Molecular diagnosis of human metapneumovirus

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Background
The recent discovery of human metapneumovirus (hMPV) as a major respiratory pathogen has been made possible by means of reverse transcriptase-PCR (RT-PCR). Studies published so far have been mostly conducted using the molecular approach.
Objective
The objective of the present study was to clarify the epidemiological and clinical features of hMPV using molecular biological techniques for its diagnosis.
Patients and methods
A total of 189 patients with suspected viral respiratory tract infections were included and their respiratory specimens were analyzed for the presence of hMPV using a Seeplex respiratory virus detection kit. Detection techniques that were applied included virus identification by transcriptase-PCR (TC-PCR), direct fluorescent antibody staining, and the rapid culture technique known as shell vial amplification using monoclonal antibodies (mAbs) of nasal wash or aspirate fluid. The epidemiological and clinical data were analyzed and the latter were represented as percentages where applicable.
Results
The study determined the presence of respiratory viruses in 61 (32.3%) of the 189 respiratory samples and showed the presence of hMPV in eight (13.1%) of the 61 samples. hMPV showed variable seasonal activity. Six patients (75%) positive for hMPV had pre-existing serious disorders. Using the shell vial cultures with mAbs, we found that non-Hodgkin lymphoma patients with the related isolated virus showed a plaque of infected cells with small syncytial formations, whereas the other seven patients showed single infected cells. The RT-PCR results of all samples from hMPV-positive patients were correlated with the results of direct fluorescent antibody staining or shell vial cultures using mAbs.
Conclusion
hMPV is a significant pathogen in immunocompromised patients with a risk for high morbidity and mortality. A combination of diagnostic workups may be useful for confirming the detection of hMPV.

Keywords:
acute respiratory illness, clinical characteristics, direct fluorescent antibody, epidemiology, human metapneumovirus, monoclonal antibodies, molecular biological approach, RT-PCR

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Introduction

Human metapneumovirus (hMPV) had been proven to be a major cause of upper and lower respiratory infections in children, adults, and the elderly [1] and infection with this virus has been detected worldwide. Severe and fatal hMPV infections have been reported in immunocompromised patients [2]. In humans clinical infection with hMPV occurs throughout life, despite the fact that most individuals sustain humoral immune responses to both hMPV [3] and human respiratory syncytial virus (hRSV) [4]. Although the cellular immune response following hRSV infection is well understood in human [5] and animal models [6], it is incompletely described in the case of hMPV infection. Similarities to hRSV suggest that $CD8^+$ T cells are likely to be necessary to resolve hMPV infection in humans [7]. A role of cytotoxic T lymphocytes in the control of hMPV infection is supported by in-vivo mouse studies showing increased hMPV titers in T cell-depleted mice [8] and protection against infection by adoptive transfer of hMPV-specific cytotoxic T lymphocytes [9] and hMPV-directed T-cell vaccines [10]. The strong association between hMPV infection and asthma in both children [11] and adults [12] and the ability of hMPV infection to exacerbate hRSV disease [13] illustrate the need to improve the understanding of hMPV-induced T-cell immunity, particularly as a prelude to therapeutic intervention. hMPV is a negative-sense nonsegmented RNA virus that has been categorized under the pneumovirus subfamily, family Paramyxoviridiae, on the basis of

genomic sequence and gene constellation [14]. The prevalence of hMPV has not been reported in Saudi Arabia. The recent discovery of hMPV as a major respiratory pathogen has been made possible by means of RT-PCR. Studies published so far have mostly been conducted using the molecular approach. The availability of specific monoclonal antibodies (mAbs) now opens the door to the routine use of direct fluorescent antibody (DFA) staining for hMPV detection in nasopharyngeal aspirates (NPAs). In addition, the documented ability of the tested mAbs to react with all four hMPV [15] subtypes proves the ability of these reagents to detect all known hMPV strains.

Patients and methods

A total of 189 patients, selected from internal male and female medical wards and from adult ICU departments of KFSH and HGH, were included in the present study from February 2011 to January 2012 as a part of the routine workup for patients presenting with suspected viral respiratory tract infections. Respiratory specimens were submitted for centrifugation, and supernatants were analyzed for hMPV using a Seeplex respiratory virus detection kit from Seegene (Seegen, Jeoul, Korea). This Seeplex system applies dual specific oligonucleotide technology, which greatly improves the specificity without any false positives. This dual specific oligonucleotide-based system is a fundamental tool that blocks the extension of nonspecifically primed templates, thereby generating a consistently high PCR specificity, even under less-than-optimal PCR conditions. Positive RT-PCR results for hMPV were verified by the Mayo Clinic Laboratory (Mayo, USA). Detection techniques that were applied included virus identification by TC-PCR, DFA staining of NPAs, and the rapid culture technique known as shell vial amplification using hMPV-specific mAbs. The clinical sample appropriate for submission to the laboratory includes nasal wash or aspirate fluid or nasopharyngeal flock swab culture [16]. The epidemiological and clinical data were analyzed and the latter were represented as percentages where applicable.

Results

The study determined the presence of respiratory viruses in 61 (32.3%) of 189 respiratory samples tested through the present workup and showed the presence of hMPV in eight (13.1%) of 61 samples. hMPV showed variable seasonal activity. A higher incidence was reported in March, August, and September (Table 1). The clinical characteristics and outcomes of hMPV infections are presented in Tables 1 and 2. Six patients (75%) positive for hMPV had pre-existing or serious underlying disorders. These disorders were leukemia, non-Hodgkin lymphoma (NHL), the Guillain–Barré syndrome, sickle cell disease, chronic obstructive pulmonary disease, and cystic fibrosis. Clinical signs and symptoms upon presentation to the hospital were cough in eight (100%) patients, fever in seven (87.5%), shortness of breath in six (75%), nasal congestion in five (62.5%), and wheezing in three (37.5%) patients. Patients with underlying leukemia, NHL, and Guillain–Barré syndrome (37.5%) presented with severe pneumonia, which fulfilled the criteria for ICU admission. One of them who had NHL (12.5%) died after 17 days of admission to the ICU. Figure 1a and b shows DFA staining of respiratory mucosal cells from two different NPAs from the patients with hMPV-positive samples. By using the shell vial cultures with mAbs, we found that NHL patients with the related isolated virus showed a plaque of infected cells with small syncytial formations (Fig. 1d), whereas the other seven patients showed single infected cells (Fig. 1c).

Discussion

The recently identified hMPV is the only member of the genus Metapneumovirus (family Paramyxoviridae, subfamily Paramyxovirinae) that also includes avian pneumoviruses A, B, C, and D, which infect humans. hMPV is responsible for a fair proportion of respiratory infections in early infancy, childhood, in the elderly, and in immunocompromised hosts [17]. In the present study, hMPV was detected in 13.1% of patients who were hospitalized with lower respiratory tract infections. These findings are slightly higher than those reported in the USA [18], Europe [19], and Australia [20]. This may be explained by the presence of a multinational population in the Makkah region. Most of the reported studies revealed that infections with hMPV were significantly higher among infants than among younger and older children [14]. Our present study reported that seven of eight patients (87.5%) were between 13 and 21 years of age. This implies that hMPV infects both adults and older children. A broad seasonal activity of hMPV showing a distinctive pattern over several years was reported by Sloots *et al.* [20]. In the present study, peak incidence was reported in March, August, and September, which reflects the seasonality of hMPV.

The present study reported that hMPV infections in immunocompromised patients were characterized by several respiratory symptoms, including cough, fever, shortness of breath, and nasal congestion. Three patients were admitted to the ICU with rapidly progressing respiratory failure, pneumonia, and culture-negative shock. hMPV was the only detected respiratory pathogen in the NPA or BAL of these three patients. One of the patients suffering from NHL died despite receiving intensive and aggressive therapy in the ICU. These figures were similar to those reported by Englund *et al.* [21]. The severity of

Table 1 Number of patients with human metapneumovirus infections each month over a year

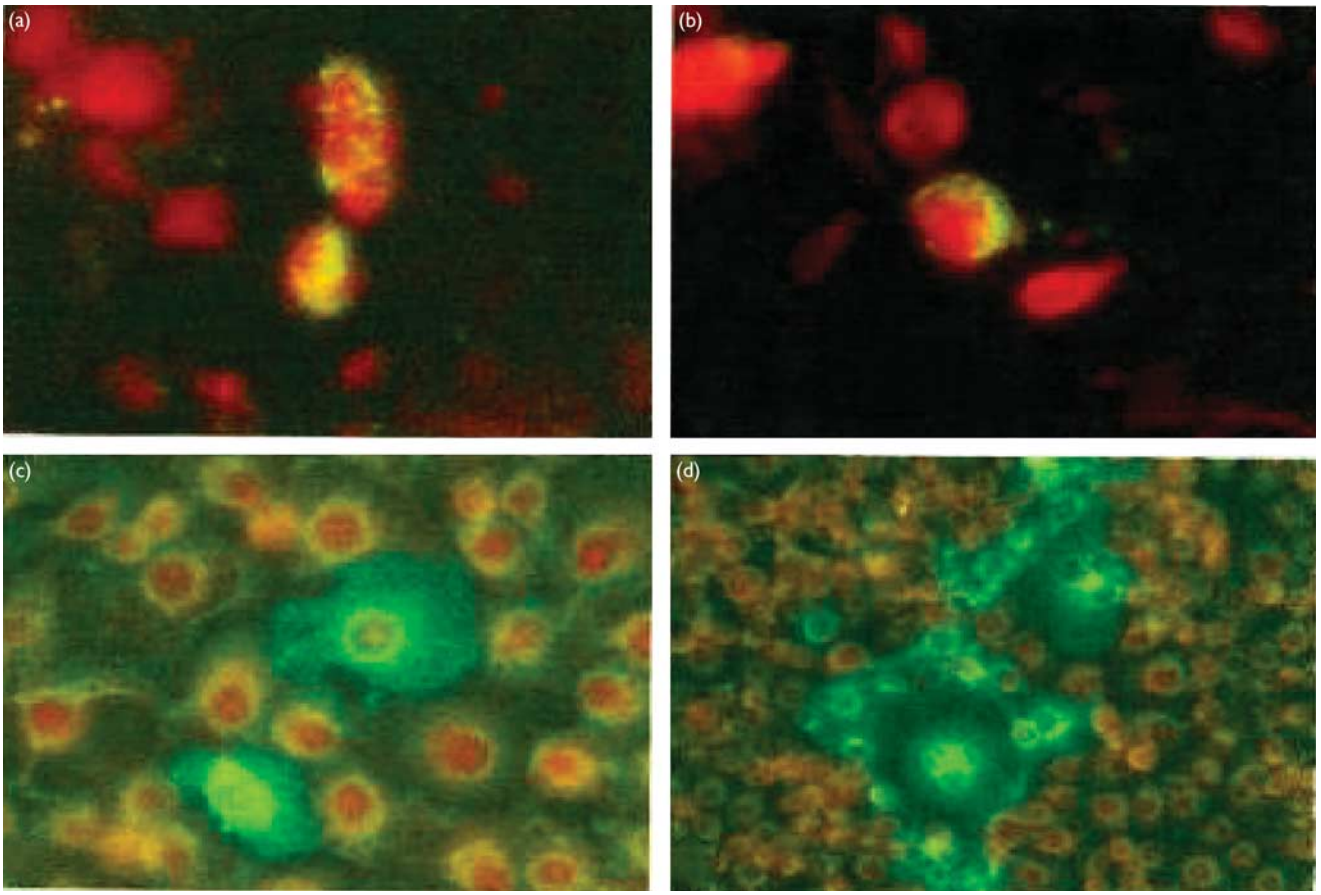
Months	February	March	April	May	June	July	August	September	October	November	December	January
Number of patients	0	3	0	0	0	0	3	2	0	0	0	0

Table 2 Clinical characteristics and outcomes of patients with human metapneumovirus infections

Age (years)	Sex	Pre-existing disorder	Symptoms and signs	Diagnosis	Department of admission	Outcome
16	M	Leukemia	Cough, fever, SOB	Pneumonia	ICU	Survived
13	M	NHL	Cough, fever, SOB, nasal congestion, wheezing	Pneumonia, respiratory failure	ICU	Died
19	F	SCD	Cough, fever, nasal congestion	Pneumonia	FMW	Survived
30	M	COPD	Cough, fever, SOB, wheezing	Pneumonia, respiratory failure	MMW	Survived
15	F	Cystic fibrosis	Cough, fever, SOB, nasal congestion	Pneumonia	FMW	Survived
21	M	GBS	Cough, fever, SOB, wheezing	Pneumonia, respiratory failure	ICU	Survived
14	M		Cough, fever, SOB, nasal congestion	Pneumonia	MMW	Survived
17	F		Cough, nasal congestion	Pneumonia	FMW	Survived

COPD, chronic obstructive pulmonary disease; FMW, female medi word; GBS, Guillain–Barré syndrome; MMW, male medi word; NHL, non-Hodgkin lymphoma; SCD, sickle cell disease; SOB, shortness of breath.

Figure 1



(a, b) Direct fluorescence antibody staining of respiratory mucosal cells from nasopharyngeal aspirates. (c, d) Human metapneumovirus (hMPV) isolation and identification in shell vial cell cultures using monoclonal antibodies of different nasopharyngeal aspirates. (c) Single infected cells from the surviving patients infected with hMPV. (d) A plaque of infected cells with small syncytial formations related to the deceased patient who had underlying non-Hodgkin lymphoma and had been infected with hMPV.

infection with hMPV associated with rapidly progressive respiratory failure in immunocompromised patients having NHL was correlated with the detection of a plaque of infected cells with small syncytial formations (Fig. 1d).

Because of the inability of nonspecific staining to detect hMPV, DFA staining was used to detect the virus in different NPAs (Fig. 1a and b). Development of mAbs against hMPV is an important advancement in the field of rapid direct diagnosis of viral respiratory tract infections. Following the introduction of hybridoma technology, mAbs against known respiratory viruses were developed and made commercially available. Since then, DFA staining using mAbs has become the most popular technique for direct diagnosis of acute respiratory infections, taking only 2–3 h to complete. Currently, DFA and molecular assays, such as RT-PCR, may be used either as alternatives or in combination for detection of respiratory

viruses in NPAs [22]. The combination of DFA and RT-PCR was applied in the present study. This combination was used to confirm the diagnosis, as in certain studies some samples had been diagnosed as negative by both RT-PCR and DFA staining, suggesting the possibility of the presence of extracellular viruses that were undetectable by DFA staining in the relevant NPAs but that were positive using hMPV-specific mAbs that react with all four hMPV subtypes [22]. In the present study, all samples from hMPV-positive patients diagnosed using RT-PCR were rechecked either with DFA staining or with shell vial cultures using mAbs.

Conclusion

hMPV is a significant pathogen in immunocompromised patients with a risk of high morbidity and mortality. Use of a combination of diagnostic workups may be useful for confirming the detection of hMPV. There are four known types of avian metapneumoviruses (A–D), with type C being the most closely related to hMPV [23]; however, we cannot exclude the existence of other as-yet-unidentified types of hMPV strains. The present study has certain limitations. It is not an accurate epidemiological research, and therefore may not represent the prevalence in the community. The frequency of viruses detected by molecular techniques is of those sampled and not of all those who are symptomatic. Moreover, the present study was not carried out on the general population; therefore, larger comprehensive studies are required to accurately detect the full spectrum of hMPV presentation and its impact on the healthcare system.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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