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Key Messages

1. Coronaviruses accounted for 1.6% (98/6272) of respiratory tract infections based on nasopharyngeal aspirate samples.
2. HCoV-OC43 was the most common coronavirus detected, followed by HCoV-NL63, CoV-HKU1, and HCoV-229E.
3. Although CoV-HKU1 infections were most often associated with the upper respiratory tract, more severe illness (pneumonia, acute bronchiolitis, and asthmatic exacerbation) may occur, especially in those with underlying disease. In young children, CoV-HKU1 infection is associated with a high rate of febrile seizures (50%).
4. CoV-HKU1 and HCoV-OC43 infections peaked in winter, in contrast to HCoV-NL63, which mainly occurred in early summer and autumn, but was absent in winter.
5. Reverse transcriptase polymerase chain reaction is useful for the rapid diagnosis of coronavirus infections.

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Epidemiology of coronavirus-associated respiratory tract infections and the role of rapid diagnostic tests: a prospective study

Introduction

Four novel causative agents of respiratory tract infections have been identified: human metapneumovirus,[1] SARS coronavirus (SARS-CoV),[2] human coronavirus NL63 (HCoV-NL63),[3,4] and coronavirus HKU1 (CoV-HKU1).[5] Coronavirus HKU1 was first isolated in Hong Kong in January 2005 from a 71-year-old Chinese patient with pneumonia.[5] It has also been found in another patient with pneumonia,[5] suggesting that this virus is associated with respiratory tract infections. The clinical spectrum of CoV-HKU1 infection and its epidemiology in relation to other coronaviruses were unknown at that time. We examined the epidemiology and clinical spectrum of CoV-HKU1, HCoV-NL63, HCoV-OC43, and HCoV-229E infections in patients hospitalised for acute respiratory illness. Specific reverse transcriptase polymerase chain reaction (RT-PCR) assays were developed for the rapid diagnosis of coronavirus infections. The molecular epidemiology of CoV-HKU1 was analysed by sequencing of selected gene targets.

Methods

This study was conducted from 16 December 2005 to 15 December 2007. All prospectively collected nasopharyngeal aspirate (NPA) samples sent to the microbiology laboratory of Queen Mary Hospital during an 18-month period (April 2004 to September 2005) were included. All NPA samples were assessed for influenza A and B viruses, parainfluenza viruses types 1, 2 and 3, respiratory syncytial virus, adenovirus by direct immunofluorescence, and metapneumovirus by RT-PCR.[6,7] Those NPA samples negative for these respiratory viruses were subject to RT-PCR for coronaviruses. Once coronavirus was detected, corresponding patients were identified and their clinical features, laboratory results, and outcomes were analysed.

A total of 6272 NPA samples from patients with acute respiratory tract infections were identified. To evaluate the specificity of the RT-PCR assays, RNA of CoV-HKU1, HCoV-OC43, HCoV-229E, HCoV-NL63, and SARS-CoV, as well as RNA extracted from 200 NPA sample positive for influenza A and B viruses, parainfluenza viruses 1-3, respiratory syncytial virus (RSV), or adenovirus antigens were also subject to RT-PCR.

Upon receipt of samples, viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAgen, Hilden, Germany) within 10 hours. Reverse transcription was performed using random hexamers and the SuperScript II kit (Invitrogen, San Diego [CA], USA).[5,8] For coronaviruses, PCR was performed using four sets of primers specifically designed to amplify CoV-HKU1, HCoV-OC43, HCoV-229E, and HCoV-NL63, targeted to the same region of the RNA-dependent RNA polymerase (*pol*) gene. The sequences of the PCR products were compared with the sequences of the *pol* genes of coronaviruses in the GenBank database. To determine the molecular epidemiology of CoV-HKU1, the complete *pol*, spike (S) and N genes of CoV-HKU1 from 13 patients were amplified and sequenced. Phylogenetic tree construction was performed using the neighbour-joining

method with GrowTree using the Jukes-Cantor correction (Genetics Computer Group).

Since a high rate of febrile seizures was noted in patients with CoV-HKU1 infection, the relative frequency of febrile convulsion, maximum temperature, duration of fever, and duration of hospitalisation in children at risk of febrile seizures (aged 6 months to 5 years) with different respiratory virus infections were analysed by review of clinical records. Comparisons were made among the various groups of patients with respiratory virus infections.

Results

Coronaviruses were detected in 98 (1.61%) of the 6272 NPA samples. Using RT-PCR, 13 were positive for CoV-HKU1, 61 for HCoV-OC43, five for HCoV-229E, and 19 for HCoV-NL63. None of the 200 NPAs positive for influenza A and B viruses, parainfluenza viruses 1-3, RSV, or adenovirus antigens was RT-PCR positive for coronaviruses. The PCR reactions were specific for the corresponding coronavirus.

No epidemiological linkage was identified in 13 cases: 10 occurred in autumn or winter (November to February) and three in spring or summer (April to July). The median patient age was 3 (range, 1.6-87) years; most patients were children. Common presenting symptoms were fever, running nose, and cough with or without sputum. All had upper respiratory infections, except for two who had lower respiratory tract infections and abnormalities on chest radiographs (one had bilateral lower zone haziness, and another had perihilar haziness). Eight patients had underlying diseases. Two patients had recent travel histories. Two patients were smokers. Five children had febrile seizures and two others with underlying epilepsy had breakthrough seizures. Febrile seizures occurred in 38% of patients with CoV-HKU1 infections, 18% of those with HCoV-NL63 infections, and 6% of those with HCoV-OC43 infections. In patients with pre-existing epilepsy, breakthrough seizures occurred in those with CoV-HKU1 and HCoV-NL63 infections. Both febrile and breakthrough seizures were more common in patients with CoV-HKU1 infections than in those with HCoV-OC43 infections (P<0.05).

The seasonality of CoV-HKU1 was compared to that of other coronaviruses. CoV-HKU1 and HCoV-OC43 mainly occurred in the autumn and winter months, whereas HCoV-NL63 mainly occurred in the summer and autumn.

A total of 629 children aged 6 months to 5 years were hospitalised for acute respiratory virus infections during the study period. The rate of febrile seizures was significantly higher in children with CoV-HKU1 infections than in those with HCoV-OC43, adenovirus, human parainfluenza virus 1, or respiratory syncytial virus infections (P<0.05). There was no significant difference in the maximum temperature

and duration of hospitalisation between CoV-HKU1 infections and other virus infections. Children with other respiratory virus infections, except HCoV-OC43, had longer duration of fever than those infected with CoV-HKU1 did (P<0.05).

Sequencing and phylogenetic analysis showed the presence of at least two genotypes which are better distinguished based on S and N genes than the *pol* gene. Seven of the 13 strains belonged to genotype A and the other six to genotype B.

Discussion

Coronavirus infections were present in 1.6% of NPA samples, with HCoV-OC43 being the most prevalent, followed by HCoV-NL63 and CoV-HKU1 and then HCoV-229E. CoV-HKU1 was responsible for 0.2% of patients with acute respiratory illness. In old people with major underlying diseases, CoV-HKU1 mostly caused pneumonia.[8] In young children with or without underlying diseases, upper respiratory tract infection was the most common presentation. CoV-HKU1 infections occasionally resulted in more severe illness (acute bronchiolitis and asthmatic exacerbation). There may be selection bias especially for adults, as only hospitalised patients were included. Young children are often admitted to hospital even for mild illness, whereas adults with mild disease are usually treated in the primary care sector. This may have over-estimated the severity of such infections in adults.

HCoV-NL63 infections appeared in early summer and peaked in autumn, but were absent in winter. Similarly, CoV-HKU1 infections increased in autumn and peaked in winter, and a few cases occurred in spring to early summer, unlike HCoV-OC43 infections that no cases were observed in the other seasons. The seasonal pattern of HCoV-229E could not be determined because of the small number of cases. Continuous studies carried out over a number of years are required to ascertain the seasonal and any possible inter-year variation in the relative incidence of the different coronaviruses.

Specific RT-PCR was useful for the rapid diagnosis of coronavirus infections. Especially for CoV-HKU1, it may provide a clue to anticipating febrile seizures in children with acute respiratory tract infections. In fact, half of the affected children had febrile seizures, which was the highest rate among other studied respiratory virus infections. Although there was no significant difference in the maximum temperature caused by CoV-HKU1 and other respiratory viruses, there was a trend for lower temperatures with the coronaviruses compared to influenza A and adenovirus. Therefore, it is unlikely that the high rate of febrile seizures associated with CoV-HKU1 and HCoV-NL63 infections can be explained by differences in the extent of fever. A novel amino acid substitution in the haemagglutinin gene of influenza A correlates with acute

encephalopathy.[9] Nonetheless, further studies with a much larger sample size are needed to evaluate the significance of the present findings. If such association between febrile seizures and CoV-HKU1 can be confirmed, investigation for specific neurotropic or epileptogenic factors in CoV-HKU1 and those respiratory viruses with a propensity to cause febrile seizures can be carried out.

At least two distinct genotypes of CoV-HKU1 were revealed by sequencing the S and N genes, whereas the *pol* gene is less discriminative for such classification. The two genotypes co-circulated during the winter, a phenomenon similar to HCoV-NL63 demonstrated in different geographical areas.[4,10,11] The S and N genes may be more useful for genotyping of CoV-HKU1.

Acknowledgements

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