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Full Length Research Paper

Human Metapneumovirus (hMPV) associated with respiratory infection in children hospitalized with acute lower respiratory tract infection in Hilla, Iraq

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Acute respiratory infection (ARI) and lower respiratory tract infection (LRI) are the main cause of childhood death world wide. Human metapneumovirus (hMPV) and respiratory syncytial virus are the most frequently implicated in childhood illness. The study aim to provide first information about circulation of hMPV in Hilla–Iraq. The current study utilized direct immunoflourescent antibody technique to identify hMPV in nasopharyngeal swabs specimens. The samples were collected from 150 infants (few days-to two years of age) who had acute respiratory tract infection and were admitted to Babylon Maternity and Children Hospital in Hilla. The results of direct immunofluorescent assays revealed that 13.3% of patients were positive for hMPV and most of them were less than 2-3 months of age. The females were more affected than male at a ratio of (1.5:1), the reinfection with multiple times was found in 5 cases during the same hMPV season. The hMPV should be considered as a serious cause of ARI as well as RSV. The study recommended a direct immunofluorescent assay as a good and reliable test for hMPV diagnosis in our country.

Key words: HMPV, ARI, LRI, DIFA, diagnosis.

INTRODUCTION

Human metapneumovirus (hMPV), a respiratory virus, has recently been recognized as an etiologic agent of respiratory tract infections in children and adults (van den Hoogen et al., 2001, Boivin et al., 2002) .The hMPV is genetically related to human respiratory syncytial virus (hRSV), Both hMPV and hRSV are grouped in the subfamily *Pneumovirinae*, of the family *Paramyxoviridae* (van den Hoogen et al., 2001). The clinical manifestations and epidemiology of hMPV and hRSV have been reported to be very similar (van den Hoogen et al., 2003, Peiris et al., 2003, Williams et al., 2004). Both viruses are separated into two groups by genetic differences (Ishiguro et al., (2004).

Several lines of evidence suggested that hMPV is a common human respiratory pathogen. Previous studies have indicated that hMPV causes mild respiratory tract infections in healthy adults (Stockton et al., 2002).

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However, it has been shown that children under 2 years of age, elderly people over 50 years old, and immunocompromised patients are at greater risk of lower respiratory tract infections, such as bronchitis, pneumonia, and bronchiolitis (van den Hoogen et al., 2004). hMPV has been revealed to be associated with acute wheezing in children (Jartti et al., 2002, Ebihara et al., 2004, Xepapadaki et al., 2004).

The immunofluorescent-antibody test of respiratory epithelial cells has been shown to be a very useful method to detect other respiratory viruses, such as hRSV, parainfluenza virus, adenovirus, and influenza virus (van den Hoogen et al., 2003). The aim of this study was to provide firsthand information about the infection with hMPV in Hilla-Iraq by detecting the virus in nasopharyngeal secretions through a direct immunofluorescence assay (DIFA).

MATERIALS AND METHODS

The nasopharyngeal swab samples were collected from 150

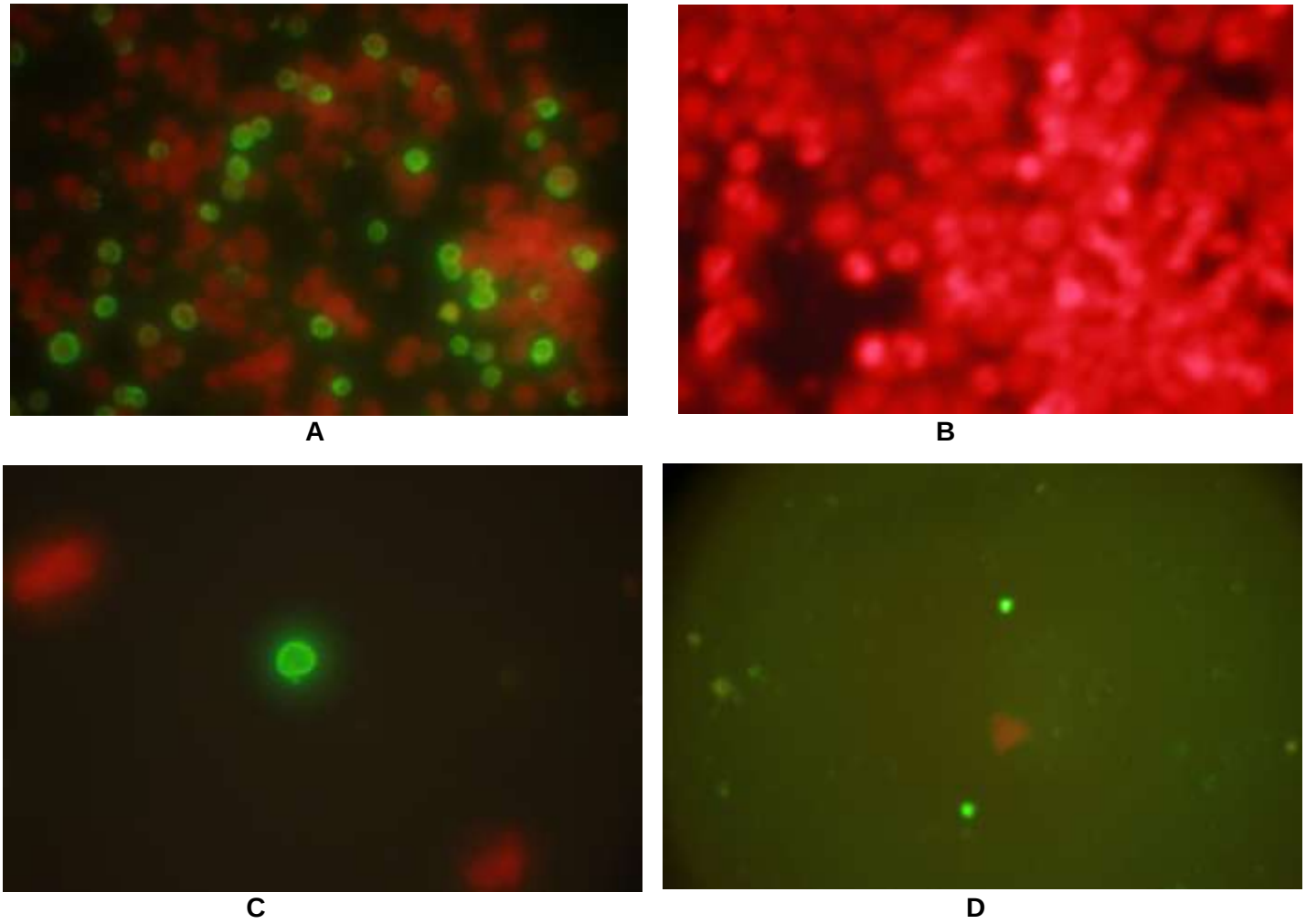

Figure 1. The results of hMPV by indirect immunoflourescent assay(IFA). A and B:positive and negative controls (supplied with the test kit on tissue culture). C :positive nasopharengeal swab sample .D-: positive nasal wash samples

150 children who were attended to in the hospital as out and in patents. The samples were collected over the period from Nov...2010 to May 2011. The Samples were placed in 2 ml of Eagle's minimum essential medium for testing by DIFA. The female-to-male ratio was 1.5 to 1. The mean age of the children was 2 years (age range, from > 1 month to 2 years). All samples were collected after informed consent was obtained from the children's parents.

Nasopharyngeal swab samples for DIFA were mixed gently to create a free cell suspension. The cell suspension was centrifuged, and the cell pellet was resuspended in 50 to 400 µl of phosphate-buffered saline (PBS). Cell smears were prepared by spotting 1 drop of the cell suspension onto a slide glass. Then, the smears were air dried and fixed in acetone for 10 min. For detection of hMPV antigens by IFA, we then added one drop of hMPV reagent, Smears were incubated for 30 min at 37°C with the anti-hMPV mouse monoclonal antibody at a dilution of 1:80 and non-immune mouse antibody as a negative control. Then incubated for 30 min at 37°C with fluorescein isothiocyanate-conjugated rabbit anti-mouse Ig (Dako, Glostrup, Denmark) at a dilution of 1:40 with 0.001% Evans blue. After incubation, they were washed twice in PBS for 10 min each time, air dried, and mounted with PBS-glycerin (1:1). We confirmed that there were at least more than 100 epithelial cells on one spot at x40 magnification under a fluorescent microscope. We examined approximately 100 to 500 epithelial cells for the presence of specific fluorescence patterns, such as granular staining and diffuse staining, in the cytoplasm.

In an attempts to obtain better DIFA specimens, different specimens (including a nasal wash alone or combined nasal and oro-pharyngeal swabs) were obtained from a two years group of patients. A small number of tracheal aspirates and one brush biopsy pulmonary specimen were also evaluated. Nasal wash specimens were adequate, but even after careful centrifugation they cont-

Table 1. The peak age for hospitalized children infected with hMPV.

Age group	No. of cases	No. of	Percent
Few days-1month	9	0	0
2-3 month	12	3	25%
4-5	33	5	15%
6-7	27	4	14.8%
8-9	15	2	13.3%
10-11	24	3	12.5%
1-2 years	30	3	10%
Total	150	20	13.3%

Table 2. The gender distribution in relation to hMPV infection.

Gender	No. of cases	No. of positive	Percentage
ale	54	7	12.9%
female	96	17	17.7%
Total	150	20	13.3%

Table 3. Type of signs and symptoms among the study group.

Type of symptoms	No. of patient	+ve to, h MPV	Percentage
Cough	50	20	40%
Dyspnea	37	5	13%
Wheezing	49	20	40%
Pneumonia	50	20	40%
Broncholitis	50	20	40%
Croup	50	14	28%
Vomiting	35	13	37%
laryngotrachitis	50	20	40%

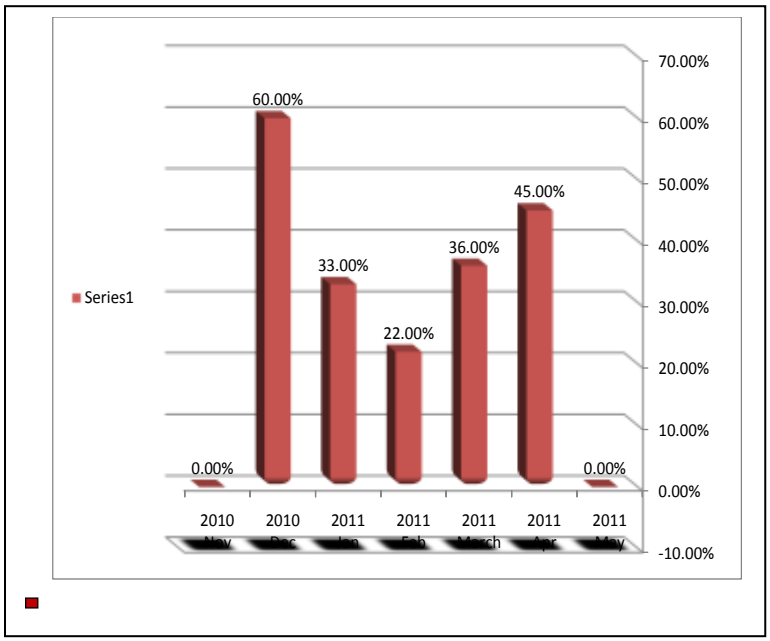

Figure 2. The seasonal pattern of hMPV infection.

ained debris such as mucus, squamous cells, leukocytes, and erythrocytes. Also, the numbers of cells obtained by nasal wash were smaller than those obtained by nasal med according to the manufacturer instruction (Chemicon (Millipore, Light diagnostics).

RESULTS

The hMPV was detected in 20 (13.3%) out of 150 child less than 2 year by direct immunoflourecsence Assay (DIFA) (Figure 1) . The hMPV was detected in 20 hospitalized children less than 2 years of age (Table 1). and oropharyngeal swabs. Test procedures were perfor- Gender distribution revealed that the females (17.7%) were affected more than male (12.9%) in a male to female ratio 1.5:1 (Table 2 .) All of children positive for hMPV by DIFA had lower respiratory tract signs and symptoms, including wheezing, dyspnea, bronchiolitis and pneumonia. Bronchiolitis was diagnosed in 20 (40%) and pneumonitis in 20 (40%) of hMPV-infected patients (Table 3). The seasonal pattern of infection showed that

the peak of infection were in December and April .The reinfection with multiple hMPV was found in five cases during the same hMPV season (figure 2).

DISCUSSION

Previous studies have suggested that hMPV added to the list of human respiratory viral pathogens substantially contributes to ARTI that lead to childrens hospitalization. (van den Hoogen et al., 2001, Jartti et. al., 2002 Nissen et.al., 2002). The positivity in the current works among the infected children and the severity of the symptoms (bronchiolitis, pneumonitis, or both) suggest that hMPV is a pathogenic respiratory virus with a wide distribution in lower and upper tract.

The current prospective study provides as the first attempts to estimate the proportion of ARI and LRI hospitalizations attributable to hMPV in a well-defined pediatric population in Hilla, Iraq .The results revealed that out of 150 patients 20 (13.3%) were positive for hMPV. This data are comparable to the study from Finland in which hMPV was detected in 8% of children (age range, 4 months to 13 years) admitted for acute wheezing. (Jartti et.al., 2002).

The current study found that HMPV disease cannot be distinguished from RSV and influenza A on clinical findings.

A serologic study from the Netherlands showed that all children >5 years of age had hMPV antibodies, which suggests a high level of transmission (van den Hoogen et al., 2003) . While the data of this study were limited to children ≥ 2 years of age, other reports suggested that illness caused by hMPV is greatest in children <2 years of age because they represented 10 (83%) of 12 of hospitalized case-patients and 24 (66%) of 36 of the hMPV detected by (IFA). During the 4-week period from mid-March to mid-April, hMPV infections were associated with 18.9% of all hospitalizations for ARI in children. These findings contrast with those for RSV and influenza A infections, which occurred mostly in January and February. Many reports suggested that seasonal outbreaks of HMPV may differ from those of other common respiratory viruses (Stockton et al., 2002, Treanor et al., 2002).

The current study supports the concept of the epidemic nature of hMPV infection and its role as a significant pathogen in severe ARI and LRI of children and the importance of using DIFA as a good tool for laboratory diagnosis for this virus in our country (Iraq). Active surveillance studies on consecutive years and in different geographic regions are needed to better define the epidemiology of hMPV in Iraq.

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