UNIVERSITI PUTRA MALAYSIA

DETECTION OF VIRUSES IN NASOPHARYNGEAL ASPIRATES OF CHILDREN ADMITTED WITH LOWER RESPIRATORY TRACT INFECTIONS AT HOSPITAL SERDANG, SELANGOR, MALAYSIA

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By

MOHAMMADREZA ETEMADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2011

Specially dedicated to,
My beloved Mother and Father,

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirement for the degree of Master of Science.

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Chair: Professor Norlijah Othman, MBBS, MRCP

Faculty: Medicine and Health Sciences

Acute lower respiratory tract infections (ALRTIs) continue to be the most important cause of infant and young children mortality worldwide, most of them occuring in developing countries including Southeast Asia and Africa. The role of viruses as major causative agents of ALRTIs in children is increasingly becoming more evident by using sensitive molecular detection methods. The aim of the study was to assess the epidemiology of respiratory viral infections among children less than five years of ages hospitalized with ALRTIs to the Hospital Serdang using conventional and molecular detection methods and to correlate these findings with demographic and clinical

features of the patients in order to determine further common viral atiologic agents. A cross-sectional study was conducted from June until December 2009 among children hospitalized with ALRTI. Nasopharyngeal aspirates were collected from 165 patients based on pre-determined inclusion and exclusion criteria. Direct immunofluorescence assay (DFA) was performed to screen the samples for the presence of respiratory syncytial virus (RSV), human metapneumovirus (HMPV), parainfluenzavirus 1-3 (PIV 1-3), influenza virus type A and B (IFV A & B), and human adenoviruses (HAdV). Negative samples tested by DFA were followed by shell vial culture (SVC), as a supplementary test to enhance the detection of these eight viruses. Viral genomes (RNA/DNA) were extracted and subsequently reverse transcription was done on RNA extracts in order to perform diagnosis using molecular methods. Hemi-nested multiplex RT-PCR was applied for detection of RSV, HMPV, IFV-A and B, PIV 1,2,3, and 4, human rhinoviruses (HRV), human enteroviruses (HEV) and human coronaviruses (HCoV) 229E and OC43. In addition, the presence of human bocavirus (HBoV) and human adenoviruses (HAdV) was investigated separately by nested PCR method. The positive samples using either method were subjected to isolation by cell culture. Vero, HEp-2, HeLa and MRC-5 cell lines were used for isolation of RSV, HAdV and HRV. Selected samples of patients diagnosed with RSV, HRV/HEV, and HAdV were subjected to the sequencing and molecular typing. Mycoplasma serology and bacterial culture were performed on blood samples. At the end of the hospitalization, the children's hospital chart was reviewed to collect demographic, clinical, laboratory and radiological investigation data using standardized protocol. The association of demographic, clinical features, hematologic factors, radiographic findings, hospital

course and severity of disease with infections due to different viruses was studied. Aetiologic agents including virus and/or bacteria were detected in 158 (95.8%) of the patients. Single virus was detected in 114 (67.9%) patients; 46 (27.9%) were co-infected with different viruses including double-virus infections in 37 (22.4%) and triple-virus infections in 9 (5.5%) cases. Approximately 70% of samples were found positive using conventional methods as compared with 96% using molecular methods. A wide range of respiratory viruses was detected in the study. RSV (50.3%), with predominance of group B (GB3 genotype), played a major role among hospitalized children. The results of this survey also showed significant burden of HRV infections (32.7%). Phylogenetic study of the VP4/VP2 region confirmed the broad genetic diversity of circulating HRV. HRV-A strains represented the majority of the detections, 22/36 (61%). Recently discovered HRV-C group was substantially implicated as etiological agent among studied patients, 14/36 (39%). Other etiological agents including HAdV (serotypes 1, 2, 3, and 6), HMPV, IFV-A, PIV 1-3, HBoV, HCoV-OC43 and HEV (B, C, and D species) were detected in 14.5, 9.6, 9.1, 4.8, 3.6, 2.4 and 1.8 percent of the samples, respectively. Ninety percent of the cases occurred in children less than 2 years. The majority of RSV infections occurred in children less than six months as compared with other virus groups. However, HRV was mainly detected in the second half of the infancy. The most common clinical presentations of ALRTI, among hospitalized children infected with the studied viruses were cough (96%), fever (85%), rhinorrhea (83%), difficulty in breathing (84%), tachypnea, chest wall crepitations (93%) and recession (80%). Children were admitted after a mean duration of three days. However, it was significantly earlier for HRV (1.9 days) than RSV (4.0

days) infections. Fever was a prominent feature of RSV and IFV infections. In this study, HRV-C infected children were more likely to have wheezing/rhonchi as compared with HRV-A. The results of the investigation also showed that antibiotics were administerd in majority of the patients 136/165, (82%). HRV-infected patients were less likely to receive antibiotics compared with RSV patients. The results of the study suggested that respiratory viral agents significantly contributed to the aetiology of ALRTIs among hospitalized children. Our results demonstrated the potential usefulness of molecular detection methods compared with conventional methods for the diagnosis of ARTIs among hospitalized children. In this study, newly discovered viruses including HMPV, HBoV and HRV-C were reported for the first time in Malaysia. Our study also highlighted that the epidemiology and clinical features were specified to certain viral agents studied.

Abstrak tesis yang telah dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains

PENGESANAN VIRUS-VIRUS DALAM ASPIRAT NASOFARIKS KANAK-KANAK DENGAN JANGKITAN PERNAFASAN BAHAGIAN BAWAH YANG DIMASUKKAN KE DALAM HOSPITAL SERDANG, SELANGOR, MALAYSIA

Oleh

MOHAMMADREZA ETEMADI

Julai 2011

Pengurusi: Profesor Norlijah Othman, MBBS, MRCP

Fakulti: Perubatan dan Sains Kesihatan

Jangkitan pernafasan bahagian bawah akut (ARLTIs) merupakan penyebab utama kematian di kalangan bayi dan kanak-kanak di seluruh dunia, kebanyakannya berlaku di negara membangun termasuk Asia Tenggara dan Afrika. Peranan virus sebagai agen penyebab ARLTIs di kalangan kanak-kanak semakin jelas dengan menggunakan kaedah pengesanan molekular yang sensitif. Tujuan kajian adalah untuk menilai epidemiologi jangkitan virus sistem pernafasan di kalangan kanak-kanak kurang daripada lima tahun yang dimasukkan ke Hospital Serdang disebabkan ARLTIs menggunakan kaedah pengesanan konvensional dan molekular serta

menghubungkaitkan penemuan ini dengan ciri-ciri demografik dan klinikal pesakit untuk menentukan kebiasaan etiologik agen virus dengan lebih mendalam. Kajian keratan rentas dilakukan dari Jun sehingga Disember 2009 di kalangan kanak-kanak hospital disebabkan ARLTIs. Aspirat nasofarinks dikumpulkan daripada 165 pesakit berdasarkan kriteria pra penentuan inklusi dan eksklusi. Ujian imunofloresense secara langsung (DFA) dilakukan pada sampel untuk mengesan kehadiran virus respirasi sinsitium (RSV), metapneumovirus manusia (HMPV), virus parainfluenzavirus 1-3 (PIV 1-3), virus influenza jenis A and B (IFV A & B), dan adenovirus manusia (HAdV). Sampel negatif diuji oleh DFA diikuti kultur shell vial (SVC) sebagai ujian tambahan untuk meningkatkan pengesanan kelapan-lapan virus ini. Genom virus (RNA/DNA) diekstrak dan diikuti dengan transkripsi berbalik ke atas ekstrak RNA untuk menjalankan diagnosis menggunakan cara molekular. Hemi-nested multiplex RT-PCR telah digunakan untuk mengesan RSV, HMPV, IFV-A dan B, PIV 1,2,3, dan 4, rhinovirus manusia (HRV), enterovirus manusia (HEV), dan coronavirus manusia (HCoV) 229E dan OC43. Tambahan pula, kehadiran bocavirus manusia (HBoV) dan adenovirus manusia (HAdV) telah disiasat berasingan menggunakan cara nested PCR. Sampel positif menggunakan kaedah-kaedah ini dilanjutkan kepada isolasi oleh sel kultur. Sel Vero, HEp-2, HeLa dan MRC-5 telah digunakan untuk isolasi RSV, HAdV and HRV. Sampel pesakit terpilih yang mengandungi RSV, HRV/HEV, dan HAdV telah dirujuk kepada jujukan dan molekular typing. Serologi mycoplasma dan kultur bakteria telah dijalankan ke atas sampel darah. Di akhir penghospitalan, carta hospital kanak-kanak diselidik untuk mengumpul data demografi, klinikal dan makmal menggunakan kaedah yang dipiawaikan. Hubungkait antara demografi, ciri-ciri klinikal,

faktor hematologi, penemuan radiografi, laporan hospital, dan keterukkan jangkitan disebabkan oleh kajian pelbagai virus telah dinilai menggunakan kaedah statistik. Agen aetiologi termasuk virus dan/atau bakteria dikesan pada 158 (95.8%) pesakit. Virus tunggal telah dikesan pada 114 (67.9%) pesakit; 46 (27.9%) pesakit telah dijangkiti oleh beberapa virus yang berlainan termasuk jangkitan dua-virus pada 37 (22.4%) pesakit dan jangkitan tiga-virus di dalam 9 (5.5%) kes. Dianggarkan 70% sampel telah dikesan positif menggunakan kaedah konvensional berbanding 96% menggunakan kaedah molekular. Virus sistem pernafasan yang meluas telah dikesan di dalam kajian ini. RSV (50.35%) dengan predominan kumpulan B (genotip GB3), memainkan peranan utama di kalangan kanak-kanak yang dimasukkan ke dalam hospital. Keputusan tinjauan ini juga menunjukkan bebanan yang signifikan oleh jangkitan HRV (32.7%). Kajian filogenetik kawasan VP4/VP2 telah mengesahkan diversiti genetik edaran HRV yang luas. Strain HRV-A mewakili majoriti pengesanan 22/36 (61%). Baru-baru ini penemuan kumpulan HRV-C telah dibabitkan sebagai agen etiologi di kalangan pesakit-pesakit yang di kaji 14/36 (39%). Agen etiologi lain termasuk HAdV (serotip 1,2,3, dan 6), HMPV, IFV-A, PIV 1-3, HBoV, HCoV-OC43 dan HEV (spesis B, C, dan D) telah dikesan masing-masing di dalam 14.5, 9.6, 9.1, 4.8, 3.6, 2.4 and 1.8 peratus sampel. Sembilan puluh peratus kes berlaku pada kanak-kanak kurang daripada dua tahun. Majoriti jangkitan RSV terdiri daripada kanak-kanak kurang dari enam bulan berbanding kumpulan virus lain. Walaubagaimanapun, HRV terutamanya dikesan pada separuh masa kedua kehamilan. Kebiasaannya, laporan klinikal ALRTIs di kalangan kanak-kanak hospital yang dijangkiti dengan virus yang dikaji merupakan batuk (96%), demam (85%), rhinorrhea (83%), kesukaran bernafas (84%), tachypnea, krepitasi

dinding dada (93%), dan retraksi (80%). Kanak-kanak telah dimasukkan ke hospital pada purata masa 3 hari. Walaubagaimanapun, ia signifikan lebih awal bagi jangkitan HRV (1.9 hari) berbanding RSV (4.0 hari). Demam merupakan ciri-ciri utama oleh jangkitan RSV dan IFV. Dalam kajian ini, kanak-kanak yang dijangkiti oleh HRV-C lebih cenderung menghadapi wheezing/ronki berbanding HRV-A. Keputusan kajian menunjukkan bahawa antibiotik yang dimasukkan ke dalam majoriti pesakit-pesakit 136/165, (82%). Pesakit-pesakit yang dijangkiti HRV kurang memerlukan antibiotic berbanding dengan pesakit RSV. Ini merupakan kajian pertama ke atas epidemiologi dan etiologi oleh panel virus sistem pernafasan di Malaysia yang lengkap. Keputusan menunjukkan agen virus sistem pernafasan menyumbang secara signifikan atiologi ALRTIs di kalangan kanak-kanak yang dimasukkan ke hospital. Keputusan kami menunjukkan potensi penggunaan kaedah pengesanan molekular berbanding kaedah konvensional untuk mendiagnos ALRTIs di kalangan kanak-kanak yang dimasukkan ke hospital. Dalam kajian ini, penemuan baru virus-virus termasuk HMPV, HBoV dan HRV-C telah dilaporkan pertama kali di Malaysia.

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I certify that an Examination Committee has met on 4 July 2011 to conduct the final examination of Mohammadreza Etemadi on his thesis entitled "Detection of Viruses in Nasopharyngeal Aspirates of Children Admitted with Lower Respiratory Tract Infections to Hospital Serdang, Malaysia" in accordance with Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

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Date: 23 August 2011

This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Superivisory Committee were as follows:

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Date: 15 September 2011

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institution.

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MOHAMMADREZA ETEMADI

Date: 4 July 2011

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