

Detection and clinical characterization of WU polyomavirus with acute respiratory tract infection in children, Guangdong of China

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Background: In 2007, Gaynor et al. discovered a previously unidentified human polyomavirus in respiratory secretions obtained from human patients with symptoms of acute respiratory tract infection. This new virus was designated WU polyomavirus. To investigate the frequency of its infections in Guangdong, China, we sought to describe the detection and clinical characterization of WU Polyomavirus with infection in children.

Methods: From July 2008 through June 2009, nasopharyngeal aspirates were collected from 771 children who were hospitalized with acute lower respiratory tract infection in Second Affiliated Hospital of Shantou University Medical College, and 82 were asymptomatic who visited the well-being clinic. WU Polyomaviruses were detected by using PCR technology and was identified by using DNA sequences. All WU polyomavirus positive specimens were screened for 9 common viruses (influenza A and B; RSV; PIV 1 and 3; human metapneumovirus; human bocavirus; adenovirus; rhinovirus) by using PCR or RT-PCR.

Results: In this study, fifteen of the 771 tested specimens with acute lower respiratory tract infection were positive for WU polyomavirus, the positive rate was 1.95%. and all of the asymptomatic children who visited the well-being clinic were negative. Positive specimens were noted for patients 2 months to 48 months of age, the median age was 18.8 months. Of the 15 WU Polyomaviruses positive patients, 9 (60%) were male, 6 (40%) were female. WU polyomavirus was the sole virus detected in 9 specimens (60%) from patients with acute respiratory tract infection. WU polyomavirus were associated with the coinfection of another respiratory virus in 6 of 15 (40%), most frequently with RSV (n=4), followed by adenovirus (n=1) and rhinovirus (n=1). The most common clinical findings in the patients with WU polyomavirus are cough, fever, wheezing. The most frequent diagnoses were pneumonia (n=8), bronchiolitis (n=4), upper respiratory tract infections (n=2) and bronchitis (n=1). A severe case was complication with viral encephalitis.

Conclusion: We suggest that WU Polyomaviruses may be a respiratory pathogen because WU polyomavirus was the sole virus detected in 9 specimens from patients with respiratory illness and all of the asymptomatic were negative. The most common clinical findings are cough and wheezing. Young children are the main target. And the pathogenetic conditions are generally mild. More comprehensive studies are required to prove these viruses are agents causing respiratory disease.

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Amplification of early genes of human Papilloma Virus targeting nine virus genotypes. Mérida, Venezuela

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Background: Subtyping of Human Papilloma Virus (HPV) by molecular biology tools may enhance cervical cytological assessments information for patient's management and for prognosis of cervical cancer evolution. The aim of this study was to subtype HPV from cervical samples of sexually active women (15-69yr-old) seen in cervic-gyn public clinic (IHAULA) of Merida State, Venezuela.

Methods: DNA-collection device (Digene[®]) were used to collect and transport 250 cervical smears, DNA were isolated using QIAamp[®] DNA Mini Kit (Qiagen[®]) and storage at -20°C until used. A Nested-PCR-Multiplex assay was standardized for amplification of early E6/E7 HPV virus. A first run included amplification of a 630bp-length region of E6/E7 gene and a second run allowed to genotype HPV virus in a multiplex format. Amplicon sizes varied from 151-457 bp for HPV-16,-18,-31,-45; and HPV-33,-6/11,-58,-52,-56. Simultaneously, HPV detection assays were performed using Hybrid-Capture II (Digene[®]) technology and an endpoint PCR assays for L1 and E6/E7 regions.

Results: HPV were detected in 27.2% samples, 94.12% (64/68) were positive for at least one of the genotypes assayed. High risk HPV were identified in 98.44% (63/64) samples; where HPV18 (50/63) was the most common genotype isolated, along with HPV16 (24/63). One or several types were detected in 56.25% (36/64) of the cases, being VPH-18-6/11 (14/64) combination the most common one.

Conclusion: We found a high frequency of cervical infection with high-oncogenic-risk HPV genotypes, especially caused by HPV18, alone or in multiple types infection.

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Development of a new genotyping system for predicting TTV genotype using evolutionary restriction map and artificial neural network

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Background: TT virus is belonging to the family Circoviridae and was discovered in 1997. On the basis of sequence variation in both coding and noncoding regions, many classification systems have been proposed. Several genotyping techniques, including DNA sequence analysis as well as primer specific PCR are used. None of the reported systems was

able to determine the TTV genotype in all major types and the common subtypes on the basis of the TTV sequence. To date, TTV genotypes have been primarily determined by sequence-based PCR products analysis techniques, including direct sequencing. Here we proposed a new simple method for genotyping based on neural network method and restriction map.

Methods: This method classifies genes into groups which are made distinct from each other by evolutionary changes in their restriction site. Our objective was to accurately predict, from complex restriction site patterns, TT virus, by use of artificial intelligence. Neural network models were constructed based on changes at restriction site in the untranslated region of virus. Models were trained, validated, and tested with 330-UTR sequence. A procedure of determining the optimal neural network parameters was proposed to speed up the training processes.

Results: The results suggested that the restriction site map is a more accurate predictor of TTV genotype. To show the ability of the model in genotyping, the internal representations developed by the networks are analyzed using principal component analysis (PCA). This analysis shows that the networks are able to discover relevant features on the basis of the association between the restriction map and the virus genotype.

Conclusion: The proposed predictor shows vigorous clusterization of both predicted and observed TTV genotype. The current study is designed to find the algorithm for genotype prediction using the restriction site information in combination with artificially created neural network. The applied properties for the model must be mighty and low time consumption. After the pattern recognition model is constructed the algorithm can be used to determine the repertory of restriction site properties required for each genotype. This complex can be defined as genotypic pattern. The pattern can be used for further correlation analysis with known and unknown viral sequence.

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Seroprevalence of human cytomegalovirus infection in Singapore

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Background: The human cytomegalovirus virus (HCMV) is a ubiquitous *betaherpesvirus*, common in all human populations, with seroprevalence of 80-100% in adults across the world. Most infections are harmless, but it can cause congenital HCMV infection in neonates or disseminated disease in the immunocompromised hosts. Till date, there has been scarce seroprevalence data of this disease in our country.

Methods: A retrospective analysis of the HCMV IgG assays performed in our laboratory was done. The period of study was from 2004 to 2007.

Results: A total of 3582 patient data were available. Duplicates and patient with missing variables (age, sex) were removed, leaving 2042 patients (median of 707 patients per year) for analysis. Across the 4 years of data, there was no significant difference seen in across the age groups, sex or

race. An overall seropositivity rate was 40% for the 1-10 year age group.

This rises gradually to 44%, 73%, 82%, 90%, 97% and 100% for each decade increase. The males and females have a similar rate of acquisition of HCMV seropositivity at age group 1-10 of 40%. However, seropositivity in females increases rapidly compared to the males in the age groups 10-20 to 40-50 before being similar again in the older age groups. This difference becomes statistically different in the 20-30 year age group ($p=0.0024$). Amongst the races, the predominant Chinese race group has a lower seropositivity in all age groups from 1-10 to 40-50 compared to the minority races of Indians and Malays combined. This difference was statistically different in the 20-30 year age group ($p=0.001$). The differences are likely related to the social habits of each race, and sex.

Conclusion: The result show a HCMV seroprevalence of 73% in the adult age group of 20-30. This prevalence is higher for females and in the minority races. This data has implications in estimating the risk for congenital CMV disease in neonates born to mothers acquiring their primary infection during pregnancy, and risk of CMV disease in transplant programmes.

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Viral load and genome integration detection: Two molecular markers for HPV persistent infection

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Background: Detection and quantification of Human Papillomavirus (HPV) are predicting factors associated with cervical cancer progression. Molecular biology techniques based upon amplification are the most sensitive methods for the detection of the viral DNA, especially in patients without apparent lesions, colposcopic changes or pathologic PAP smears. The aim of this study was to determine HPV infection, genotypes and viral load, in a cohort of 250 women in Mérida State (2008-2009) comparing a novel SYBR Green® quantitative real-time (q-RT) polymerase chain reaction (PCR) technology targeting the late-L1 viral genes with an end-point-PCR assay. Additionally, in order to detect HPV integration, we amplified viral E6/E7 ORFs as the target region by end-point PCR. In this assay, consensus primers including HPV18 and HPV16 genotypes were tested.

Methods: Samples were collected in transport medium device (Digene®) and DNAs were isolated using QIAmp® DNA isolation Kit (QIAGEN®). Pap smear was performed by Papanicolaou tech.

Results: End-point PCR of L1 region was applied to 125 samples, 20% (71/125) were positive for HPV and there was a moderate agreement ($K=0.46$) with the SYBR Green® q-RT PCR assay. Amplification of early E6/E7 region identi-