Molecular Screening of KI and WU Polyomaviruses among Patients with Chronic Kidney Disease and Urinary Tract Infections

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Abstract

KIPyV and WUPyV were recognized based on commercial standard and PCR techniques. To occurrence of KIPyV and WUPyV DNA among chronic kidney disease (CKD) and UTI patients using VP2 gene for detection of KIPyV and VP1 gene for WUPyV. Molecular assay results of this study revealed the presence of KIPyV in 6 (5.3%) of CKD patients and 3 (2.7%) of UTI patients while the presence of WUPyV was 1 (0.88%) in CKD patients and negative result in UTI patients. WUPyV DNA and KIPyV DNA was not detected in plasma of healthy persons. The distribution of KIPyV and WUPyV according to gender among CKD showed a no significance difference among both sexes in which (*P value* = 0.27) and (*P value* = 0.36) respectively while the distribution of KIPyV and WUPyV among urinary tract infections showed a no significance difference among both sexes in which (*P value* = 0.13) and (*P value* = 0.79) respectively .The current study survey about both viruses show that female was higher than male in KIPyV in which 2 (3.22%) of KIPyV DNA were detected in male and 4 (7.84%) in female out of 113 (100%) among chronic kidney disease patients while in urinary tract infection, patients revealed a higher percentage rate of KIPyV in female than male in which 0 (0%) of KIPyV DNA were in male and 3 (4.54%) in female out of 111 (100%) while doesn't detect any isolate of WUPyV in both sexes.

Keywords: *KIPyV, Chronic kidney disease, WUPyV, Urinary tract infections.*

Introduction

Polyomaviruses are another type of emerging pathogens that generally cause infection to the urinary tract of humans [(1)]. Primary infection of polyomavirus happens in childhood and it persists for the whole life of the persons, especially in the epithelial cells of the kidneys and urinary tract as well as leukocytes in the blood [(2)] . In the secretions of children with acute respiratory symptoms using high-throughput sequencing technologies, two forms of polyomaviruses have recently been identified, one known as Karolinska Institute Polyomavirus (KIPyV) and the other named Washington University polyomavirus (WUPyV) [(3)].

The site of persistent infection of these two viruses across the lifespan still unclear [(4)] . These two viruses are closely related to each other than to SV40, BK and JC, but, as shown by the amino acid identity and phylogenetic analysis ratio, they vary greatly from each other [(5)] . Seroepidemiological surveys have shown that the initial infection with KIPyV and WUPyV that occurs early in life could be via the respiratory and oral-fecal pathways, similar to BK and JC polyomaviruses [(6)] . A high rate of co-infection with other major respiratory viruses, such as influenza viruses, parainfluenza viruses, adenoviruses, respiratory syncytial viruses, rhinoviruses,

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human coronaviruses, human bocaviruses, and human metapneumoviruses is typically associated with the identification of the genome of these two viruses in respiratory tract samples [7] .

In the view of the above mentioned introduction, the design of this study to determine the presence of KIPyV and WUPyV in patients suffering from UTI and CKD in AL-Najaf city-Iraq.

Materials and Method

A total number of 224 clinical samples were collected from patients who suffer from chronic kidney disease (CKD) and urinary tract infections (UTI) at age range (15-80 years) and 100 healthy persons as controls at age range (25–65) years. All subjects were admitted to one of the biggest Hospital including Al-Sadr Medical City as well as some chief clinical laboratories in Al-Najaf City/Iraq within the time two months started in January 2020. The patients in this study included 107 males and 117 females while healthy controls was 50 males and 50 females. Blood plasma were collected from each participant was used to extract DNA for detection of KIPyV and WUPyV.

The complete genomic DNA of 113 patients with CKD and 111 of patients with UTI was extracted employing a traditional kit of the entire genomic DNA extraction (iNtRoN, Biotech. Inc., Korea), wheresoever, the extraction was performed based on the guidance of manufacture corporation.The nucleic acid was conserved under -20^{o}C state using the deep freezing device, the PCR technique was employed to examine and detect all the genes described in table (1) that suggested by Gozalo-Margüello *et al*.(2015)[8]. The process of gel document (Cleaver, United Kindom), demanded to check and distribute the migration of PCR bands applying 1% agarose (iNtRoN, Biotech. Inc., Korea), back dyeing the gel with ethidium bromide at 0.5 µg/ml concentration.

Table (1) Primers of viral detection

Virus	Primer	Sequence (5' - 3')	Product size bp
KIPyV	VP2-F	CGTCATACTTGCCCGAGTTG	378-bp
	VP2-R	CATCTTTGGGCAGGCTTGAA	
WUPyV	VP1-F	GCCGTACCACTGTCAGAAGA	546-bp
	VP1-R	TCTGCAGTTATCATTGCGGC	

Statistical Analysis

The SPSS V.24 program was used in statistical analysis of the data. Chi- Square was used to compare and extract duplicates and percentages

Result and Discussion

This study revealed that WUPyV and KIPyV can be detected in both plasma samples from UTI and chronic kidney disease patients , but these viruses were not found in plasma of healthy blood donors. A higher prevalence of KIPyV was observed compared with WUPyV (9/10 vs. 1/10).

Detection of KI and WU polyomavirus in plasma of both groups occurred by using sensitive molecular techniques which include conventional PCR as showed in figure (1). To our knowledge, there is limited studies have been published on KIPyV and WUPyV using conventional PCR in UTI and chronic kidney disease

patients and this considered the first study that conducted locally . Co-infection with other pathogens has been reported in 74% of KIPyV patients, 68% to 79% of WUPyV patients, and 10% of KIPyV and WUPyV co-infections in the absence of other respiratory viruses [9]. In this study, we investigated the KIPyV and WUPyV in the absence of respiratory tract infections.

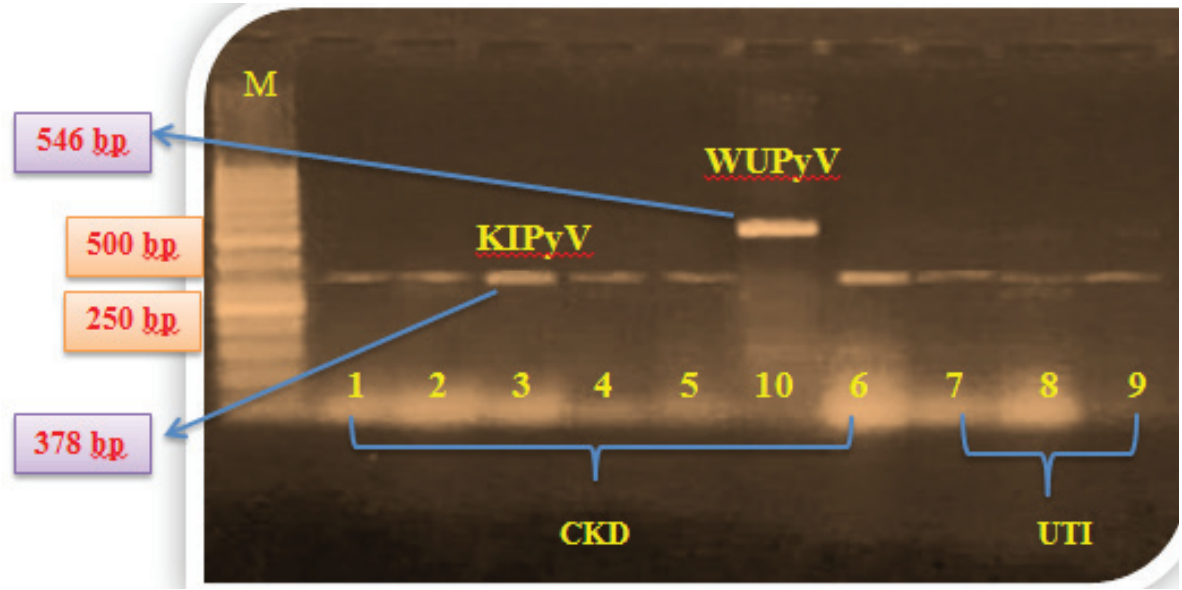

Figure 1. Agarose gel electrophoresis image that show the PCR product analysis of KIPyV VP2 gene and WUPyV VP1 gene in CKD and UTI samples. PCR product was analysis by 1% agarose gel . Where M: marker (50bp – 1000bp), lane (1,2,3,4,5) showed positive bands to KIPyV VP2 (378 bp) in CKD samples, Lane(10) represent positive band to WUPyV VP1 (546 bp) in CKD samples, while lane (7,8,9) represent positive bands to KIPyV VP2 (378 bp) in UTI samples.

Neither WUPyV DNA nor KIPyV DNA was detected in plasma of healthy persons in the current study as shown in Table (2). This results is similar to Csoma *et al*., (2011)[10] which dosent detect these virus in healthy subjects. It also in line with recent study conducted by Kamminga *et al.,* (2019)[11] which detected only one of WUPyV DNA in healthy blood donors but contrast to Šroller *et al.,* (2015)[12] which revealed about 58% of KIPyV in serum of healthy blood donors as well as to Song *et al*., (2016)[13] which reported 12.5% of KIPyV VP1 DNA in blood specimens from healthy individuals. The role of the KIPyV and WUPyV in the pathogenesis of the urinary tract and their effect on kidney function is not well explained. Some investigations have shown the presence of both these viruses in asymptomatic people [14,15].

Prevalence of KIPyV and WUPyV in study groups

The KIPyV DNA was detected in (6 out of 113) or (5.3%) in patients with chronic kidney disease, and (3 out of 111) or (2.7%) in patients with urinary tract infection while in control group was (0 out of 100). There was no significant difference (Chi-square = 3.5 ; P value = 0.16) among study groups (CKD patients, UTI patients and control group) as shown in Table (2)

Table(2). Prevalence of KIPyV and WUPyV in study groups

Viruses		Control Group (N= 100)	UTI Patients (N= 111)	CKD Patients (N= 113)	Chi Square P value
KIPyV	Positive	0 (0%)	3 (8.20%)	6 (5.3%)	3.5 0.16 NS
	Negative	100 (100%)	108 (91.8%)	107 (94.7%)	
WUPyV	Positive	0 (0%)	0 (0%)	1 (0.8%)	0.77 0.67 NS
	Negative	100 (100%)	111 (100%)	112 (99.2%)	

The present study detected 6 (5.3%) out of 113 (100%) of KIHPyV DNA in chronic kidney disease patients. These result is similar to Csoma *et al*., (2011) [10] which detect (7/195) to KIHPyV in plasma of renal transplant patients and to Csoma *et al*., (2014)[16] which revealed the occurrence of KIHPyV in plasma of renal transplant patients also to Badura *et al*., (2014)[17] and Hansen-Estruch *et al*., (2018)[18]. On the other hand the present result is incompatible to Porrovecchio *et al*., (2013)[19] which seen that All plasma samples from transplant patients were negative for KIPyV DNA. The present study revealed about 3 (2.7%) out of 111 (100%) were positive to KIHPyV DNA in plasma of urinary tract infection patients. These result is similar to csoma *et al*., (2012)[20] which detected KIPyV DNA in two plasma samples from non-pregnant women out of 200 plasma samples and to Csoma *et al*., (2015)[21] but is unsimilar to Limam *et al.,* (2020)[22] which not detected any KIPyV out of 112 from Tunisian patients.

The WUPyV DNA was detected in (1 out of 112) or (0.88%) in plasma of patients with chronic kidney disease and (0 out of 111) in plasma of patients with urinary tract infection while in control group was (0 out of 100). There was no significant difference for WUPyV detection and distribution (Chi-square = 0.77 ; P value = 0.67) as shown in table (2) . The current study detected 1(0.88%) out of 113 (100%) of WUPyV DNA in plasma of chronic kidney disease patients while dosent detect any isolate of WUPyV in plasma of urinary tract infections patients. The result of both groups is compatible to with Csoma *et al*., (2011)[10] which detect 0.8 of WUPyV in blood from renal transplant patients as well as to csoma *et al*., (2012)[20] which dosent detect WUPyV DNA in plasma of pregnant and non- pregnant women as well as to Limam *et al*., (2020)[22] which not detected WUPyV out of 112 from Tunisian patients but is incompatible to Csoma *et al*., (2014) which record from 9.1% and 5.3% of WUPyV in plasma samples in addition to Aghamirmohammadli *et al*., (2020)[4] which revealed 1.5% of WUPyV in children under 5 years of age in Tehran, Iran. The low detection of WUPyV in samples from chronic kidney disease and UTI groups may be related to the seasonal distribution and environmental conditions which are important for WUPyV transmission.

Table (3) show the differences in the detection of KIPyV and WUPyV DNA by conventional polymerase chain reaction (PCR) among CKD patients classified by gender. This table shows the positive KIPyV DNA was detected in (7.84 %) and (3.22 %) for female and

male patients respectively . There was no significant difference (Chi-square = 1.19 ; *P* value = 0.27) among male and female according to KIPyV detection and distribution. These result is contrast to prezioso *et al*., (2019)[23] which detected KIPyV in 12/31 thalassemic. patients (6 females/6 males) as well as incompatible to Al-Obaidi *et al.,* (2018)[24] which reported 27 (43.55) in male and 4 (44.44) in female of JCPyV in patients with kidney transplantation.

The same table shows the positive WUPyV DNA was detected in (0%) and (1.61 %) for female and male patients respectively. There was no significant difference for WUPyV detection and distribution (Chi-square = 0.83 ; P value = 0.36) . These result is similar to Sharp *et al*., (2009)[25] which recorded only one of WUPyV from a 41-year-old man but un similar to Neske *et al*., (2010) [26] which reported 49.4% of WUPyV were in male and 46.3%of KIPyV in female in plasma of German blood donors. The plasma viral loads of KIPyV and WUPyV in both gender with this disease might be detectable and their impact on the kidney remains to be determined

Table(3). Prevelance of KIPyV and WUPyV in chronic kidney disease patients according to gender .

		CKD patients		Chi Square P value
		Male	**Female**	
KIPyV	Postive	2 (3.22%)	4 (7.84%)	1.19 0.27 NS
	Negative	60 (96.7%)	47 (92.1%)	
Total		62 (100%)	51 (100%)	
WUPyV	Postive	1 (1.61%)	0 (0%)	0.83 0.36 NS
	Negative	61 (98.3%)	51 (100%)	
Total		62 (100%)	51 (100%)	

Table (4) show the differences in the detection of KIPyV and WUPyV DNA by conventional polymerase chain reaction (PCR) among UTI patients classified by gender . This table shows the positive KIPyV DNA was detected in (4.54 %) and (0 %) for female and male patients respectively while dosent detect any isolate of WUPyV in both male and female. There was no significant difference (Chi-square = 2.23 ; P value = 0.13) among male and female according to KIPyV detection and distribution nor for WUPyV detection and distribution (Chi-square = 0.07 ; P value = 0.79). These result is similar to Bialasiewicz *et al*., (2009)[27] which dosent detect WUPyV from different biological samples include blood, urine, and CSF. The high rate of KIPyV among female in UTI group may due to the physiology

of genital tract in female and the incidence of bacterial infection that possibly regard the risk factor for infection with KIPyV .

Table(4). Prevalence of KIPyV and WUPyV in urinary tract infections patients according to gender

		UTI patients		Chi Square P value
		Male	Female	
KIPyV	Postive	0 (0%)	3 (4.54%)	2.23 0.13 NS
	Negative	45 (100%)	63 (95.4%)	
Total		45 (100%)	66 (100%)	
WUPyV	Postive	0 (0%)	0 (0%)	0.07 0.79 NS
	Negative	45 (100%)	66 (100%)	
Total		45 (100%)	66 (100%)	

NS : Non-significant

Conclusions

The study describes the prevalence of these two viruses in patients with CKD and UTI without respiratory symptoms in Iraq, which demonstrates how these two viruses can be applied as part of virological screening when suspected of etiology of the viral urinary tract. and indicate that a candidate respiratory pathogen, KIPyV and WUPyV in the urinary tract can also be identified.

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