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Research Article

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Human Herpes virus 8 Infection in Egyptian Hemodialysis Patients: Prevalence and Risk Factors

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ABSTRACT

Patients on maintenance hemodialysis (HD) have a high risk of blood-borne viral infections. Blood-borne transmission of Human herpes virus 8 (HHV-8) may exist. The possibility of high incidence of post transplantation Kaposi sarcoma (KS) might be the result of infection with HHV-8. The aim of this study was to determine the prevalence of HHV-8 in 60 uremic Egyptian patients on regular HD by polymerase chain reaction (PCR) compared to 30 healthy controls and study the role of associated risk factors. HHV-8 DNA was detected in peripheral blood mononuclear cells by PCR using primer sets located in the HHV-8 open reading frame 9-1(ORFK9-1). The prevalence of HHV-8 was significantly higher (p=0.029) in HD patients compared to the control group. The detection rate of HHV-8 DNA was significantly higher in older age (p=0.026), and longer duration of HD (p= 0.025). HHV-8 DNA detection rates were insignificantly different with patient sex, history of blood transfusion, patient education and residence. High prevalence of HHV-8 infection in HD patients supports the possibility of virus transmission in those patients via HD, or uremic patients are at risk of reactivation of HHV-8-latent infection.

Key words*: HHV-8, Blood transmission, PCR, Hemodialysis.*

INTRODUCTION

Human herpes virus 8 (HHV-8) was first described in 1994. It was also called Kaposi sarcoma-associated herpes virus (KS*HV*) as it was identified as the underlying infectious cause of Kaposi sarcoma (KS)[1]. In 2010, *HHV-8* was declared a Group 1 carcinogenic agent by the International Agency for Research on Cancer, highlighting its public health significance[2].

The *HHV-8* viral genome comprises about a 140-kb long unique region flanked by multiple terminal repeat sequences. The virus is difficult to cultivate, therefore, diagnosis of infection rests on demonstration of antibodies to the virus or detecting viral nucleic acid in clinical specimens[3].

HHV-8 prevalence varies between different geographic regions and sub-populations. Worldwide seroprevalence of *HHV-8* varies: generally low to moderate for populations in Western countries and Asia[4]. Adult *HHV-8* seropositivity is very high in eastern and central Africa (70%–90%), where KS is endemic, and lower in southern and northern Africa (10%–40%), including Egypt, where KS is more rare[5].

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The transmission modes of *HHV-8* may also differ in different geographic areas and subpopulations; sexual and nonsexual transmissions have been described. It could be transmitted through contact with saliva. Blood-borne transmission may exist, especially among intravenous drug users and blood receivers[6]. Atkinson *et al.*[7] have reported an association between *HHV-8* seroprevalence and intravenous drug use, suggesting that blood-borne transmission might occur .

The possibility of blood-borne *HHV-8* transmission has not been directly studied in Africa, where *HHV-8* prevalence was estimated to range from 20% to 80% in adults[8]. *HHV-8* DNA was detected more readily and at higher levels in the blood of Africans with asymptomatic *HHV-8* infection than in people with *HHV-8* infection from elsewhere, which may suggest a higher transmission risk associated with blood transfusions in Africa[9].

Hemodialysis patients are known to be highly susceptible to viral infection, and may be at increased risk of developing KS from the increased risk of exposure to *HHV-8* infection[10]. Patients with end-stage renal disease (ESRD) are immunocompromised and are at high risk of catching *HHV-8* infection being exposed to multiple intravenous injections during treatment.

In Egypt the prevalence of ESRD is presumed to be increasing. Hemodialysis (HD) represents the main mode of renal replacement therapy for ESRD with more than 300 active hemodialysis centers with more than 200 hemodialysis machines, and more than 10,000 HD patients. The prevalence rate of ESRD in Egypt was 250 per million population in 2002, 260 per million population in 2005, 308 per million population in 2006 and it became 367 per million population in 2007[11,12]. Shaheen and Al-Khader[13] reported that prevalence of chronic renal failure in Egypt is lower than the true due to under reporting. So, it would be valuable to determine the prevalence of HHV-8 among HD patients in Egypt.

Spira *et al.*[14] reported in their evaluation of *HHV-8* serologic assays, no single serologic assay is completely sensitive and specific. Assays based on individual *HHV-8* proteins and peptides appear to sacrifice some sensitivity. Patients can sometimes react to one viral epitope and not another, suggesting that different antibody profiles can develop during the course of *HHV-8* infection[15]. So, using PCR technique provides more confirmatory data for *HHV-8* positivity than serological method.

This study aimed to determine the prevalence of *HHV-8* in Egyptian patients on regular HD by PCR compared to healthy controls and study the role of associated risk factors.

SUBJECTS AND METHODS

This study was conducted on 60 uremic patients on regular HD at Haemodialysis Unit of Benha University Hospital, Egypt and 30 healthy subjects matched with patients' age and sex as controls. Written consent was taken from each participant. The study was approved by the local ethics committee of Benha University Hospitals.

Sample: 5 ml of venous blood were collected by venepuncture under complete aseptic technique from all patients and controls. Buffy coat containing human mononuclear cells were isolated from heparinised venous blood by standard techniques of Histopaque (density = 1077 g/cm3) gradient centrifugation (400g, 30 min, room temperature). It was carefully removed by pasture pipette and washed three times in hanks balanced salt solution[16] and stored at -70ºC till used in DNA extraction and amplification.

Genomic DNA extraction from buffy-coat samples was performed using The Thermo Scientific Gene JET Viral DNA and RNA Purification Kit (Thermo Fisher scientific, Australia). The extracted DNA concentration was detected through measurement by UV spectrophotometer. Readings were taken at wave lengths of 260 and 280 nm. Purified DNA samples were immediately used in the amplification step.

DNA amplification: Amplification of ORFK9-1 genomic sequence was generated using the following primers: forward-5'-GTCTCTGCGCCATTCAAAAC-3' and the reverse: 5'-CCGGACACGACAACTAAGAA-3'[17].ORFK9-1 region is specific to the *HHV-8* lineage encoding the viral interferon regulatory factor 1[18].

The reaction was performed in a final volume of 50µL consisting of 25µL of Dream Green Taq PCR Master Mix (2x) (Fermentas, Germany), five µL of template DNA, 0.5µM concentration of each primer (Fermentas, Germany), water (nuclease free) to a final volume of 50 µL. All reagents were prior vortexed, and finally 25 µL of mineral oil were added to the reaction mixture.

Reaction was carried out in Thermal Cycler (Biometra, Germany) with the following steps: Initial denaturation step at 95ºC for 3 minutes, forty repeated cycles of: denaturation at 95ºC for 30 seconds,

annealing at 60°C for 30 seconds and extension at 72°C for 1 minute followed by final extension step at 72°C for 15 minutes then hold at 4°C. The size of the PCR product was 138 bp. 10 µL of each amplified DNA and 100 bp ladder (molecular weight marker) (Fermentans, Germany) were separated on 1.5% agarose gel containing 0.3µg/ ml of ethidium bromide. The bands were visualized using UV transilluminator (Biometra, Germany) (254nm).

Statistical analysis

The collected data were presented as number and percentages for categorical variables while continuous variables were expressed as mean and standard deviation. SPSS software (version 16) was used to calculate St, "t" test, Fisher's exact test, Monte Carlo exact method was used to calculate Fisher's exact test for tables larger than 2x2. Microstate software was used to calculate "Z" test and Goodness of fit test. $P<0.05$ was considered significant.

RESULTS

This study was conducted on 60 uremic patients on regular HD and 30 healthy volunteers as control group. Uremic patients were 47 males and 13 females and their age ranged between 25-65 years. Controls were 23 males and 7 females and their age ranged between 26-65 years. Patients were classified according to the disease responsible for renal failure. They were 34 (56.7%) with unknown etiology, 5 (8.3%) with hypertension, 12 (20%) with diabetic nephropathy, 8 (13.3%) with chronic glomerulonephritis and 1 (1.7%) with autoimmune glomerulonephritis. Patients' and controls' data were summarized in Table. 1.

No significant difference was detected between patients and controls groups as regard to age, sex and history of blood transfusion, p values were 0.39, 0.85 and 0.21 respectively. Within HD patients, renal failure of unknown etiology was the most prominent cause for hemodialysis compared to all other underline etiology ($p<0.001$), Table 1.

Figure 1 showed gel electrophoresis of amplified samples (positive and negative PCR samples). The prevalence of *HHV-8* was significantly higher ($p=0.029$) in HD patients compared to the control group, as out of 60 HD patients, 13 patients (21.7%) had *HHV-8*-positive DNA. Among 30 control subjects, *HHV-8*-positive DNA was detected in 1 person (3.3%), Figure 2 showed the results of PCR.

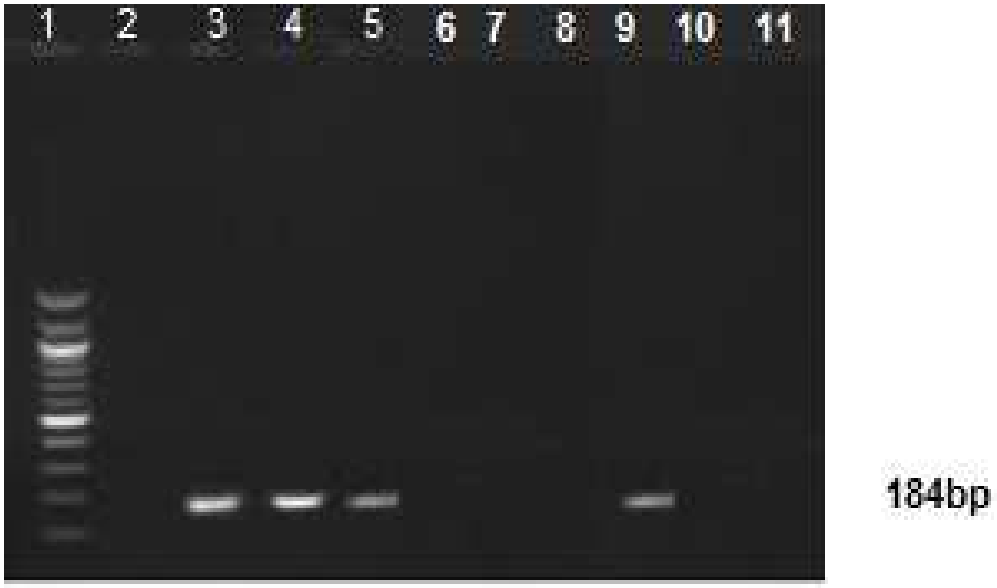
The detection rate of *HHV-8* DNA was significantly higher in older age of HD patients $p=0.026$. It was also significantly higher in patients with longer duration of HD, $p= 0.025$. There were no association with patient sex or history of blood transfusion and the detection rate of *HHV-8* DNA ($p=0.26$ and 0.115, respectively), Table 2.*HHV-8* DNA recovery rate was higher in illiteracy patients and those with low levels of education than higher levels and those lived in rural areas than urban. However, these differences were statistically insignificant ($p = 0.72$ and 0.33 respectively), Table 2.

The results showed that the prevalence of *HHV-8* DNA positivity in HD patients was higher (5 out of 12; 41.7%) in patients with diabetes mellitus as the primary cause of renal failure when compared with *HHV-8* DNA positive in patients with other causes of renal failure together (8 out of 48; 16.7 %). However, this difference was statistically insignificant ($p = 0.32$), Table 3.

Table 1: Demographic and clinical data of hemodialysis (HD) patients and controls

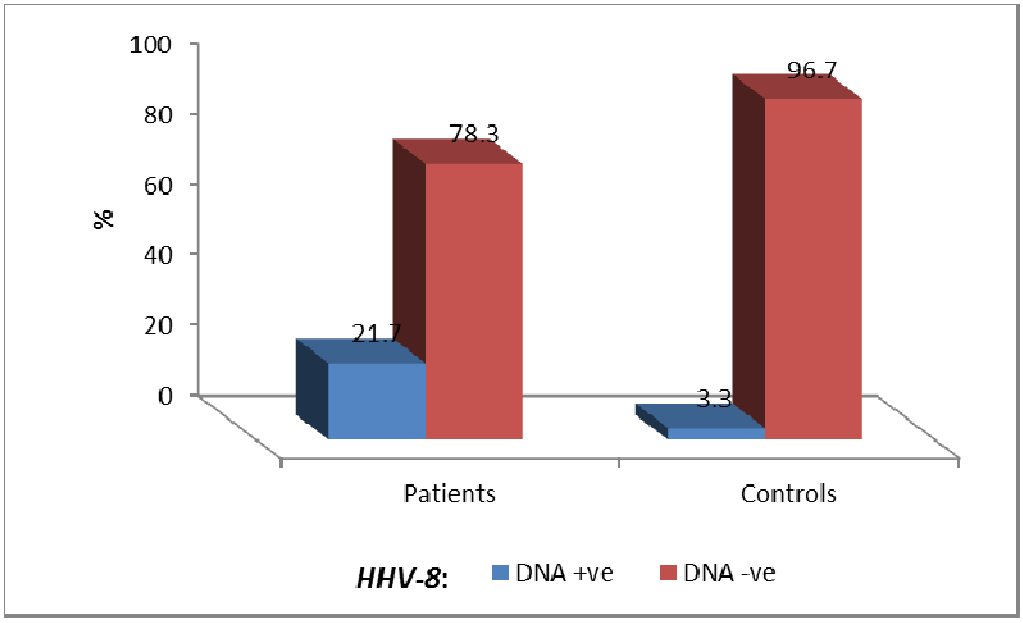
	HD patients N=60	**Control group N=30**	**Test of sig.**	**p value**
Age(years)	54±6 (25-66)	53 ±3 (26-65)	St t = 0.86	0.39
Male / Female	47/13	23/7	Z=0.18	0.85
History of bl. Transfusion (%)	**3/60**	**0/30**	Z=1.24	0.21
Duration of HD (month)	14.74±5.95	------	-------	-----
Etiology of renal failure **Unknown etiology** **Diabetic** **Chronic glomerulonephritis** **Hypertensive** **Auto immune glomerulonephritis**	34(56.7%) 12(20%) 8(13.3%) 5(8.3%) 1(1.7%)	- - - - -	**Goodness of fit test=55.8**	**<0.001-**
Total	**60**	**30**		-

Fig.1: Gel electrophoresis of amplified product (184 base pair) of *human herpes virus -8 (HHV-8)* ORFK9-1 sequence



Lan1 shows DNA ladder. Lane 2 shows negative control. Lanes 3, 4, 5, 9 show positive specific band of *HHV-8*. Lanes 6,7,8,10,11 are negative.

Fig. 2: Prevalence of *human herpes virus-8 (HHV-8)* among hemodialysis patients and controls. Fisher's exact test was used to calculate p value



The prevalence of *HHV-8* is significantly higher (p= 0.029) in HD patients than healthy controls.

Table 2: Prevalence of human herpes virus 8 (*HHV-8)* among hemodialysis (HD) patients and controls in relation to different variables

	HD patients			Control		
	HHV-8 DNA$^+$	*HHV-8* DNA$^-$	Total	*HHV-8* DNA$^+$	*HHV-8* DNA$^-$	Total
Age (years)						
25-44	0(0%)	15(100%)	15(100%)	0(0%)	6(100%)	6(100%)
45-65	13(32.6%)	32(67.3%)	45(100%)	1(4.2%)	23(95.8%)	24(100%)
		p=0.026 (S) *****			p=1.0	
Gender						
Male	12(25.5%)	35(74.5%)	47(100%)	1(4.3%)	22(95.7%)	23(100%)
Female	1(7.7%)	12(92.3%)	13(100%)	0(0%)	7(23.3%)	7(100%)
		p=0.26			p=1.0	
History of Blood transfusion	2(66.7%)	1(33.3%)	3(100%)	0(0%)	0(0%)	0(0%)
Yes	11(19.3%)	46(80.7%)	57(100%)	1(3.3%)	29(96.7%)	30(100%)
No		p=0.115			p=1.0	
Duration of HD	2 (7.4%)	25(92.6%)	27(100%)	0(0%)	0(0%)	0(0%)
0-24 months	11(33.3%)	22(66.7%)	33(100%)			
≥25 months		p=0.025 (S)		0(0%)	0(0%)	0(0%)
Education	4(21.1%)	15(78.9%)	19(100%)			
Illiteracy	5(20%)	20(80%)	25(100%)	0(0%)	7(100%)	7(100%)
Elementary school	3(33.3%)	6(66.7%)	9(100%)	1(9.1%)	10(90.9%)	11(100%)
Secondary school	1(14.3%)	6(85.7%)	7(100%)	0(0%)	7(100%)	7(100%)
University		p=0.72		0(0%)	5(100%)	5(100%)
					p=0.59	
Resident						
	10(27%)	27(73%)	37(100%)	1(6.2%)	15(93.8%)	16(100%)
Rural	3(13.1%)	20(86.9%)	23(100%)	0(0%)	14(100%)	14(100%)
Urban		p=0.33			p=1.0	

Monte Carlo exact method was used to calculate Fisher's exact test for tables larger than 2x2.*****P<0.05 was considered significant.

Table 3: Distribution of human herpes virus 8 (*HHV-8)* among hemodialysis (HD) patients with various etiologies

Parameter	*HHV-8* DNA$^+$	*HHV-8* DNA$^-$	Total
Unknown etiology	5(14.7%)	29(85.3%)	34 (100.0%)
Diabetic	5(41.7%)	7(58.3%)	12 (100.0%)
Chronic glomerulonephritis	2(25%)	6(75%)	8 (100.0%)
Hypertensive	1(20%)	4(80%)	5 (100.0%)
Auto immune glomerulonephritis	0(0%)	1(1.6%)	1(100.0%)

Fisher's exact test (Monte Carlo exact method) was used p =0.32

DISCUSSION

Transmission of *HHV-8* by hemodialysis has not been demonstrated. Although the majority of epidemiological studies have documented iatrogenic KS mostly among *HHV-8*-seropositive patients who had undergone renal transplantation, the organ per se as well as blood transfusions could not be excluded as routes of virus transmission/acquisition in these patients[19].

It was found that post-transplant KS in transplant recipients from *HHV-8* endemic areas appears to occur mainly in individuals who were already *HHV-8* infected before transplantation[20]. The risk of *HHV-8* and KS may differ according to the ethnic background of patients. Differences in the prevalence of *HHV-8* and KS may depend on the study population and a combination of geographic, behavioral and genetic risk factors. Outside of Africa, *HHV-8* seroprevalence rates roughly match the geographic distribution of KS[21].

Infection with *HHV-8* and development of KS has been observed to be associated with induced immune suppression. Immune suppression is hypothesized to exert a significant effect on the prevalence of *HHV-8* as it increases the rate of *HHV*- 8 positivity due to increased viral replication in infected individuals[22].The dialysis setting has been recognized as a high-risk environment for the transmission of blood-borne infections. There is a high risk of indirect and direct transmission of infectious agents in chronic hemodialysis, as vascular access is needed on a regular basis[23].

There are very little data on the prevalence of *HHV-8* in Egypt. Hence, it would be useful to evaluate the extent of the problem in HD patients in Egypt.

This study aimed to determine the prevalence of *HHV-8* in Egyptian patients on regular HD by PCR compared to healthy controls and study the role of associated risk factors.

Our results revealed that the prevalence of *HHV-8* is significantly higher (p=0.029) in HD patients (21.7 %) than controls (3.3%). This result is in consistent with Al-Otaibi *et al.*[24] who found that the detection rate of anti-*HHV-8*-IgG was 16.7% versus 0.4% ($p < 0.001$) and that of *HHV-8*-DNA was 4.2% versus 0.4%, ($p < 0.05$) between the HD patients and the apparently healthy people. They reported that blood is thus a likely vehicle for transmission of *HHV-8,* possibly contributing to the high risk of *HHV-8* infection in patients undergoing HD and to KS following immunosuppression after renal transplantation.

This result also agrees with Hsu *et al.*[10] who detected a prevalence of *HHV-8* antibodies in HD patients in Hualien to be higher than that in normal blood donors (19.5% versus 3%). Caterino-de-Araujo *et al.*[19] recorded a similar high prevalence of *HHV-8* infection in HD patients (22.9%) in São Paulo, Brazil.

Zavitsanou *et al.*[25] reported a lower prevalence (7.2%) in HD patients. Luppi *et al.*[26] reported a prevalence of 9.5% of *HHV-8* in HD patients from northern Italy. Almuneef *et al.*[27] did not find a significant difference between patients with end-stage renal failure and healthy controls (p=0.14).

Our results are supported by Hladik *et al.*[6] who found that risk of seroconversion was significantly higher among recipients of *HHV-8*-seropositive blood than among recipients of seronegative blood (excess risk, 2.8%; $p<0.05$), and the increase in risk was seen mainly among patients in whom seroconversion occurred 3 to 10 weeks after transfusion (excess risk, 2.7%; P=0.005), a result consistent with the transmission of the virus by transfusion.

The present study demonstrated that detection rate of *HHV-8* DNA was significantly higher in older age of HD patients (p=0.026). This result is consistent with Almuneef *et al.*[27] who reported that *HHV-8* seropositive individuals were on average 10 years older than seronegative subjects (55.3 years vs. 46.9 years). They also revealed that the strongest association of *HHV-8* infection was with increasing age. Olsen *et al.*[28] showed a linear increase in *HHV-8* seroprevalence with age from childhood to adolescence. Mbulaiteye *et al.*[29] reported that among adult men and women *HHV-8* seropositivity was higher among older participants (>45 years of age) compared with younger participants (15–24 years of age).

Zavitsanou *et al.*[25] reported that patients 50 years and younger had an increased probability to experience seroreversion to *HHV-8* antibodies than patients older than 50 years, and this finding was statistically significant. This could be attributed to the impaired defense mechanisms of middle-aged and elderly patients with renal failure, particularly related to the combined effect of advancing age, uremia, and hemodialysis treatment[30].These investigators support that the greater prevalence at older ages could be related to easier recognition of infection with advancing age.

This study also showed that there was also significantly higher *HHV-8* percentage in patients with longer duration of HD (p= 0.025).This finding could be explained by the fact that longer duration of HD could increase the chance of possible viral transmission via repeated multiple injections, reactivation of latent infection, or impaired defense mechanism.

HHV-8 DNA recovery rate was higher in illiteracy patients and those with low levels of education than higher levels and those lived in rural areas than urban. However, these differences were statistically insignificant (p = 0.72 and 0.33 respectively). These finding go in hand with Mbulaiteye *et al.*[29] who reported that *HHV-8* seropositivity was 14.2% in rural Egypt and associated with higher age and lower education.

The rate of *HHV-8* infection in the present study is higher in males (25.5%) than in females (7.7%) but this difference is statistically insignificant (p=0.25). This finding is in contrast with Sheldon *et al.*[20] who

reported a higher prevalence in women (12.9%) than men (4%) and explained this difference by the small number of positive individuals. However, no significant difference was detected by Cattani *et al.*[31] in rates of *HHV-8* in males and females. Olsen *et al.* [28] stated that the rate of *HHV-8* infection in men and women is generally similar but KS is more common in males.

Several studies reported an association between *HHV-8* prevalence and intravenous drug use, suggesting possible blood borne transmission[7, 32.]The present results showed that the percentage of *HHV-8* DNA positivity in HD patients was higher (5 out of 12; 41.7%) in HD patients with diabetes mellitus compared to other causes of renal failure (8 out of 48; 16.7 %). However, this difference was statistically insignificant (p = 0.32). It could be explained by partial state of immunosuppression which helps in increased viral replication in infected patients.

To our knowledge, this study is the first to determine the prevalence of *HHV-8* infection in Egyptian HD patients.

In conclusion, High prevalence of *HHV-8* infection in HD patients supports the possibility of virus transmission in those patients via HD or uremic patients are at risk of reactivation of *HHV-8*-latent infection. Additional follow-up studies of a larger sample of individuals are recommended to confirm the role of long term hemodialysis treatment as a transmission route of *HHV-8* infection. A screening program in hemodialysis units could be proposed.

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