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HERPES GROUP VIRUSES: A SEROPREVALENCE STUDY IN HEMODIALYSIS PATIENTS

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SUMMARY – Herpes group viruses (herpes simplex virus, HSV; varicella-zoster virus, VZV; cytomegalovirus, CMV; and Epstein-Barr virus, EBV) remain an important cause of morbidity in immunocompromised persons. The aim of the study was to analyze the prevalence of HSV-1, HSV-2, VZV, CMV and EBV in patients undergoing hemodialysis. During a three-year period (2013-2015), 152 consecutive serum samples from hemodialysis patients and 150 healthy subjects (control group) were tested for the presence of IgM/IgG antibodies to herpes group viruses. Serologic tests were performed using a commercial enzyme-linked immunosorbent assay (ELISA) or enzyme-linked immunofluorescent assay (ELFA). Hemodialysis patients showed significantly higher CMV IgG seropositivity compared to controls (88.2% *vs*. 78.7%, p=0.011). In addition, seroprevalence rates of HSV-1 and VZV were higher in hemodialysis patients; however, these differences did not reach statistical significance (85.5% *vs*. 80.0%, p=0.054 and 99.3% *vs*. 96.0%, p=0.051, respectively). The prevalence of HSV-2 and EBV was similar in both groups (12.5% *vs*. 12.7%, p=0.137 and 98.0% *vs*. 95.3%, p=0.113, respectively). There was no difference in IgG seropositivity according to gender and place of residence. Logistic regression showed that older age was a significant predictor for CMV and EBV IgG seropositivity (increase in age by one year: CMV OR=1.055; 95%CI=1.030-1.080 and EBV OR=1.075, 95%CI=1.023-1.130).

Key words: *Herpesvirus 1, human; Herpesvirus 3, human; Cytomegalovirus, herpesvirus 4, human; Renal dialysis; Croatia*

Introduction

Members of the herpesvirus family including herpes simplex virus (HSV) type 1 and 2, varicella-zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are distributed worldwide. Prevalence rates vary significantly among regions and population groups[1,2]. Herpesviruses are usually spread by direct contact with infected saliva (HSV, CMV and EBV). VZV is the exception and is spread by airborne transmission, while sexual transmission is important for HSV type 2 and CMV[3]. Moreover, herpesviruses can be transmitted by blood transfusion and organ transplantation[4]. The unique feature of these viruses is the ability to establish a latent, nonproductive infection with the life-long capacity for reactivation to productive, lytic infection[1].

In immunocompetent persons, primary infections are usually asymptomatic or presented as mild, self-limited disease, i.e. gingivostomatitis or blisters (HSV) and infectious mononucleosis syndrome (CMV and EBV)[3,5,6]. Clinical manifestations of VZV infection are chickenpox or varicella (primary infection) and shingles (reactivation)[3]. In immunocompromised persons such as transplant recipients, patients with malignant diseases and HIV-infected patients, herpesviruses can cause severe, sometimes fatal disease[7,8]. In this

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population group, common clinical symptoms include retinitis, pneumonitis, hepatitis, esophagitis, meningitis and encephalitis[1,9]. It has been documented that hemodialysis patients have impaired immune response, which predisposes them to herpesvirus infections. Infections in these patients may be due to a primary infection or, more commonly, by reactivation of latent virus or reinfection with exogenous virus (CMV)[10,11].

There are many published studies on the prevalence of herpes group viruses in the general population; however, there are few recent data on hemodialysis patients.

The aim of this study was to analyze the prevalence of herpes group viruses in the Croatian patients with end-stage renal disease undergoing chronic hemodialysis.

Subjects and Methods

During a three-year period (2013-2015), 152 consecutive serum samples from hemodialysis patients were tested for the presence of HSV-1/2, VZV, CMV and EBV IgM and IgG antibodies at two large Croatian medical institutions (Croatian Institute of Public Health and Zagreb University Hospital Center). Control group included 150 asymptomatic persons presenting for routine testing (antenatal screening, couples undergoing medically assisted reproduction, or elective preoperative check-up) with no symptoms of acute febrile disease. The mean age of hemodialysis patients was 53.4±14.5 (range 20-78) years, whereas the mean age of control subjects was 46.1±13.2 (range 19-87) years.

Serologic tests were performed using a commercial enzyme-linked immunosorbent assay (ELISA) (Sekisui Virotech, Rüsselsheim, Germany) or enzyme-linked immunofluorescent assay (ELFA) (Vidas; Biomerieux, Marcy l'Etoile, France). VZV, CMV and EBV IgM/IgG positive samples were further tested for IgG avidity using ELISA (Euroimmun, Lübeck, Germany) for confirmation of recent infection. HSV-1/2 IgM/IgG positive samples were confirmed using a western blot test (Euroimmun, Lübeck, Germany).

Statistical analysis

Seroprevalence rates were expressed with 95% confidence intervals (CI). Differences between groups were compared using χ^2 of Fisher exact test. The strength of association between outcomes (seropositivity to herpesviruses) and potential risk factors (hemodialysis, gender, age and setting) was assessed using multiple logistic regression: adjusted odds ratios and 95%CI are presented. Statistical analysis was performed using STATA/IC ver. 14.1 (StataCorp LP, USA). The level of statistical significance was set at $p<0.05$.

Results

The overall prevalence of IgG and IgM antibodies to herpes group viruses in hemodialysis patients and control subjects is presented in Table 1 a,b. Hemodialysis patients showed significantly higher CMV IgG seropositivity compared to controls (88.2% *vs*. 78.7%, p=0.011). Although higher HSV-1 (85.5% *vs*. 80.0%, p=0.054) and VZV (99.3% *vs*. 96.0%, p=0.051) seroprevalence rates were also recorded in hemodialysis patients, these differences did not reach statistical significance. The prevalence of HSV-2 and EBV was similar in both groups (12.5% *vs*. 12.7%, p=0.137, and 98.0% *vs*. 95.3%, p=0.113, respectively). Acute VZV infection (IgM positive) was detected more commonly in the control group (4.7% *vs*. 0.7%, p=0.029). There was no difference in the prevalence of other herpesvirus infections between patients on hemodialysis and control subjects: HSV-1 0% *vs*. 2.0%, p=0.121; HSV-2 1.3% *vs*. 0%, p=0.252; CMV 2.0% *vs*. 0.7%, p=0.253; and EBV 2.6% *vs*. 2.0%, p=0.278. Using IgG avidity, recent primary CMV and EBV infection was detected in two control group subjects. In all other IgM/IgG positive hemodialysis patients and control subjects, IgG antibodies of high avidity were detected indicating recurrent infection.

The IgG seropositivity to herpes group viruses according to participant characteristics is presented in Tables 2 and 3. There was no difference in IgG seropositivity between genders. According to age, a sharp increase in CMV prevalence was observed in hemodialysis group, from 62.5% in patients aged less than 30 years to 90.5% in patients aged 31-39 years; thereafter the prevalence remained stable (83.9%-92.9%). In the control group, seroprevalence was bimodal with an increase in seropositivity from 46.7% in <30 age group to 71.1% in 31-40 age group and from 72.4% in 41-50 age group to 95.2% in 51-60 age group. High preva-

Table 1 a,b. Seroprevalence of herpes group viruses in hemodialysis patients and control subjects

(a)

Group	N	HSV-1 IgM n/% 95%CI		HSV-2 IgM n/% 95%CI		VZV IgM n/% 95%CI		CMV IgM n/% 95%CI		EBV IgM n/% 95%CI	
Hemodialysis	152	0/0	0.0-2.4	2/1.3	0.2-4.7	1/0.7	0.1-3.6	3/2.0	0.4-5.7	4/2.6	0.7-6.6
Controls	150	3/2.0	0.4-5.7	0/0	0.0-2.4	7/4.7	1.9-9.4	1/0.7	0.1-3.7	3/2.0	0.4-5.7
p value		0.121		0.252		0.029		0.253		0.278	

(b)

Group	N	HSV-1 IgG n/% 95%CI		HSV-2 IgG n/% 95%CI		VZV IgG n/% 95%CI		CMV IgG n/% 95%CI		EBV IgG n/% 95%CI	
Hemodialysis	152	130/85.5	78.9-90.7	19/12.5	7.7-18.8	151/99.3	96.4-99.9	134/88.2	81.9-92.8	149/98.0	93.4-99.6
Controls	150	120/80.0	72.7-86.1	19/12.7	7.8-19.1	144/96.0	91.5-98.5	118/78.7	71.2-84.9	143/95.3	90.6-98.1
p value		0.054		0.137		0.051		0.011		0.113	

HSV = herpes simplex virus; VZV = varicella-zoster virus; CMV = cytomegalovirus; EBV = Epstein-Barr virus

lence rates of VZV and EBV were found in all age groups in both hemodialysis patients and control group (98.5-100% *vs*. 96.4-100%, and 87.5-100% *vs*. 89.5-100%, respectively). HSV-1 seroprevalence was stable in different age groups among hemodialysis patients (83.9-88.9%), whereas in control subjects it increased progressively by the age of 40 years (53.3-93.1%) and remained stable thereafter. In hemodialysis patients, HSV-2 seropositivity was highest in the youngest age group (25.0%) and varied from 4.8% to 16.7% in other age groups. In the control group, an increase in seropositivity was found starting from 31-40 group to 51-60 group (7.9%-21.4%) (Fig. 1). There was no difference in seropositivity between the residents of urban and rural areas.

Results of logistic regression (Table 4) showed older age to be a significant predictor for CMV and EBV IgG seropositivity (increase in age by one year: CMV OR=1.055; 95%CI=1.030-1.080; and EBV OR=1.075, 95%CI=1.023-1.130).

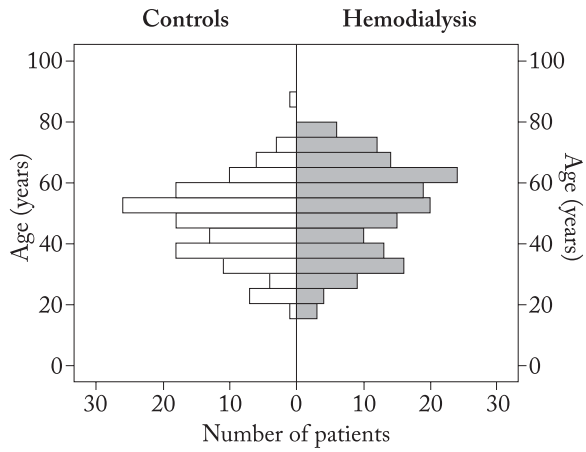

Fig. 1. Distribution of study participants according to age.

Discussion

The results of this study showed a significantly higher CMV seropositivity rate in hemodialysis patients compared to healthy subjects (88.2% *vs*. 78.7%), which is similar to the results of an earlier Croatian study (2010-2012; 90.7% *vs*. 81.9%)[12]. Similar results have been reported from German (83.0% *vs*. 63.7%-73.9%) and Turkish (99.6% *vs*. 82.9%) studies[13,14], whereas in a Dutch study, the percentage of CMV seropositive hemodialysis patients (68.7%) was in the range of the prevalence reported in the general population[15]. A higher prevalence among hemodialysis patients could be explained by the acquisition of CMV through repeated blood transfusions or exposure to CMV through contaminated equipment during hemodialysis procedure.

Although this study found higher seroprevalence rates of HSV-1 and VZV in hemodialysis patients, these differences were not significant (HSV-1 85.5%

Table 2. Prevalence of IgG antibodies to herpes group viruses in hemodialysis patients according to patient characteristics

	N/%	HSV-1 n/% 95%CI		HSV-2 n/% 95%CI		VZV n/% 95%CI		CMV n/% 95%CI		EBV n/% 95%CI	
Gender:											
Male	101/66.4	86/85.1	76.7-90.9	13/12.9	7.6-21.0	101/100	96.4-100	88/87.1	79.0-92.4	100/99.0	94.6-99.9
Female	51/33.6	44/86.3	73.6-93.4	6/11.8	5.3-24.1	50/98.0	87.0-99.7	46/90.2	75.8-95.9	49/96.1	86.5-99.5
Age group:											
<30 yrs	8/5.3	7/87.5	42.3-98.5	2/25.0	5.6-65.2	7/87.5	42.3-98.5	5/62.5	26.3-88.6	7/87.5	42.3-98.5
31-40 yrs	21/13.8	18/85.7	62.9-95.5	1/4.8	0.6-28.5	21/100	83.9-100	19/90.5	67.8-97.7	21/100	83.9-100
41-50 yrs	31/20.4	26/83.9	66.1-93.3	4/12.9	4.8-30.3	31/100	88.8-100	26/83.9	66.1-93.3	30/96.8	79.6-99.6
51-60 yrs	36/23.7	32/88.9	73.4-95.9	6/16.7	7.5-32.9	36/100	90.3-100	32/88.9	73.4-95.9	36/100	90.3-100
60+ yrs	56/36.8	47/83.9	71.7-91.5	6/10.7	4.8-22.1	56/100	93.6-100	52/92.9	82.2-97.3	55/98.2	88.0-99.8
Setting:											
Urban	125/82.2	106 /84.8	77.3-90.1	2/1.6	0.4-6.3	124/99.2	94.4-99.9	108/86.4	79.1-91.4	123/98.4	93.7-99.6
Rural	27/17.8	24/88.9	70.0-96.5	2/7.4	1.8-26.0	27/100	87.2-100	26/96.3	77.0-99.5	26/96.3	77.0-99.5

HSV = herpes simplex virus; VZV = varicella-zoster virus; CMV = cytomegalovirus; EBV = Epstein-Barr virus

Table 3. Prevalence of IgG antibodies to herpes group viruses in control group according to subject characteristics

	N/%	HSV-1 n/% 95%CI		HSV-2 n/% 95%CI		VZV n/% 95%CI		CMV n/% 95%CI		EBV n/% 95%CI	
Gender:											
Male	86/57.3	49/76.6	64.5-85.5	11/12.8	7.2-21.8	85/98.8	92.0-99.8	70/81.4	71.6-88.4	84/97.7	91.0-99.4
Female	64/42.7	71/82.6	72.9-89.3	8/12.5	6.3-23.3	59/92.2	82.4-96.8	48/75.0	62.8-84.2	59/92.2	84.2-96.8
Age group:											
<30 yrs	15/10.0	8/53.3	28.4-76.7	0/0	0-21.8	15/100	78.2-100	7/46.7	23.2-71.6	14/93.3	62.8-99.1
31-40 yrs	38/25.3	27/71.1	54.5-84.3	3/7.9	2.5-22.2	35/92.1	77.8-97.5	27/71.1	54.5-83.4	34/89.5	74.7-96.1
41-50 yrs	29/19.3	27/93.1	75.6-98.3	3/10.3	3.3-28.2	29/100	88.1-100	21/72.4	53.2-85.8	27/93.1	75.6-98.3
51-60 yrs	42/28.0	37/88.1	74.0-95.0	9/21.4	11.4-36.7	42/100	91.6-100	40/95.2	82.4-98.8	42/100	91.6-100
60+ yrs	26/17.4	21/80.8	60.6-92.0	4/15.4	5.7-35.2	23/88.5	69.0-96.4	23/88.5	69.0-96.4	26/100	86.8-100
Setting:											
Urban	128/85.3	102/79.7	71.7-85.8	16/12.5	7.8-19.5	122/95.3	89.9-97.9	103/80.5	72.6-86.5	121/94.5	89.1-97.8
Rural	22/14.7	18/81.8	59.5-93.2	3/13.6	4.3-35.7	22/100	84.6-100	15/68.2	45.9-84.4	22/100	84.6-100

HSV = herpes simplex virus; VZV = varicella-zoster virus; CMV = cytomegalovirus; EBV = Epstein-Barr virus

Table 4. Logistic regression for the risk of IgG seropositivity to herpes group viruses

	HSV-1 OR 95%CI		HSV-2 OR 95%CI		VZV OR 95%CI		CMV OR 95%CI		EBV OR 95%CI	
Hemodialysis *vs*. controls	1.368	0.722-2.592	0.901	0.437-1.858	6.430	0.725-56.965	1.681	0.850-3.324	1.913	0.461-7.941
Male *vs*. female	0.729	0.388-1.369	0.998	0.490-2.030	0.312	0.057-1.688	0.613	0.315-1.195	0.580	0.152-2.205
Age (one year increase)	1.021	0.999-1.043	1.013	0.987-1.038	1.026	0.971-1.085	1.055	1.030-1.080	1.075	1.023-1.130
Urban *vs*. rural setting	0.764	0.388-1.369	1.300	0.479-3.525	1.259	0.148-10.670	0.964	0.403-2.303	0.546	0.065-4.576

HSV = herpes simplex virus; VZV = varicella-zoster virus; CMV = cytomegalovirus; EBV = Epstein-Barr virus

vs. 80.0%; and VZV 99.3% *vs*. 96.0%). A study from Ahvaz (Iran) showed positive HSV IgG antibodies in 93.33% of kidney recipients and 77.27% of donors[16]. Seroprevalence rates in Croatian hemodialysis patients were high in all age groups (HSV-1 83.9%-88.9% and VZV 98.5%-100%). Similar VZV seropositivity was noted in some other countries such as The Netherlands where 97.9% of transplant recipients and 96.8% of pre-transplant patients were found to be seropositive[17]. In Scotland, hemodialysis patients showed a similar seroprevalence of 98.8%[18]. An Iranian study found a very high overall VZV seropositivity (97.9%), with a significant difference between age groups: 88.8% in patients aged ≤40 years, 98.2% in patients aged 41-55 and 100% in patients aged ≥56 years[19].

The prevalence of HSV-2 was similar in both tested groups in our study (hemodialysis 12.5% and controls 12.7%). Studies from other countries showed a wide range of HSV-2 prevalence among hemodialysis patients, from 5.4% in Iran[20] to 29% in Kosovo[21]. In addition, a Kosovo study found that hemodialysis patients had about a three-fold greater higher risk of being HSV-2 seropositive than blood donors[21].

Our study showed EBV to be highly prevalent in both hemodialysis patients (98.0%) and adult healthy population (95.3%). Two Iranian studies addressed the prevalence of EBV infection in hemodialysis patients. A study conducted from 2005 to 2008 showed 100% EBV seropositivity in both potential renal transplant donors and transplant recipients[22]. A more recently published study (2011) showed a higher, although not statistically significant difference in EBV IgG seroprevalence between re-transplant patients (70%) and kidney donors (52%)[23].

The present study found no significant difference in herpes group virus seroprevalence between genders, which is similar to the results of many other studies conducted in hemodialysis patients[23-25]. However, a slightly higher CMV seroprevalence in women was noted in some studies[26], whereas a Sudanese study has reported a slightly higher seroprevalence in men (97.9% *vs*. 93.3%)[27].

Our results showed that older age was a significant predictor for CMV and EBV seropositivity. Age-related increase in the seroprevalence of CMV/EBV antibodies has also been shown in some studies[15], whereas others found a higher, but not significant difference in seropositivity in older patients[26]. In contrast, studies from Urmia (Iran) and Gezira (Sudan) showed no relationship of age with CMV seroprevalence[27,28].

In conclusion, results of this study indicate that herpes group viruses are widely distributed in Croatia. Being on hemodialysis is a significant risk factor for CMV seropositivity. In addition, older age is a significant predictor for both CMV and EBV seropositivity.

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Sažetak

SEROPREVALENCIJA NA VIRUSE IZ HERPES GRUPE U BOLESNIKA NA HEMODIJALIZI

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Virusi iz herpes grupe (herpes simpleks virus, HSV; varičela-zoster virus, VZV; citomegalovirus, CMV; Epstein-Barrov virus, EBV) su značajan uzrok smrtnosti u imunokompromitiranih osoba. Cilj rada bio je analizirati učestalost HSV-1, HSV-2, VZV, CMV i EBV u bolesnika koji se liječe hemodijalizom. Tijekom trogodišnjeg razdoblja (2013.-2015.) ispitano je ukupno 152 uzastopno pristiglih uzoraka seruma bolesnika na hemodijalizi te 150 uzoraka seruma zdravih osoba (kontrolna skupina) na prisutnost herpes virusnih IgM/IgG protutijela. Serološko testiranje učinjeno je pomoću komercijalnog dijagnostičkog imunoenzimnog testa (ELISA) ili imunoenzimnog testa s fluorescentnom detekcijom (ELFA). Bolesnici na hemodijalizi bili su značajno češće CMV IgG seropozitivni u odnosu na kontrolnu skupinu (88,2% prema 78,7%, p=0,011). Nadalje, seroprevalencija HSV-1 i VZV također je bila viša u bolesnika na hemodijalizi, no statistička značajnost nije dostignuta (85,5% prema 80,0%, p=0,054; 99,3% prema 96,0%, p=0,051). Učestalost HSV-2 i EBV protutijela nije se razlikovala između skupina (12,5% prema 12,7%, p=0,137, odnosno 98,0% prema 95,3%, p=0,113). IgG seroprevalencija nije se razlikovala u odnosu na spol i mjesto prebivališta. Rezultati logističke regresije pokazali su da je starija životna dob značajan čimbenik rizika za CMV i EBV seropozitivnost (porastom dobi za jednu godinu CMV OR=1,055; 95%CI=1,030-1,080; EBV OR=1,075, 95%CI=1,023-1,130).

Ključne riječi: *Herpesvirus 1, humani; Herpesvirus 3, humani; Citomegalovirus, herpesvirus 4, humani; Hemodijaliza; Hrvatska*