Detection of human coronavirus strain HKU1 in a 2 years old girl with asthma exacerbation caused by acute pharyngitis

Razieh Amini1, Fatemeh Jahanshiri2, Yasaman Amini2,3, Zamberi Sekawi2 and Farid Azizi Jalilian2,4*

Abstract
Respiratory viral infections can trigger asthma attack which may lead to sever morbidity. In this report, using molecular methods, we show the chronological association between human coronavirus - HKU1 infection and asthma exacerbation in a two years and seven months old asthmatic girl who was not under treatment and was otherwise healthy.

Keywords: Respiratory viral infection, Asthma, Coronavirus

Introduction
Asthma is a chronic respiratory disease characterized by hypersensitivity and inflammation of the airways with clinical symptoms of dyspnea, wheezing, coughing, reversible episodes of bronchoconstriction, shortening of breath, chronic eosinophilic inflammation, mucus hypersecretion and tightening of the chest [1,2]. The exacerbation of asthma symptoms and the level of asthma severity have shown to be related to genetic susceptibility to exposure to various environmental triggers [3]. Inflammatory triggers of asthma include pets, dander, dust, mold, pollen, and viral respiratory infections and non-inflammatory triggers include tobacco smoke, exercise, weather changes, stress, and air pollution [4,5]. The contribution of viral infections to the occurrence of asthma exacerbations has been of broad interest for the past 20 years [6]. In this regard, it is believed that viral respiratory tract infections with respiratory syncytial virus and rhinoviruses are the major causes of acute asthma exacerbations in children [7-10]. Besides, the involvement of coronaviruses (hCoV) in asthma exacerbation has been recently revealed with the implementation of molecular methods such as the reverse transcriptase polymerase chain reaction (RT-PCR) [12]. Here, we report the first case of an asthma exacerbation triggered by acute pharyngitis event in a child caused by HCoV-HKU1 infection.

Case report
An almost three-year old girl, was admitted to the pediatric ward of Serdang Hospital (Malaysia) with a history of two days cough, runny nose and shortness of breath. Prior to the hospitalisation, she was given one dose of Salbutamol nebulizer in a private clinic for her respiratory problem. Her symptoms partially relieved. She had been diagnosed with bronchial asthma at the age of 18 months when she first presented with 2 episodes of wheezing and breathing difficulty following an upper respiratory infection which had responded well to the bronchodilator nebulization. Subsequent to the above episode, she continued to have intermittent symptoms such as nocturnal cough and occasional wheeze 2-3 times per week. However, she had not been given any
prophylactic medications for asthma. The symptoms were triggered by changes in temperature, dust, common cold and cigarette smoke. Her father is a chronic smoker. There was no history of allergy but she has strong family history of asthma. There was no history of exercise-induced cough or wheeze. On admission, she was afebrile but tachypneic with respiratory rate of 50 breaths per minute with intercostal and subcostal recession. There was an audible wheeze. Her vital signs except the increased respiratory rate were within normal limits. There was no skin eczema. The patient’s throat was inflamed without tonsillar enlargement. Chest X-ray showed hyperinflation. No pneumonic changes were observed and cardiac size was normal. Generalized rhonchi on lungs auscultation could be heard.

During hospitalization she did not required oxygen. She was diagnosed with acute exacerbation of bronchial asthma due to acute pharyngitis with underlying moderate persistent asthma. She was treated with regular Salbutamol nebulisation and a five day course of oral prednisolone. She was prescribed with asthma medications including Fluticasone MDI 250 mcg to be taken twice a day and Salbutamol MDI 200 mcg to be used when needed via aerochamber device. Tamiflu syrup was also prescribed for her flu-like symptoms. An asthma education was provided to the parents. She was hospitalized for 5 days. Her blood tests revealed Hb of 12.8 g/dl, total white count of 16.2 × 10^9/l with predominantly neutrophil 70%, lymphocyte 21% and 7% of eosinophil. The platelet count was 390 × 10^9/l. Her blood urea and electrolytes were within the normal limit. The level of C-Reactive Protein was 15 mg/L.

**Laboratory diagnosis**

To investigate the potential causative infectious virus, the patient’s respiratory sample (NPA) was collected at the pediatrics department Serdang Hospital, Malaysia and sent to the Clinical and Molecular Virology Center (CMVC) of University Putra Malaysia. As a part of routine virological screening, DNA and RNA were extracted using viral genomic extraction kit (GeneAll, Korea) and screened for 15 respiratory viruses including influenza A and B viruses, human respiratory syncytial virus A and B viruses, para influenza 1, 2, 3 and 4 viruses, Boca virus, human metapneumovirus (hMPV), adeno virus, rhinovirus, enterovirus and coronaviruses OC43/ HKU1 and NL-63/ 229E, by using a highly sensitive commercial multiplex (seplex RV15 ACE) RT-PCR kit (Seegene, Korea) which yielded positive result for coronavirus OC43/HUK1. To differentiate between coronavirus OC43 and coronavirus HKU1, a one-step RT-PCR amplification was carried out using RT-PCR kit (Promega, USA) with the following primer sets: HKU1( Sense 5-ACCAATCTGAGCGAAATTACCAAAC-3) and antisense 5-CGGAAACCTAGGGATAGCTT-3)[11] and OC43 (Sense 5-CGATGAGGCTATTCGACTAGGT and antisense 5-CTTGCTGAGGTTTAGTGCCAT) [13] (Figure 1).

As shown in Figure 1, RT-PCR results for HKU-1 was positive. This result was confirmed by neucleotide sequencing. To exclude bacterial infections such as mycoplasma and chlamydia pneumonia, ELISA and bacterial culture were carried out on the patient’s samples which all yielded negative results.

**Discussion**

Human coronaviruses are known as the second cause of common cold [14]. Recently, HCoVs strains including 229E, OC43, NL63 and HKU1 have been shown to be associated with more severe acute lower respiratory tract infection such as pneumonia in both infants and immunocompromised patients [15,16]. These strains were also detected from asthmatic adults with ARI [17] but their relation to asthma exacerbation remains unclear [18]. It has also been shown that infection with HCoV-NL63 can preset as asthma exacerbation, croup , high fever and febrile seizures in children [19].

Another Human coronavirus strain which has been successively detected in respiratory specimen worldwide is strain HKU1. This strain was first detected by Woo et
al (2005) in Hong Kong [20]. In a study on the prevalence of recently identified viruses in children with acute wheezing, HCoV-HKU1 was not detected [21]. Kuypers et al., (2007) showed that HUK1 was the most common subtype of coronaviruses found in children [22]. In a prospective longitudinal cohort study of young children, NL63 and OC43 strains were shown to be associated with a burden of LRTI. Bronchiolitis, pneumonia and asthma were the most important diagnosis in these patients [23]. It has also been reported that all four strains of coronaviruses are associated with both URI and LRI in children and also OC43 and NL63 strains are responsible for the most cases of bronchiolitis, pneumonia and asthma exacerbation in these patients [24]. In this report for the first time we detected HCoV HKU1 in a child with asthma exacerbation triggered by acute pharyngitis. However, more detailed microbiological investigations especially molecular techniques are required to confirm the relationship between HCoV HKU1 infection and asthma attack. Our findings suggest the necessity of considering HCoV HKU1 as a potential cause or precursor of asthma exacerbation in children.

Consent
Written informed consent was obtained from the patient’s parents for publication of this Case Report. A copy of written consent is available for review by the Editor- in- Chief of this journal.

Competing interests
The authors declare that they have no competing interests.

Authors’ contribution
RA carried out the molecular studies and participated in the sequence alignment and drafted manuscript, FJ performed the ELISA and participated in drafted the manuscript, YA carried out the clinical diagnosis and participated in the design of the study, ZS performed the bacterial culture and participated in the clinical diagnosis, FAJ conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgments
This research was partially supported by the Malaysian Ministry of Science, Technology and Innovation (NO202-01-04 SF0633). We dedicate this work to the memory of our friend and colleague Norrashidah Abd Wahab, MD. The authors would like to thank Dr.Kausar, Dr.Hafizah, Lim AH Cheng and Rashidah Husan for their assistance during this project.

Author details
1Department of Molecular Medicine, Faculty of Medicine, Hamadan University of Medical Sciences, HUMS, Hamadan, Iran. 2Department of Medical Microbiology, Faculty of Medicine and Health Sciences, 43400 UPM Serdang, Selangor, Malaysia. 3Department of Infectious Disease, Masih Daneshvari hospital, Tehran, Iran. 4Department of Medical Microbiology, Faculty of Medicine, IUM University of Medical Sciences, 69316 IUMS, Ilam, Iran.

Received: 27 July 2011 Accepted: 10 July 2012 Published: 3 August 2012

References
1. Just J. Asthma in children. Rev Pat 2011, 61:699–708.
2. Buse WW, Lemanske FF Jr. Asthma. N Engl J Med 2001, 344:350–362.
3. Wilhelm M, Meng YY, Rull RP, English P, Balmes J, Ritz B: Environmental public health tracking of childhood asthma using California health interview survey, traffic, and outdoor air pollution data. Environ Health Perspect 2008, 116:1254–1260.
4. Sarafino EP, Dillon JM: Relationships among respiratory infections, triggers of attacks, and asthma severity in children. J Asthma 1998, 35:497–504.
5. Weiss ST, Horner A, Shapiro G, Steenberg AL: The prevalence of environmental exposure to perceived asthma triggers in children with mild-to-moderate asthma: data from the Childhood Asthma Management Program (CAMP). J Allergy Clin Immunol 2001, 107:634–640.
6. Dulek DE, Peebles RS Jr. Viruses and asthma. Boehm Chem Acta 2011, 1810:1080–1090.
7. Wark PA, Gibson PG: Asthma exacerbations. Pathogenesis, Thorax 2006, 61:909–915.
8. Freymuth F, Vabret A, Blouard J, Toutain F, Verdon R, Pettijean J, Gouarin S, Duhamel JF, Guillois B: Detection of viral, Chlamydia pneumoniae and Mycoplasma pneumoniae infections in exacerbations of asthma in children. J Clin Virol 1999, 13:131–139.
9. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisford E, et al.: Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med 2008, 178:667–672.
10. Rakes GP, Angel J, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, Platt-Mills TA, Heymann PW: Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. J Allergy and clinical Immunol. Am J Respir Crit Care Med 1999, 159:785–790.
11. Vabret A, Dina J, Gouarin S, Pettijean J, Corbet S, Freymuth F: Detection of the new human coronavirus HKU1: a report of 6 cases. Clin Infect Dis 2006, 42:634–639.
12. Jartti T, Waris M, Niesters HG, Allander T, Ruuskanen O: Respiratory viruses and acute asthma in children. J Allergy Clin Immunol 2007, 120:216. author reply 217.
13. Arden KE, Chang AB, Lambert SB, Nissen MD, Sloots TP, Mackay IM: Newly identified respiratory viruses in children with asthma exacerbation not requiring admission to hospital. J Med Virol 2010, 82:1458–1461.
14. Lau PW, Cook N, Andrews H, Bracka A, Myint SH: Detection of human papillomavirus types in balanitis xerotica obliterans and other penile conditions. Genitourin Med 1995, 71:228–230.
15. Gerna G, Campanini G, Rovida F, Percivalle E, Sarasini A, Marchi A, Baldanti F: Genetic variability of human coronavirus OC43-, 229E-, and NL63-like strains and their association with lower respiratory tract infections of hospitalized infants and immunocompromised patients. J Med Virol 2006, 78:539–542.
16. Kim YJ, Boeckh M, Englund JA: Community respiratory virus infections in immunocompromised patients: hematopoietic stem cell and solid organ transplant recipients, and individuals with human immunodeficiency virus infection. Semin Respir Crit Care Med 2007, 28:222–242.
17. Kister A, Avila PC, Roussin S, Wang D, Ward T, Iagi S, Schnurr D, Ganem D, Defilisi J, Boushey HA: Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. J Infect Dis 2007, 196:817–825.
18. Leung TF, To MY, Yeung AC, Wong YS, Wong GW, Chan PK: Multiplex molecular detection of respiratory pathogens in children with asthma exacerbation. Chest 2010, 137:348–354.
19. Chua SS, Chan KH, Chu RW, Kwan SW, Guan Y, Poon LL, Peiris JS: Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. Clin Infect Dis 2005, 40:1721–1729.
20. Woo PC, Lau SK, Chu CM, Chan KH, Tsio HW, Huang Y, Wong BH, Poon RW, Cai JH, Luk WK, et al.: Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol 2005, 79:884–885.
21. Chung JY, Han TH, Kim SW, Kim CK, Hwang ES: Detection of viruses identified recently in children with acute wheezing. J Med Virol 2007, 79:1238–1243.
22. Kuypers J, Martin ET, Heugel J, Wright N, Morrow R, Englund JA: Clinical disease in children associated with newly described coronavirus subtypes. Pediatr Infect Dis J 2007, 119970–76.
23. Talbot HK, Shepherd BE, Crowe JE Jr, Griffin MR, Edwards KM, Podsiad AB, Tollefson SJ, Wright PF, Williams JV: The pediatric burden of human coronaviruses evaluated for twenty years. Pediatr Infect Dis J 2009, 28:682–687.

24. Dominguez SR, Robinson CC, Holmes KV: Detection of four human coronaviruses in respiratory infections in children: a one-year study in Colorado. J Med Virol 2009, 81:1597–1604.

doi:10.1186/1743-422X-9-142

Cite this article as: Amini et al: Detection of human coronavirus strain HKU1 in a 2 years old girl with asthma exacerbation caused by acute pharyngitis. Virology Journal 2012 9:142.