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Molecular Diagnosis of Human Metapneumovirus

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Abstract

BACKGROUND;The recent discovery of Human metapneumovirus (hMPV) as a major respiratory pathogen has been made possible by means of RT-PCR. Studies thus far published have mostly been conducted using the molecular approach.
OBJECTIVES: Clarification of epidemiological and clinical features and using molecular biological techniques for diagnosis of hMPV.
PATIENTS AND METHODS: 189 patients with suspected viral respiratory tract infections were included and respiratory specimens were analyzed for hMPV by Seeplex respiratory virus detection kit. Detection techniques that applied, include virus identification by TC-PCR, DFA staining and the rapid culture technique known as shell vial amplification using MAbs of nasal wash or aspirate fluid. The epidemiological and clinical data were analyzed and the later were represented as percentages where applicable.
RESULTS:The study determined 61(32.3%) respiratory viruses in the 189 respiratory samples and showed presence of hMPV in 8 (13.1 %) of 61 samples. hMPV showed variable seasonal activity. Six patients (75%) with positive hMPV had preexisting serious disorders. By using the shell vial cultures with monoclonal antibodies (MAbs), the related isolated virus of the patient with NHL, showed a plaque of infected cells with small syncytial formations, while that of the other seven patients showed single infected cells. All samples of hMPV positive patients with RT-PCR were correlated whatever with DFA staining or shell vial cultures by MAbs.
CONCLUSION: hMPV is a significant pathogen in immunocompromised patients with a risk of high morbidity and mortality. Using combination of diagnostic workup may be useful to confirm detection of hMPV.

Keyword: hMPV-Epidemiology-Clinical characteristics-Molecular biological approach-DFA-MAbs-RT- PCR-Acute respiratory illness

Introduction

Human metapneumovirus (hMPV) is a major cause of upper and lower respiratory infections in children and adults[1]. hMPV infection has been detected worldwide and has been proved to be the cause of upper as well as lower respiratory tract infections in young children, adults as well as the elderly. Severe and fatal hMPV infections have been reported in immuno compromised patients[2]. Clinical infection with hMPV in humans occurs throughout life, despite the fact that most individuals sustain humoral immune responses to both hMPV[3] and hRSV (human respiratory syncytial virus)[4]. Whilst the cellular immune response following hRSV infection is well-understood in humans[5] and in animal models[6] , in hMPV infection it is incompletely described. 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 (CTLs) in the control of hMPV infection is supported by in vivo mouse studies showing increased hMPV titres in T cell-depleted mice [8], and protection against infection by adoptive transfer of hMPV-specific CTLs[9] and hMPV-directed T-cell vaccines[10]. The strong association between hMPV infection and asthma in both childern[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 prelude to therapeutic intervention. Human metapneumovirus is a negative-sense non segmented RNA virus that has been categorized in the pneumovirus subfamily, family Paramyxoviridiae, based on 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 thus far published have mostly been conducted using the molecular approach. The availability of specific MAbs now opens the door to the routine use of DFA (Direct fluorescent antibody) 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

In the present study, 189 patients were included from February, 2011 to January, 2012, from internal male and female medical wards as well as from adult ICU-departement of KFSH and HGH, as a part of routine work up

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for patients presented with suspected viral respiratory tract infections. Respiratory specimens were submitted for centrifugation and supernatants were analyzed for hMPV by Seeplex respiratory virus detection kit from Seegene. This Seeplex system applies dual specific oligonucleotide (DPO) technology which produce greatly improve specificity without any false-positive. This DPO-based system is a fundamental tool that blocks extension of nonspecifically primed templates, thereby generating consistently high polymerase chain reaction (PCR) specificity, even under less- than-optimal PCR conditions. Positive RT-PCR for hMPV was verified by the Mayo Clinic Laboratory (United States). Detection techniques that applied include virus identification by transcriptase polymerase chain reaction (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 include nasal wash or aspirate fluid, nasopharyngeal flock swab culture[16]. The epidemiological and clinical data were analyzed and the later were represented as percentages where applicable.

Results

The study determined 61(32.3%) respiratory viruses in the 189 respiratory samples tested through the present work up and showed that the presence of hMPV in 8 (13.1 %) of 61 samples. Human metapneumovirus showed variable seasonal activity. The high incidence was in March, August and September, (Tab.1). Clinical characteristics and Outcome of hMPV infections were showen in the table 1. Six patients (75%) with positive hMPV had preexisting or underlying serious disorders. These disorders were leukemia, Non-Hodgkine lymphoma (NHL), Guillain-Barre´ syndrome(GBS), sickle cell disease, COPD, Cystic fibrosis. Clinical symptoms and signs at presentation to the hospital were cough in 8 (100%) patients, fever in 7 (87.5%), shortness of breath in 6 (75%), nasal congestion in 5(62.5%), wheezes in 3(37.5%). Patients with underlying leukemia, NHL and GBS (37.5%), were presenting with severe pneumonia which fulfill criteria of intensive care unit (ICU) admission. One of them who had NHL (12.5%), died after seventeen days of admission to ICU . Figure 1(A-B), showed direct fluorescent antibody(DFA) staining of respiratory mucosal cells from two different NPAs from the patients with hMPV-positive samples. By using the shell vial cultures with monoclonal antibodies (MAbs), the related isolated virus of the patient with NHL, showed a plaque of infected cells with small syncytial formations (Fig.1-D), while that of the other seven patients showed single infected cells (Fig.1-C).

Tab.1: Number of Patients with hMPV Infections Each Month Over A year.

Month	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
No. Of Patie-nts	**0**	**3**	**0**	**0**	**0**	**0**	**3**	**2**	**0**	**0**	**0**	**0**

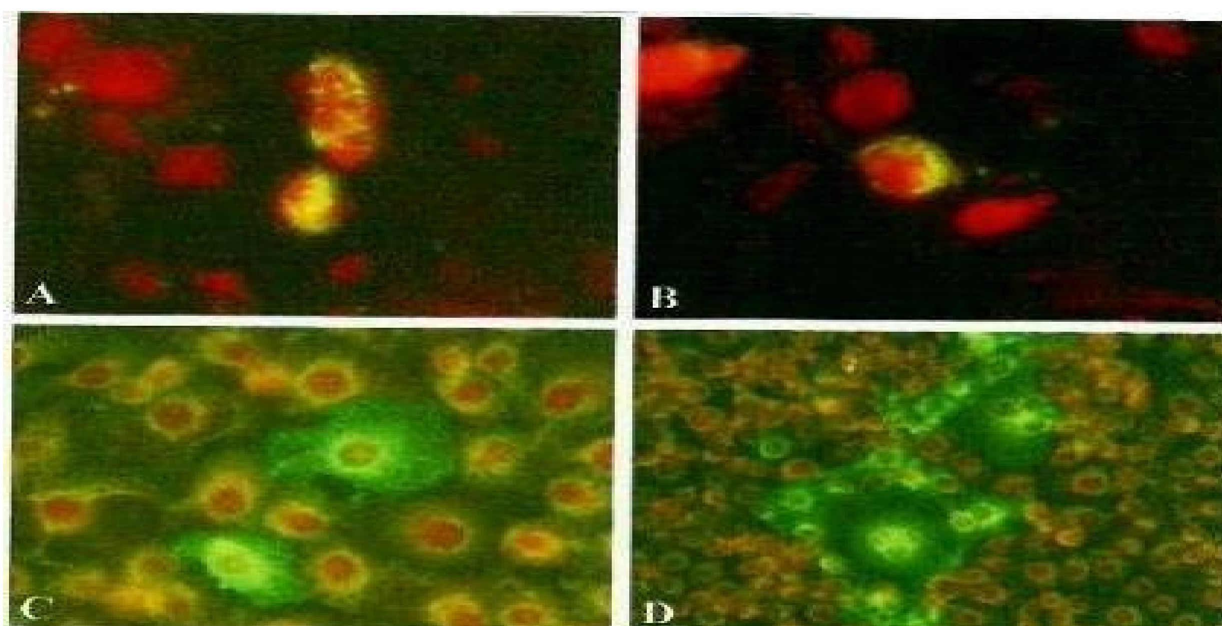
hMPV = Human metapneumovirus.

Tab.2: Clinical Characteristics and Outcomes of Patients with hMPV Infections.

Age(yrs)	Sex	Preexisting disorder	Symptoms & 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

SCD=Sickle cell disease GBS=Guillain-Barre´ syndrome SOB=Shortness of breath

Figure (1): A & B are showing DFA staining of respiratory mucosal cells from NPAs. C & D are showing hMPV isolation and identification in shell vial cell cultures by using MAbs of different NPAs. (c); Single infected cells related to the survived patients infected with hMPV.(D); A plaque of infected cells with small syncytial formations related to the died patient with underlying NHL and infected with hMPV.



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Discussion

The recently identified hMPV is the only member of the genus Metapneumovirus (family Paramyxoviridae, subfamily Paramyxovirinae), which also includes avian pneumoviruses A, B, C, and D, that infects humans. hMPV is responsible for a fair proportion of respiratory infections in early infancy and childhood, and also in the elderly and in immuno compromised hosts[17]. In the present study, hMPV was detected in 13.1% of those hospitalized with lower respiratory tract infections (LRTIs). These finding are slightly higher than those reported in United states[18], Europe[19], and Australia[20]. This may be explained by the presence of multi nationality population in Makkah region. Most of the reported studies revealed that infections of hMPV were significantly higher among children in comparison to that among younger and older children[14]. Our present study reported seven patients (87.5%) from eight were between 13-21 years old. That means the hMPV infects also adults as well as older children. A broad seasonal activity of hMPV with distinctive pattern in different years was reported by Sloots et.al.[20] In the present study, the peak incidence was in March, August and September, this reflects that the seasonality of hMPV is becoming evident.

The present study reported that hMPV infections in immuno compromised patients were characterized by several respiratory symptoms, including cough, fever, shortness of breath, nasal congestion. Three patient were admitted to ICU with rapidly progressive respiratory failure with pneumonia and culture-negative shock. hMPV was the only detected respiratory pathogen in NPA or BAL in those three patients. One of them with NHL, died inspite of intensive and aggressive therapy in ICU. These figures were similar to those reported by Englund et.al.,2006[21]. The severity of infection with hMPV-associated with rapidly progressive respiratory failure in immunocompromised patient having NHL, was correlated with detection of a plaque of infected cells with small syncytial formations (Fig.1-D). This can be explained by the severity of infection.

Due to problems of nonspecific staining which can not detect hMPV, direct fluorescent antibody staining was used to detect the virus from different NPAs (Fig.1-A,B). Development of MAbs to hMPV is an important advance in the field of rapid direct diagnosis of respiratory tract viral infections. Following the introduction of hybridoma technology, MAbs to known respiratory viruses were developed and made commercially available. Since then, DFA staining using MAbs has become the most rapid technique for direct diagnosis of acute respiratory infections, taking only 2 to 3 h to perform. 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], and the later was applied in the present study. This combination was used to confirm the diagnosis, as certain studies reported that some samples were diagnosed as negative by both RT-PCR and DFA stextracellular virus not detectable by DFA staining in the relevant aiming, suggesting the possibility of the presence of NPAs but were positive by hMPV-specific MAbs which react with all four hMPV subtypes[22]. In the present study, all samples of hMPV positive patients with RT-PCR were rechecked either with DFA staining or Shell vial cultures by MAbs.

Conclusion

hMPV is a significant pathogen in immuno compromised patients with a risk of high morbidity and mortality. Using combination of diagnostic work up may be useful to confirm detection of hMPV. Since avian metapneumoviruses belong to four different types (A through D) and type C is the closest to hMPV[23], 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 so may not represent the prevalence in the community and the frequency of viruses detected by molecular techniques are of those sampled and not of all those who are symptomatic. Also, the present study was not carried out on the general population, So more large comprehensive studies are in need to detect the accurate full spectrum of hMPV presentation and its impact on the health care system.

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