Human Washington University Polyomavirus in Patients with Respiratory Tract Infection in Kuwait

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Significance of Study

- In this study performed in Kuwait, circulating genotypes of the Washington University (WU) polyomavirus found among hospitalized patients with viral respiratory tract infections (RTI) were type Ia and IIb. Knowledge on the role of these circulating WU polyomavirus genotypes in RTI patients is still indefinable, and this article can contribute to the advancement in this field and be a starting point for future studies.

Keywords

Phylogenetic analysis · Respiratory tract infections · Washington University polyomavirus

Abstract

Objective: To determine Washington University (WU) polyomavirus strains circulating among hospitalized patients with respiratory tract infections (RTI) in Kuwait. **Materials and Methods:** Samples from 459 hospitalized children and adult RTI patients were screened for respiratory viruses by polymerase chain reaction from April 2013 to April 2016. The VP2 gene from WU virus (WUV)-positive samples was sequenced and subjected to phylogenetic analysis. **Results:** Of the 459 hospitalized RTI patients, 18 (3.9%) patients were positive for WUV infection. WUV infection was common among children aged ≤ 11 years (9 patients, 50%). Among the 18 WUV-infected hospitalized patients, viral co-infection was detected in 9 patients (50%). The most common viruses associated with mixed infection were respiratory syncytial virus and human rhinovirus (2 patients, 11.1% each). Of the

18 WUV-infected patients, 4 were sequenced and subjected to phylogenetic analysis. The circulating strains belong to type Ia and IIb. **Conclusion:** This study enabled us to detect WUV among hospitalized RTI patients. Co-infection with other respiratory viruses was notable. Two circulating WUV genotypes (Ia and IIb) were identified among hospitalized RTI patients in Kuwait.

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Introduction

Although viruses are the major cause of respiratory tract infections (RTI), the etiology of many suspected RTI cases remains unknown. However, since 2001, several human viruses have been identified from respiratory samples using various molecular methods [1–6], e.g., human metapneumovirus [1], human coronavirus [2], human bocavirus [3], and the human KI polyomaviruses and the human Washington University virus (WUV) [4–6].

These viruses were initially isolated from individuals with respiratory tract diseases, most of whom were children. Newly discovered viruses such as WUV [4–6] might be responsible for many respiratory tract illnesses whose cause has remained a mystery for decades.

The WUV is a novel member of the genus of polyomavirus in the polyomaviridae family. It is a double-strand deoxy nucleic acid (DNA) virus with nonenveloped and icosahedral capsid [7]. Two human polyomavirus species called BK and JC viruses were first identified in 1971 from the urine sample of a kidney allograft recipient with chronic pyelonephritis and advanced renal failure [8] and from the brain sample of a patient with progressive multifocal leukoencephalopathy [9]. The KI virus was detected in nasopharyngeal aspirates and feces from RTI patients in Sweden [4]. Also, Gaynor et al. [5] described the detection and molecular characterization of WUV in patients with acute RTI.

Previously, the role of newly discovered viruses was studied in hospitalized patients with RTI using highly sensitive molecular techniques [10]. In this article, the phylogenetic analysis indicates that the circulating WUV genotypes among patients with viral RTI in Kuwait belong to Ia and IIb.

Materials and Methods

Study Area and Study Population

A total of 459 hospitalized children and adult patients with RTI were screened for WUV during a 3-year period from April 2013 to April 2016 in respiratory samples collected from the Mubarak Al-Kabeer Hospital, Kuwait.

Nasopharyngeal aspirates were sampled from patients with RTI admitted to medical wards, intensive care unit (ICU), or pediatric ICU (PICU). Samples were delivered to the Virology Laboratory (Department of Microbiology, Faculty of Medicine, Kuwait University) within 2 h to decrease possible degradation of the viral nucleic acid. Ethical permission to perform this research study was granted by the Health Science Center and the KIMS (Kuwait Institute for Medical Specialization) Joint Committee on the Protection of Human Subjects in Research. Clinical data were obtained from the patients' medical records.

Amplification of the WUV VP2 Typing Region and Phylogenetic Analysis

Molecular detection of respiratory viruses including WUV was performed as described earlier [10]. Genomic DNA was extracted from WUV-positive samples and stored at -80°C . Polymerase chain reaction (PCR) was carried out as follows: 3 μL of the extracted DNA were mixed with 12.5 μL of PCR mix that was composed of high-fidelity AmpliTaq Gold DNA Polymerase, 250 U (0.625 U/ μL), GeneAmp PCR Gold Buffer, 15 mM Tris/HCl, pH 8.05, 50 mM KCl, dNTP, 200 μM each, 2.5 mM MgCl_2 (Applied Biosystems, Foster City, CA, USA), and 0.4 μM each of WUV

Table 1. Demographic and clinical features of patients infected with Washington University polyomavirus

	Patients, <i>n</i>
Sex	
Male	9 (50%)
Female	9 (50%)
Age	
≤11 years	9 (50%)
12–30 years	3 (16.7%)
>30 years	6 (33.3%)
Median age, years	13
Hospital admission	
Medical ward	12 (66.7%)
Intensive care unit	1 (5.6%)
Pediatric intensive care unit	5 (27.8%)
Diagnosis	
Upper respiratory tract infection	7 (38.9%)
Lower respiratory tract infection	11 (61.1%)
Nationality	
Kuwaiti	10 (55.6%)
Non-Kuwaiti	8 (44.4%)

primers [11]. The primer sequences were as follows: WUT forward 5'-GGTACTCCCCATTATGCAGCC-3' and WUT-reverse 5'-GGTTGGAGGGGCTGCAA-3'. PCR amplification was carried out as described previously [12].

The samples were analyzed using sequencing analysis software (v 3.7; Applied Biosystems, Foster City, CA, USA). The WUV sequence alignment was performed with sequences that were present in the GenBank database (under accession Nos. EU711058, EU711057, EU711056, EU711055, EU711054, FJ890982, FJ890981, NC_009539, EU358769, EU358768, EU296475, EF444554, EF444553, EF444552, EF444551, and EF444550). The best available nucleotide substitution model was chosen using the Find-Model application [13]. Epidemiological classification of WUV types was carried out as described before [11].

The evolutionary history was established using the neighbor-joining method [14]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) were displayed next to the branches [15]. Bootstrap values <50% were masked. The tree was made to scale, with branch lengths in similar units as those of the evolutionary distances used to complete the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and were in the units of the number of base substitutions per site. Evolutionary analyses were conducted using Molecular Evolutionary Genetics Analysis version 5 (MEGA5) [16, 17].

Statistical Analysis

A descriptive statistical analysis using SPSS (version 20.0; SPSS, Chicago, IL, USA) was performed initially to identify the characteristics of the study population. Statistical analysis was done by χ^2 test for categorical variables. Statistical significance was set at a value of $p \leq 0.05$.

Fig. 1. Black circles represent Washington University polyomavirus (WUV) strains obtained in this study. A phylogenetic tree was constructed for each of the 3 WUV types using the sequence of the VP2 region of WUV isolates from Kuwait. The numbers next to the branch points of the tree are bootstrap values (1,000 replicates).

Results

Of the 459 hospitalized children and adult RTI patients (351 children and 108 adults), 18 (3.7%) patients (9 males and 9 females) were diagnosed with WUV infection. Their age ranged from 1 month to 73 years. Of the 18 WUV-infected patients, 10 (56%) were Kuwaiti, and 8 (44%) were non-Kuwaiti (Table 1).

The 18 cases of WUV infection were distributed among various age groups; children aged ≤ 11 years: 9 (50%), 12–30 years: 3 (16.6%), and >30 years: 6 (33.3%). The median

age of the patients infected was 13 years. No significant difference was observed regarding the age of patients infected with WUV ($p > 0.05$). Among the 18 patients infected with WUV, 12 (66.7%) were admitted to medical wards and 6 (33.3%) to ICU and PICU. No significant difference in prevalence was noted between WUV-infected patients admitted to medical wards and ICU and PICU ($p > 0.05$). Nine WUV-infected patients (50%) were co-infected with both respiratory syncytial virus and human rhinovirus, followed by human bocavirus, human polyomavirus, HCoV-229E (2 patients, 11.1% each), and 1 pa-

Fig. 2. Seasonal distribution of respiratory tract infections in hospitalized patients positive for Washington University virus from April 2013 to April 2016.

tient was co-infected with influenza virus A (5.6%). Lower respiratory tract infections (LRTI) (pneumonia and bronchopneumonia) were detected in 11 hospitalized patients with WUV infection (61.1%), and upper respiratory tract infection (URTI) (bronchitis) was detected in 7 patients (38.9%). All 9 WUV-infected children (aged ≤ 11 years) were diagnosed with LRTI. No significant difference in prevalence was noted between URTI and LRTI patients with WUV infection ($p > 0.05$). Also, the incidence of co-infection was statistically insignificant between LRTI and URTI patients ($p > 0.05$).

Four genomic WUV DNAs were sequenced from the 18 WUV-positive samples, and phylogenetic trees were constructed (Fig. 1). As can be seen from Figure 1, 3 of the circulating strains belong to type Ia and the 4th belongs to type IIb. The circulating WUV strains among Kuwaitis belong to the type Ia and among non-Kuwaitis (Indians) to IIb. The Indian patient is a resident of Kuwait for the past 10 years and did not leave Kuwait for the past 6 months, so the WUV strain IIb is a circulating strain in Kuwait.

WUV was detected throughout the year except for March and April. The highest incidence (27.8%) was detected during the month of February (Fig. 2).

Discussion

This study investigated the circulating WUV strains among hospitalized patients with viral RTI in Kuwait. WUV was identified in 3.9% of the hospitalized patients

with RTI from 2013 to 2016, which is similar to previously reported data [10]. In other studies, the WUV detection rate reported in respiratory samples ranged from 2 to 34.9% [5, 18–22]. The data from this study support the worldwide distribution of WUV as a respiratory virus.

A high co-infection rate (50%) was noted among WUV-infected patients in the current study. Notably, similar results were obtained by other studies with a co-infection rate extending from 47 to 83% [6, 19, 20–22]. On the other hand, 9 hospitalized patients with a single WUV infection (50%) were diagnosed with URTI or LRTI [19]. Although this study established the presence of the WUV as a single viral infection in 50% of the infected patients, the pathogenic potential of WUV in respiratory tract diseases remains unanswered due in part to high co-infection rates with other viruses and a deficiency in definite clinical correlations [24, 25]. Co-infection would be an important area of further research to explore the underlying clinical patterns of mixed infections with other viruses.

From the 18 WUV-positive samples, only 4 genomic WUV DNAs were sequenced and phylogenetic trees constructed. This was probably due to the inconsistent collection of specimens or low viral load. Pneumonia and bronchopneumonia were more common (61%) than URTI (39%) among WUV-infected patients. Comparable results were reported among WUV-infected infants and young children from China [26].

Our data demonstrated that positive cases were detected throughout the year (except for March and April), with the highest peak in February (Fig. 2). In related studies, WUV infection has been identified in winter and early spring or summer [5, 27, 28], while research from Thailand showed no seasonal distribution [29]. In China, the bulk of positive cases were detected in autumn and winter, with a sharp peak in November [26]. A more recent study from Shenzhen in China reported high peaks from March to May [30]. A full assessment of incidence, seasonality, and age distribution, mainly centered on epidemiological data, will be essential for medical decision-making, particularly decisions associated with the prescription of antiviral treatments, restriction of antibiotic therapy, and application of infection control policies.

Conclusion

The WUV was detectable among RTI patients, especially those diagnosed with bronchitis, pneumonia, and bronchopneumonia. Mixed viral infections with WUV

were noticeable. Two genetic WUV lineages are circulating in Kuwait. To elucidate the role of WUV in RTI and to provide extensive knowledge on the genetic diversity of this virus, future investigations should be in case-control studies including larger patient cohorts.

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