoma, with calcineurin inhibitors correlating with a heightened risk of Kaposi's sarcoma development when compared to alternative immunosuppressive regimens[6].

SUBJECTS AND METHOD:

A case-control study was carried out at the Department of Kidney Transplantation in Ghazi AL-Hariri Hospital for Surgical Specialties/Medical City from April 2023 to October 2023. Ethical approval and informed consent were procured from all participants. A comprehensive medical history was acquired from each patient through a structured questionnaire, with the exclusion of individuals who had received antiviral therapy within six months prior to the sample collection date. A total of eighty (80) blood samples were obtained from individuals aged 25-65, comprising both patients and healthy subjects. The patient cohort included 40 males and females who had undergone kidney transplant surgery within six months preceding the sample collection and were undergoing immunosuppressive therapy. Conversely, the control cohort encompassed 40 males and females from the healthy population. The detection of anti-HHV8 IgG and IgM antibodies was conducted using ELISA kits (produced by SunLong Biotech company/ the Catalogue Number:SL2685Hu. Made in China for IgG) (kit produced by MYBIOSOURCE/ CatLog Number : MBS2801962. Made in China for IgM), the analysis carried out at Baghdad Medical Laboratories.

Statistical analysis

The estimated glomerular filtration rate (eGFR) was computed using the CKD-EPI formula. Continuous variables were reported as means and standard deviations, while categorical variables were expressed as frequencies and percentages. The difference between means for normally distributed variables was assessed using the Welch's t-test, and the disparity between categorical variables was evaluated employing either the $\chi 2$ test with Yates' correction or Fisher's exact test, contingent on the specific context. A P-value below 0.05 was regarded as statistically significant. Data processing, visualization, and statistical analysis were carried out utilizing R software packages, including dplyr, gt_summary, and ggplot (R version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS:

In a comparative analysis involving 40 cases with kidney transplants and 40 controls, the study examined various demographic and clinical characteristics. The mean age ± SD of cases was (43.7 ± 12.3 years) and for the controls was (41.7 ± 11.7 years).

The analysis of HHV-8 IgM serum levels in cases and controls revealed that 4 (10%) of cases tested positive, compared to none in the control group. A significantly higher percentage of positive HHV-8 IgG results was found among 9 (22.5%) cases, compared to 2 (5%) controls, with a p-value of 0.023, as demonstrated in Table (1).

Table 1: HHV8-IgM status in cases and control groups.

HHV-8 ELISA results		Cases No. (%)	Control No. (%)	P-value*
HH8-IgM	Positive	4 (10)	0 (0)	0.12
	Negative	36 (90)	40 (100)	
HH8-IgG	Positive	9 (22.5)	2 (5.0)	**0.023**
	Negative	31 (77.5)	38 (95.0)	
Total		40 (100)	40 (100)	
***Fisher's exact test**				

An association was found between HHV8-IgG positivity and IgM status (p = 0.03). Specifically, 3 (75%) of HHV8-IgM positive cases exhibited HH8-IgG positivity, contrasting with 6 (16.7%) in the HH8-IgM negative group. Table (2).

Table 2: Association between HHV8-IgM, and HHV8 IgG.

HH8-IgG No. (%)	HH8-IgM No. (%)		P-value*
	Positive	Negative	
Positive	3 (75.0)	6 (16.7)	**0.03**
Negative	1 (25.0)	30 (83.3)	
Total	4 (100)	36 (100)	
***Fisher's exact test**			

The distribution of HHV-8 infected cases among age groups shows that the most common age category for IgM and IgG-positive cases was 40-49 years old, with frequencies of 50% and 44%, respectively, as illustrated in Figure (1).

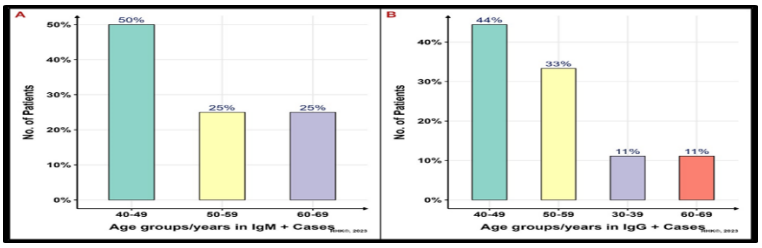

Figure 1: Percentage of age categories in HHV8 IgM antibodies (A) and HHV8 IgG antibodies (B) positive cases with kidney transplantation.

Regarding sex distribution in both IgM, and IgG positive patients with renal transplantation. Males represented the majority of the cases in both condition (IgM +:75%, and IgG +: 56%). As illustrated in figure (2).

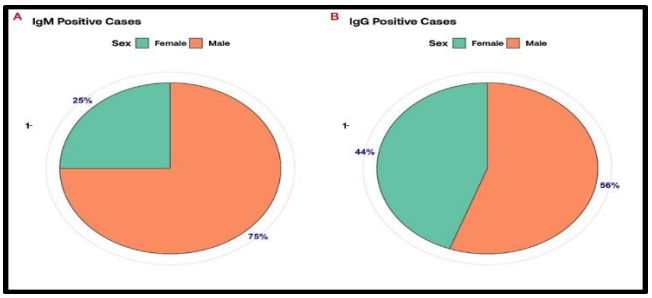

Figure 2: Percentage of sex in IgM (A) and IgG (B) positive cases with kidney transplantation.

In Table (3), a comparison was made between Human Herpes Virus 8 (HHV-8) positive and negative cases in relation to some risk factors. The study revealed no statistically significant difference in the frequency of blood transfusion between the two groups (p=0.2). Similarly, tattooing exhibited no significant association with HHV-8 status, with 30.0% in HHV-8 positive cases and 13.3% in negative cases (p=0.3). It is noteworthy that the positive cases encompassed both HHV-8 IgG antibody-positive and HHV-8 IgM antibody-positive cases. the frequency of history of dental surgery was 7 (17.5%) in cases compared to 12 (30.0%) in controls.

Table 3: Risk factors of HHV8 transmission.

Risk factor	HH8- Positive No. (%)	HH8- Negative No. (%)	P-value*
Blood transfusion			0.2
Yes	10 (100)	23 (76.7)	
No	0 (0.0)	7 (23.3)	
Tattooing			0.3
Yes	3 (30.0)	4 (13.3)	
No	7 (70.0)	26 (86.7)	
History of a dental surgery			0.2
Yes	7 (17.5)	12 (30.0)	
No	33 (82.5)	28 (70.0)	
Total	10 (100)	30 (100)	
***Fisher's exact test**			

The investigation assessed viral co-infections in cases positive for HBsAg and HCV-IgG. The findings revealed that 4 out of 10 (40%) cases with a positive HHV-8 showed HBsAg positivity, whereas 8 out of 30 (26.7%) HHV-8-negative cases were HBsAg positive. Notably, no statistically significant difference was observed, with a p-value of 0.5. Similarly, HCV-positive cases exhibited a higher percentage in HHV-8-positive individuals, with 2 out of 10 (20%) cases compared to HHV-8-negative individuals, in which only one case out of 30 (3.3%) was HCV-positive, yielding a p-value of 0.15. These findings are summarized in table (4). It is important to clarify that the positive cases encompass both HHV-8 IgG and IgM antibodies.

Table 4: Association of HBV and HCV co-infections with HHV-8.

HHV-8	HBV (HBsAg positive) No. (%	HCV-IgG positive No. (%)
Positive	4 (33.3)	2 (66.7)
Negative	8 (66.7)	1 (33.3)
Total	12 (100)	3 (100)
P-value*	0.5	0.15
***Fisher's exact test**		

Table (5) demonstrates nearly identical baseline test results for renal function, with no significant variances observed in blood urea, serum creatinine, and glomerular filtration rate (GFR). The positive cases delineated in this table encompass instances of both HHV-8 IgG antibody positivity and HHV-8 IgM antibody positivity.

Table 5: Renal function test results in relation to HHV-8 positive cases.

Baseline tests Mean ± SD *Normal value	HH8- Positive	HH8- Negative	P-value*
Blood urea *7-20 mg/dL	30.4 ± 3.4	34.1 ± 7.0	0.11
Serum creatinine *0.7-1.3 mg/dL	1.6 ± 0.2	1.6 ± 0.3	0.8
GFR *> 60 ml/min/1.73 m^2	38.5 ± 6.0	41.4 ± 9.6	0.3
Total	10 (100)	30 (100)	
***Two tailed t-test**			

The presence of dysuria, gross haematuria, fever, and skin manifestations in association with HHV-8 infection demonstrated no significant differences between HHV-8 infection and the patient's symptoms as observed in Table (6).

Table 6: Post transplants symptoms in association with HHV-8.

Patient's symptoms	HH8- Positive No. (%)	HH8- Negative No. (%)	P-value*
Dysuria			
Yes	1 (10.0)	3 (10.0)	>0.9
No	9 (90.0)	27 (90.0)	
Gross haematuria			
Yes	1 (10.0)	0 (0.0)	0.3
No	9 (90.0)	30 (100)	
Fever			
Yes	1 (10.0)	3 (10.0)	>0.9
No	9 (90.0)	27 (90.0)	
Skin manifestation			
Yes	0 (0.0)	0 (0.0)	1.000
No	10 (100)	30 (100)	
Total	10 (100)	30 (100)	
***Fisher's exact test**			

DISCUSSION:

The development of transplant-associated Kaposi's sarcoma (KS) is frequently attributed to the reactivation of human herpesvirus 8 (HHV-8) in previously infected patients. It has been estimated that HHV-8 reactivation occurs at a significantly higher frequency—approximately 60-200 times more—in patients who have undergone organ transplantation due to their compromised immune status [7]. In the present study, 22.5% of the cases examined yielded positive results for HHV-8 IgG. Furthermore, 10% of the cases showed acute HHV-8 infection indicated by IgM positivity. Among the patients who tested positive for HHV-8 IgG, 3 were found to also harbour HHV-8 IgM, which implies recent seroconversion from acute infection to subsequent chronic or latent infection, with a p-value of 0.03. Only one patient exhibited positive HHV-8 IgM antibodies along with negative HHV-8 IgG, indicating early exposure relevant to an acute phase of infection. Notably, 6 patients demonstrated positive HHV-8 IgG and negative IgM, suggesting a prior infection that warrants further evaluation to determine if the patients were undergoing asymptomatic reactivation or if the virus had entered a latent state.

In 2019, a previous study conducted in Iraq revealed that the rate of HHV8 IgG antibodies in screened blood donors at the Iraqi National Centre for Blood Transfusion was higher than the results obtained in our study [8]. Our present study found that the rate of HHV-8 infection was higher in men than in women. These findings suggest that men may be more susceptible to HHV-8 infection than women, as there was greater male participation in our study. This is consistent with the results of a study conducted by Somayeh Shokri in Iran in 2019 (1).

The age distribution of HHV-8 infected cases showed that the most common age category for IgM and IgG positive cases was 40-49 years old, with a frequency of 50% for IgM positive cases and 44% for IgG positive cases. This was attributed to the fact that most patients who had undergone kidney transplantation were in this age group. A study conducted in Iran in 2007 revealed a statistically significant seropositivity in the middle age group [9]. Regarding marital status, our study showed no association between the incidence of HHV-8 infection and being married or single, indicating that the risk of sexual transmission of this virus among kidney transplant patients was not a concern. Similar results were found in a study in Egypt that observed no risk of HHV-8 infection based on marital status[10]. However, a study conducted in Kenya in 2019 found a positive association between HHV-8 infection and married individuals. This was also noted in a recent study conducted in Iraq in 2023 by the Department of Dermatology at Baghdad Medical City [11]. The association between blood transfusion and HHV-8 infection has been extensively investigated due to the prevalence of blood transfusion receipt among patients, with 82.5% having undergone transfusions to address anemia, a common manifestation in advanced chronic kidney disease (CKD), particularly in individuals undergoing dialysis. A similar lack of association was noted in a study conducted in Tunisia in 2023, which found no relevant link between the development of HHV-8 infection and blood transfusion [12]. Furthermore, we did not find any

association between HHV-8 infection and tattooing in our study, as only a small number of patients had tattoos due to their social environment. This finding is consistent with another study conducted in Greece in 2007, which also reported no association between HHV-8 infection and having tattoos [13].

The potential transmission of HHV-8 infection through dental procedures is deemed to be a significant risk factor for viral spread via saliva. Also a highly positive result was obtained in a study conducted in Iraq in 2019 in the saliva of drug related immunosuppressed patients [14]. A 2020 study conducted in Iran revealed a noteworthy correlation between HHV-8 presence and the usage of removable dentures, suggesting a potential association between HHV-8 detection and denture use[15]. The co-infection of HHV-8 with other viruses such as HBV, HCV, CMV, and HIV has been extensively documented in patients due to their shared route of transmission. A study conducted in Taiwan in 2021 also revealed that chronic HBV infection was correlated with a reduced risk for HHV-8 seroconversion [16,17].

Assessment of baseline kidney function in HHV-8 infected patients is imperative for monitoring post-transplant complications. The limited correlation between infected and non-infected individuals undergoing kidney transplantation in this study may be attributed to the inadequate duration of sample collection and testing, which may have failed to capture potential deterioration in renal function test results (18). Symptoms observed following a kidney transplant may be indicative of infections that could culminate in organ rejection and HHV-8 infection.

CONCLUSION:

A considerable proportion of renal transplant recipients presented with latent HHV-8 infection, placing them at a considerably elevated risk of reactivation and subsequent organ rejection. Implementation of pre- and post-kidney transplant HHV-8 screening protocols holds intrinsic value in mitigating adverse outcomes such as the onset of Kaposi's sarcoma and transplant failure.

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