Silent reactivation of Varicella Zoster virus in hemodialysis patients. *A management dilemma*

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ABSTRACT

Objectives: To determine the seroprevalence of Varicella Zoster virus (VZV) immunoglobulin (Ig) G and IgM antibodies among hemodialysis patients (HDP). Additionally, the presence of VZV viral DNA has been investigated for possible reactivation status.

Methods: Sera from 265 individuals were collected and tested. The study was carried out from November 2023 to February 2024. Enzyme linked immunosorbent assay was accomplished to determine the VZV IgG and IgM antibody levels. The viral DNA was tested by qPCR.

Results: We found a significantly higher proportion of Host defense peptides (HDP) have positive antibody levels compared to healthy controls (HC) (92.0% of HDP have positive immune responses compared to 79.1% of HC, p=0.005). Patients with both hypertension and diabetes showed lower VZV seropositivity (25.6%) than those without (74.4%). Only 3 HDP had positive IgG and IgM levels (1.9%, n=3), while 14 patients (100%) had negative IgG and IgM levels. Additionally, all IgM-positive patients appeared to have detectable viral DNA of the VZV.

Conclusion: In conclusion, significantly higher VZV seroprevalence and antibody levels in HDP indicate greater viral exposure than HC. Screening of hemodialysis (HD) to VZV serologically or at the molecular level is most important to avoid the consequences of viral reactivation, especially in those with asymptomatic HDP.

Keywords: Varicella-zoster virus, hemodialysis patients, reactivation, immunosuppression

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Varicella, frequently recognized as chickenpox, is initiated by Varicella Zoster Virus (VZV) infection, and depicted by fever, viremia, and skin affected with distributed vesicular lesions.[1] VZV is of the genus Varicellovirus.[2]

When immunity to VZV weakens due to aging or immunosuppression, the virus can reactivate and develop herpes zoster (shingles).[3]

Patients with end-stage renal disease (ESDR) are at high morbidity and mortality rates due to their weakened immunity.[4] Therefore, investigating those HDPs is one of the important medical issues that researchers are investigating nowadays.[5] Moreover, the spread of VZV among these immunocompromised patients can be lethal.[6] Those on chronic hemodialysis can acquire kidney transplantation. Thus, following them up is critical to avoid reactivation of the virus. Immunization against varicella can afford long-term protection.[7]

We aimed in the current study to investigate the prevalence of anti-VZV IgG and IgM antibodies in hemodialysis (HD). Moreover, we tested them for the presence of viral DNA to determine possible reactivation status. Therefore, we will test the hypothesis of silent reactivation in asymptomatic host defense peptides (HDP).

Methods. This study was carried out between November 2023 and February 2024 at Tibah University, Al Madina Al Munawara, Saudi Arabia. This study was approved by the Institutional Review Board National. The study has been conducted following the principles of the Helsinki Declaration. A 5 ml blood were taken and then the serum was separated. Informed consent was obtained from all the subjects. Sera were then separated and assayed on the same date of collection.

Enzyme linked immunosorbent assay for the detection of IgG and IgM antibodies. Both anti-VZV IgG and IgM antibody levels were measured for the HDP, whereas for HC only anti-VZV IgG antibody levels were measured. The assay was then performed using a fully automated system according to the manufacturer's instructions. The process was briefly 100 µL per well of diluted (1:100) samples, and undiluted controls were added to the plate. Following the addition, of the diluted samples and controls, the plates were incubated for 45 minutes at 37°C. After the incubation, the plates

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were washed four times, and an addition of 100 µL of conjugate (either IgG or IgM) was to each well, and then the plates were incubated again for 45 minutes at 37°C followed by washing the plates four times. Finally, the substrate was distributed at 100 µL /well. Following 15 minutes, the reaction was stopped with 100 µL of stop solution. The absorbance (O.D.) was read at 450/620 nm within 30 minutes.

Quantitative real-time PCR (qRT-PCR) of VZV. We used the VZV PCR Kit, and QIAamp® DNA Mini Kit (QIAGEN). For detailed assay protocol, it is recommended to visit the manufacturer's website.

Statistical analysis. We analyzed the collected data using SPSS Statistics for Windows version 20.0 (IBMCorp, Armonk, NY, USA). Released 2011 was used in the analysis. Descriptive statistics for continuous data are presented as mean ± (SD) and median. Categorical variables are presented as frequencies (percentages). Fisher's exact test was used to assess the associations between 2 categorical variables. The normality of the distribution of all continuous variables was assessed using the Shapiro-Wilk test. Mann-Whitney test was used to compare medians of reactive and non-reactive groups. Simple linear regression analysis was used to discover factors associated with IgG levels in HDP. A significance level of 95% was used in this study.

Results. The total number of participants included in this study was 265 (174 patients on HD and 91 healthy controls) (Table 1). Most patients were >50 years old (n=78, 96.3%), whereas only 3 HCs (3.7%) were within this age group. Causes of renal failure reported among patients were hypertension (82.8%), diabetes mellitus (34.5%) and both hypertension and diabetes mellitus (23.6%). Mean anti-VZV IgM levels among patients was 0.06 (0.09) (median: 0.04 [0.02-0.07]), whereas mean anti-VZV IgG levels was 2.60 (1.26) (median: 2.77 [1.73-3.31]).

Measurement of anti-VCZ IgG and IgM antibodies among HDP. Median IgG concentration was significantly higher among HDP compared to HC 9.03 (6.26-11.9) versus (vs.) 5.35 (2.63-8.37), p=0.001.

A significantly higher proportion of HDP included in this study have positive immune response compared to healthy participants (79.1% of HC have positive immune response compared to 20.9% with negative immune response; 92% of HDP have positive immune response compared to 8% with negative immune response, p=0.005). Additionally, a significantly lower proportion of HDP reported that hypertension and diabetes mellitus are the cause of renal failure and have a positive immune response compared to patients with no hypertension and diabetes mellitus (25.6% vs. 74.4%, p=0.042). Detailed data concerning the association between sample characteristics and biomarkers in relation to IgG status among HDP are presented in Table 2. Data indicated that only three HDP had positive IgG and IgM levels (1.9%, n=3), while 14 patients (100%) had negative IgG and IgM levels.

Associations of anti-VCZ IgG concentration in HDP and different variables. Different variables such as age, gender, and cause of renal failure are associated with IgG concentration. Thus, we performed a simple linear regression analysis.

The analysis indicated that age, gender, cause of renal failure, albumin, Hg, and random glucose level were not associated with IgG concentration in HDP (Table 3).

Table 1 - Hemodialysis patients and healthy controls characteristics.

Variables	Hemodialysis patients (n=174)	Healthy controls (n=91)	Total (n=265)
Age group			
18- 30 years	8 (4.60)	29 (31.9)	37 (14.0)
31- 40 years	16 (9.20)	27 (29.7)	43 (16.2)
41- 50 years	72 (41.4)	32 (35.2)	104 (39.2)
>50 years	78 (44.8)	3 (3.30)	81 (30.6)
Gender			
Male	107 (61.5)	48 (52.7)	155 (58.5)
Female	67 (38.5)	43 (47.3)	110 (41.5)
Cause of renal failure[§]			
Hypertension	144 (82.8)	-	144 (82.8)
Diabetes mellitus	60 (34.5)		60 (34.5)
Hypertension+ Diabetes mellitus	41 (23.6)		41 (23.6)
Others[†]	11 (6.32)		11 (6.32)

Numbers presented in table are frequencies (percentages). [§]total number of observations is not equal to 174. [†]Other causes of renal failure include kidney stones, systemic lupus erythematosus, glomerulonephritis, renal hypoplasia.

Discussion. Patients affected by ESRD show immune dysfunction pretentious by immunodepression which contributes to the high incidence of infections they are at high risk of reactivation. Most people experience primary infection as varicella in infancy and would stay latently infected, with scarce reactivation in a stage of shingles.[8] In the current study, we found a significantly higher seroprevalence rate (92% vs 79.1%) and higher median antibody levels (9.03 vs 5.35) VZV IgG in HDP compared to controls. These findings indicate previous VZV exposure and viral replication in the HDP group. That was most likely due to more frequent healthcare contact and impaired cell-mediated immunity (CMI) associated with renal failure. A study similar to ours in Riyadh, Saudi Arabia found 85% of HDP were VZV IgG positive compared to 92%

Table 2 - Association between sample characteristics and biomarkers in relation to IgG status among hemodialysis patients (N=174).

Variables	Non-reactive IgG <1.6 mg/dl (n=14)	Reactive IgG ≥ 1.6 mg/dl (n=160)	*P*-value
Age group, n (%)			
18- 30 years	1 (7.14)	7 (4.38)	0.070
31- 40 years	4 (28.6)	12 (7.50)	
41- 50 years	4 (28.6)	68 (42.5)	
>50 years	5 (35.7)	73 (45.6)	
Gender, n (%)			
Male	9 (64.3)	98 (61.3)	1.000
Female	5 (35.7)	62 (38.8)	
Cause of renal failure, n (%)			
Hypertension, yes	10 (71.4)	134 (83.8)	0.267
Hypertension, no	4 (28.6)	26 (16.3)	
Diabetes mellitus, yes	2 (14.3)	58 (36.3)	0.142
Diabetes mellitus, no	12 (85.7)	101 (63.1)	
Hypertension+ Diabetes mellitus, yes	0 (0.00)	41 (25.6)	0.042*
Hypertension+ Diabetes mellitus, no	14 (100)	119 (74.4)	
Others, yes[§]	2 (14.3)	9 (5.63)	0.217
Others, no	12 (85.7)	151 (94.4)	
	Biomarkers		
Albumin, g/dl, mean ± SD Median (IQR)	3.54 ± 0.29 3.60 (3.20- 3.80)	3.58 ± 1.21 3.50 (3.30- 3.73)	0.543
Hemoglobin, g/dl, mean ± SD Median (IQR)	10.7 ± 1.55 10.8 (0.05- 11.8)	11.0 ± 1.59 11.0 (10.1- 11.8)	0.531
Random glucose, mg/dl, mean ± SD Median (IQR)	134 ± 21.0 132 (117- 148)	138 ± 27.4 130 (119- 166)	0.604

[§]Other causes of renal failure include kidney stones, systemic lupus erythematosus, glomerulonephritis, renal hypoplasia. Fisher's exact test and Mann-Whitney test were used to obtain data presented in table. SD: standard deviation, IQR: interquartile range, IG: immunoglobulin

Table 3 - Simple linear regression analysis of associations of IgG ratio in hemodialysis patients (N=174).

Variables	Beta	Standard error	*P*-value	95% Confidence Interval	R-square
Age	0.03	0.03	0.284	-0.02 to 0.08	0.01
Gender	-0.81	0.66	0.224	-2.12 to 0.50	0.01
Cause of renal failure					
Hypertension	0.46	0.85	0.593	-1.23 to 2.14	0.00
Diabetes mellitus	0.76	0.68	0.262	-0.58 to 2.10	0.01
Hypertension+ Diabetes mellitus	1.34	0.75	0.077	-0.15 to 2.83	0.02
Others	0.19	1.33	0.887	-2.43 to 2.81	0.00
Biomarkers					
Albumin, g/dl	-0.14	0.28	0.618	-0.69 to 0.41	0.00
Hemoglobin, g/dl	-0.13	0.20	0.54	-0.53 to 0.28	0.00
Random glucose, mg/dl	0.01	0.01	0.490	-0.02 to 0.03	0.00

SD: standard deviation, IQR: interquartile range, IG: immunoglobulin

of HC. 9 An estimated 0.2% experience the clinical presentation of reactivation to 2% of the population, most of whom are elderly adults.

The onset of HZ is thought to be linked to a weakness in CMI. Therefore, reactivation of the latent VZV would be increased.[10] More than 95% of immunocompetent persons aged over fifty years are expected to get infected with VZV and are therefore at risk of developing HZ. As a result of age-dependent reductions in VZV-specific CMI, immunosuppressive treatment, and underlying medical conditions would be connected to reactivation.[11]

Our lower seroprevalence may reflect geographical differences in viral circulation. The control group has low seroprevalence may be due to lower infection as previously known due to improved health care in the healthy group and better infection control measures.

Surprisingly, HDP with both hypertension and diabetes showed lower VZV seropositivity (25.6%) than those without (74.4%), despite having multiple immune-weakening comorbidities.[12]

We showed that a significantly higher seropositive proportion of HDP (92%) was included in this study compared to HC (79.1%). Only 1.9% (n=3) of HDP demonstrated a probability of reactivation or new infection of VZV infection per positive IgM. This aligns with known VZV pathogenesis of latency after primary infection until later reactivation as herpes zoster. 13 Interestingly, those IgM-positive patients also showed detectable viral DNA for VZV in their samples. Therefore, they most likely had developed a condition of reactivation. Monitoring and detecting the VZV DNA is of impending importance for diagnosis and clinical management of VZV-infected patients especially in those with immunosuppressed conditions such as HDP.[14]

Because none of the patients displayed varicella or zoster, it was probable that asymptomatic reactivations of VZV happened to those patients. Infection control during dialysis may limit VZV transmission. Thus, vaccination against zoster is linked to recalling CMI to VZV to combat zoster from happening.[15] Thus, insisting patients on chronic hemodialysis get vaccinated is one of the most efficient tools to avoid the state of reactivation.

These findings support vaccination against VZV earlier in chronic kidney disease before starting dialysis when immunity is more intact. The 8% of HDP still VZV-seronegative are an important target population for routine screening and vaccination. Pre-transplant vaccination is especially critical to prevent disseminated VZV infection with subsequent immunosuppression. The increased VZV antibody levels we observed also raise concerns about waning immunity over time in HDP. Periodic VZV IgG monitoring could identify patients needing booster vaccination.

Study limitations. This study include a lack of vaccination history and one Saudi region-based study. Additionally, small healthy control samples compared to the patients was another limitation.

In conclusion, we found significantly higher VZV seroprevalence and IgG antibody levels in HDP, indicating greater viral exposure and replication compared to HC. Most importantly, those with IgM seropositive status showed a case of asymptomatic reactivation, which would be a potential source of infection and increase their possibility of developing HZ condition. Furthermore, our study added application values to the decision-makers in terms of revising the infection control measures used in hemodialysis centres and considering serological and molecular methods to screen and check the presence of different VZV markers.

Little is known regarding VZV reactivation in asymptomatic HDP. Thus, conducting such a study was of clinical importance to avoid the consequences of reactivation. Our study emphasizes the importance of paying sufficient attention to the possible occurrence of silent reactivation of VZV in HDP. Therefore, these patients are at high risk of developing reactivation due to their impaired immunity. Additionally, future investigations should be conducted to avoid such a medical problem. Furthermore, a higher diffusion of VZV vaccination should be promoted by nephrologists in these HDPs particularly those with future transplant chances.

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