การตรวจหา Human metapneumovirus antigen ในน้ำล้างหลังโพรงจมูก ด้วยวิธี immunofluorescence assay

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บทคัดย่อ

เชื้อฮิวแมนเมตะนิวโมไวรัส เป็นไวรัสก่อโรคระบบทางเดินหายใจที่ได้ถูกแยกเมื่อไม่นานนี้ โดยมีความสำคัญที่ก่อให้เกิดโรคติดเชื้อในระบบทางเดินหายใจส่วนบนและส่วนล่างของเด็กและผู้ใหญ่ แม้ว่าการตรวจวินิจฉัยการติดเชื้อเอชเอ็มพีวีทางห้องปฏิบัติการจะเชื่อถือการตรวจโดยใช้เทคนิคทางอณูชีวโมเลกุล แต่มีข้อจำกัดเรื่องราคา การตรวจวินิจฉัยด้วยวิธีอื่น เช่น การตรวจด้วยเทคนิคอิมมูนเรืองแสง (Immunofluorescence assay, IFA) ยังคงมีความต้องการ เนื่องจากมีราคาถูกและให้ผลตรวจที่รวดเร็ว ดังนั้น วัตถุประสงค์ของการศึกษานี้เพื่อทำการตรวจประเมินวิธีการทดสอบด้วยวิธีอิมมูนเรืองแสงชนิดไดเรคและการหาความชุกของการติดเชื้อเอชเอ็มพีวี ในโรงพยาบาลศิริราช ประเทศไทย โดยใช้ตัวอย่างตรวจจากน้ำล้างหลังโพรงจมูกจำนวน 655 ตัวอย่างจะถูกเก็บใน viral transport media (VTM) และนำส่งมายังห้องปฏิบัติการจุลชีววิทยา โรงพยาบาลศิริราช ในระหว่างเดือนพฤษภาคม 2555 ถึง เดือนเมษายน 2556 ขณะที่ตัวอย่างจำนวน 475 ตัวอย่างจะถูกเก็บระหว่างเดือนกรกฎาคม 2556 ถึง เดือนมิถุนายน 2557 ค่าความไวของวิธีอิมมูนเรืองแสงจะถูกเปรียบเทียบกับปฏิกิริยาลูกโซ่โพลีเมอเรสในสภาพจริง โดยพบว่ามีค่าความไวร้อยละ 95.5 และความจำเพาะร้อยละ 94.7 เมื่อเทียบกับปฏิกิริยาลูกโซ่โพลีเมอเรสในสภาพจริง ความชุกของการตรวจพบเชื้อเอชเอ็มพีวีในเดือนพฤษภาคม 2555 ถึง เดือนเมษายน 2556 พบร้อยละ 3.8 (25/655) และเดือนกรกฎาคม 2556 ถึง เดือนมิถุนายน 2557 พบร้อยละ 5.7 (27/475) ค่าความชุกของเชื้อเอชเอ็มพีวีจะพบสูงที่สุดในเด็กอายุน้อยกว่า 5 ปี และจะพบมากในช่วงเดือนสิงหาคม 2555 ถึง เดือนกุมภาพันธ์ 2556 และเดือนสิงหาคม 2556 ถึง เดือนมกราคม 2557 ซึ่งอยู่ในช่วงปลายฤดูฝนต้นฤดูหนาวของประเทศไทย

คำสำคัญ: ฮิวแมนเมตะนิวโมไวรัส น้ำล้างหลังโพรงจมูก อิมมูนเรืองแสง ความไวและจำเพาะ ความชุก

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Detection of human metapneumovirus antigen in nasopharyngeal washing specimens by immunofluorescence assay

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Abstract

Human Metapneumovirus (hMPV), a recently identified respiratory virus, has emerged as an important etiologic agent of upper and lower respiratory tract infections in children and adults. Although laboratory diagnosis of hMPV infection relied on molecular biology technique, cost of testing is a limitation of this test. The alternative method such as immunofluorescence assay (IFA) is required because it is cheaper and faster. Thus, aim of this study was to evaluate a direct antigen test by IFA and determine prevalence of hMPV infection in Siriraj hospital, Thailand. A total of 655 nasopharyngeal washing specimens were collected in viral transport medium and sent to Microbiology laboratory, Siriraj Hospital during May 2012 to April 2013 and 475 nasopharyngeal washing specimens were collected in July 2013 to June 2014. The sensitivity and specificity of IFA was compared to real time PCR. The sensitivity and specificity of IFA were 95.5%, 94.7%, respectively, when compared to real time PCR. The prevalence of hMPV during 2012-2013 was performed by using IFA and found that it was 3.8% (25/655) during May 2012 to April 2013 and 5.7% (27/475) during July 2013 to June 2014. The highest prevalence of hMPV infection was detected in children aged less than 5 years. Peaks of detection were found in August 2012 to February 2013 and August 2013 to January 2014, which is the rainy and winter season in Thailand.

Keywords: human metapneumovirus, nasopharyngeal washing specimens, immunofluorescence, sensitivity and specificity, prevalence

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Introduction

Human metapneumovirus (hMPV) belongs to family *Pneumoviridae* genus *Metapneumovirus*. The primary target for hMPV is young children, immunocompromised hosts, and patients who have underlying conditions. Since hMPV is genetically related to respiratory syncytial virus (RSV), hMPV and RSV are grouped in family *Pneumoviridae*. The clinical impact and epidemiology is very similar to RSV infection and the clinical sign alone cannot distinguish these two viruses.[1,2] The symptoms of hMPV infection in young children vary from mild upper to severe lower respiratory tract diseases. hMPV can trigger asthma in adults and young children. The epidemic season of hMPV in Europe and USA is from winter to early spring. In Thailand, hMPV is circulated during May-September.[3] Previous study has been shown that children under 2 years of age, elderly people over 50 years old and immunocompromised have greater risk of lower respiratory tract infections.[4,5,6]

Laboratory diagnosis of hMPV infection was relied on molecular biology technique, immunofluorescence and viral isolation. Rapid and accuracy of hMPV diagnosis in laboratory are required for infection control. Molecular biology technique was a fast and reliable method for hMPV detection but the cost was expensive. Although, the isolation of hMPV by cell culture was a gold standard method for viral diagnosis, this method was very difficult because it grew very slow and showed weak cytopathic effect.[7] Indirect immunofluorescence antibody (IFA), an alternative method, is an antigen detection method which could stain hMPV antigen on infected epithelial cells. The sensitivity of IFA is lower than RT-PCR, required appropriate respiratory epithelial cell number in samples, time consuming, experience technician and a fluorescence microscope but simple and inexpensive method.[7,8] Thus, IFA is the alternative method for first line detection with simple laboratory procedure.

Objective

To evaluate sensitivity and specificity of IFA for hMPV detection compared to real time PCR method and investigate the prevalence of hMPV during May 2012 through April 2013 and July 2013 to June 2014 by using IFA.

Materials and Methods

Ethics statement

The study was conducted in accordance with the Siriraj Institutional Review Board (SIRB) [Exempt. 565/2560 (EC1)].

Clinical samples

Nasopharyngeal washing (NPW) specimens from 655 of suspected patients with acute respiratory infection during May 2012 through April 2013 were collected in viral transport media (VTM) and sent to Microbiology laboratory, Department of Microbiology, Faculty of Medicine, Siriraj Hospital for respiratory viruses detection.

Total of 475 of samples were not showed positive to influenza A and B viruses, parainfluenza 1, 2, 3 viruses, respiratory syncytial virus (RSV) and adenovirus (group specific) by IFA. Of these samples were used for hMPV detection by using IFA.

Specimens preparation

NPW samples were prepared by adding 5-7 sterile beads and mixed thoroughly to decrease viscosity. The specimens were centrifuged at 2,500 rpm 4°C for 15 minutes. Supernatants were aspirated and used for virus isolation. Pellets were prepared for indirect immunofluorescence tests by washing with 5 ml of PBS and centrifuged at 1,500 rpm 4°C for 10 minutes. Supernatants were discarded and 10 µl of pellet were dropped onto slides. Slides were air-dried for 2 hours and fixed with cold acetone for 15 minutes. Slides were kept in -20°C until use.

Indirect immunofluorescence test (IFA)

Slides were stained by adding monoclonal antibody specific to hMPV (cat. no. 6002RUO, Chemicon. Temecuta, CA) and incubated at 37°C for 30 min in moisture chamber. Slides were washed with PBS for 15 min before incubation with anti-mouse immunoglobulin labeled with FITC (cat. no. 3105, Chemicon. Temecuta, CA) at 37 °C for 30 min. Slides were washed with PBS for 15 min and rinsed with distilled water. A positive hMPV virus antigen presented by apple-green fluorescence color cell was examined under fluorescence microscope.

hMPV detection by real time PCR

Total viral RNA was extracted from a 200 µl of processed NPW by using Nuclisens Magnetic Extraction Reagents (Biomerieux, France). The hMPV was detected by real time PCR according to the manufacturer's recommended procedures with Multiplex real time PCR (Allplex™ Respiratory Full Panel (Seegene, Korea)).

Statistical analysis

The sample size calculation was done by using nQuery Advisor software.

Results

A total of 60 nasopharyngeal washing specimens were collected during 2013 and used for IFA evaluation by comparing with real time PCR. Twenty-one PCR positive and IFA positive samples was demonstrated. One PCR positive and IFA negative samples was detected and considered false negative for IFA. Two PCR negative and IFA positive were detected and considered false positive for IFA. The analytical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of hMPV by IFA was 95.5%, 94.7%, 91.3% and 97.3%, respectively (Table 1).

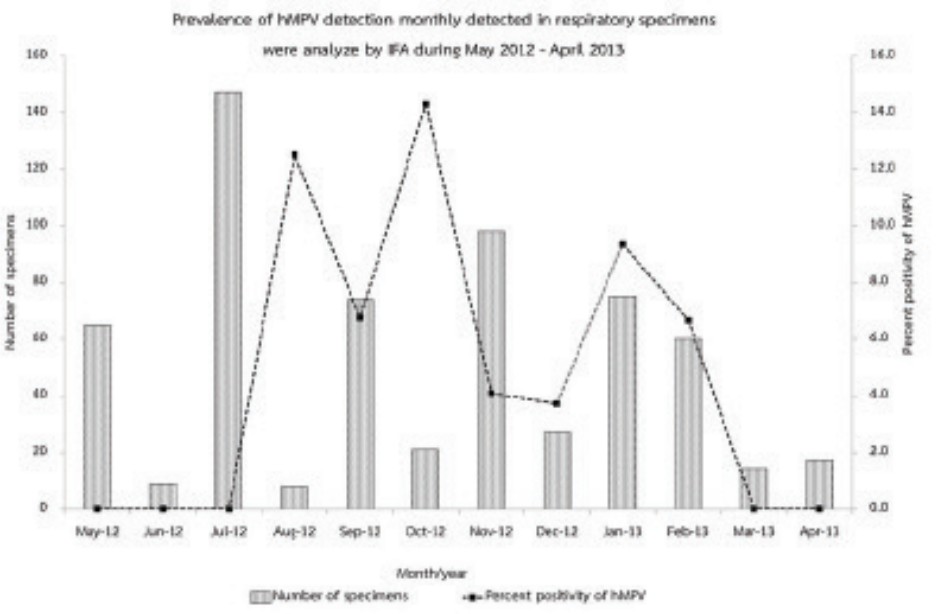
Table 1 hMPV detection by IFA compared to the real time PCR result

IFA	Total	Real time PCR	
		Positive	Negative
Positive	23	21	2
Negative	37	1	36
Total	60	22	38

During May 2012-April 2013 and Jul 2013-June 2014, a total of 1,130 nasopharyngeal washing specimens were collected and tested for respiratory viruses. Of these, 655 specimens (May 2012-April 2013) were selected randomly for hMPV detection by using IFA and real time PCR methods. The monthly prevalence of infected hMPV detection was analyzed by IFA and shown in Figure 1. The prevalence of hMPV in this period was 3.8% (25/655). Peaks of hMPV were demonstrated in August 2012 (12.5%, 1/8), October 2012 (14.3%, 3/21), and January 2013 (9.3%, 7/75) (Figure 1A).

Another period (Jul 2013-June 2014), a total of 475 specimens were investigated for hMPV by IFA compared with real time PCR. The prevalence of hMPV in this period was 5.7% (27/475). Peaks of hMPV were demonstrated in August 2013 (13.6%, 3/22), November 2013 (28.6%, 2/7), January 2014 (4.2%, 3/72), and May 2014 (1.1%, 1/89) (Figure 1B).

(A)



(B)

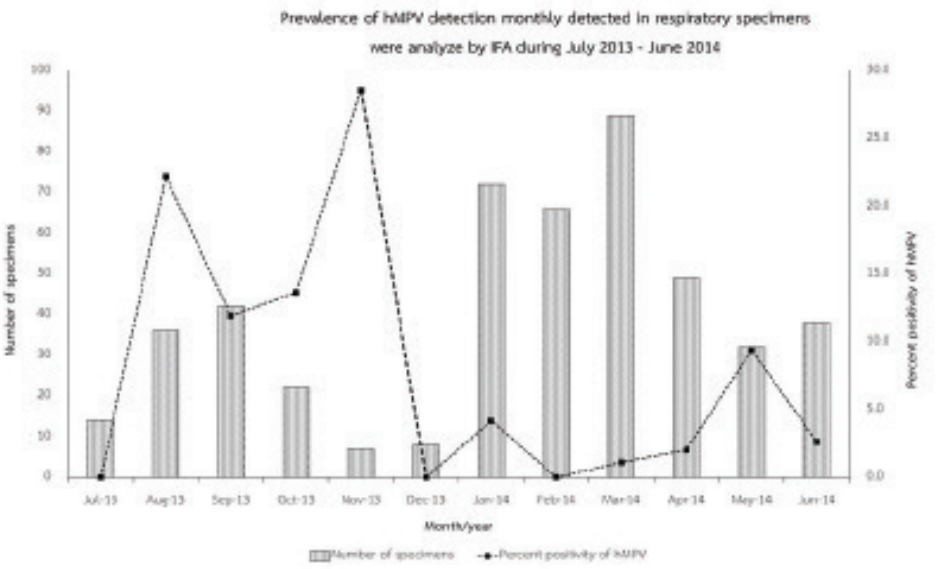


Figure 1 Prevalence of hMPV detection in respiratory samples analyzed by IFA during May 2012-April 2013 (A) and Jul 2013-June 2014 (B)

Table 2 Age of patients infected hMPV during May 2012-April 2013 and Jul 2013-June 2014

Age (year)	May 2012-April 2013	Jul 2013-June 2014
≤5	18 (72%)	23 (85.2%)
>5-10	2 (8%)	0
>10-15	0	3 (11.1%)
>15-20	0	0
>20	0	1 (3.7%)
Unknown	5 (20%)	0
Total	**25 (100%)**	**27 (100%)**

A total number of 52 positive infected hMPV detection by IFA were aged from 26 days to 80 years; mean, 4 years. Patients who younger than 5 years was the most frequently affected by hMPV (78.8%) followed by aged 5 through 10 years (3.8%) (Table 2).

Discussion

The epidemic of hMPV was increasing after hMPV discovered. Most cases were detected in children aged less than 5 years. The method for hMPV detection was relied on molecular biology technique but its cost was expensive. Another method such as IFA

was an alternative method for viral diagnosis and widely use in laboratory. Thus, this study evaluated hMPV by IFA method. The result showed that the sensitivity and specificity of hMPV by IFA-based assay comparing to real time PCR assay was 95.5% and 94.7%, respectively. A previous study from Japan reported the sensitivity and specificity of the IFA was 73.3% and 97.0% respectively.[8] Another study from Reina showed direct antigen detection in the clinical sample by the IFA with sensitivity of 70-75% against the real time PCR.[1] Our study demonstrated higher sensitivity of IFA detection than previous study. This might be caused by the different clone of antibody used in IFA. The specificity of our test was comparable to previous study. In addition, two samples showed weakly positive by IFA but negative by real time PCR. This false positive results by IFA might be caused by misleading interpretation from non-specific background and cross reactivity to RSV. The genetic analysis between hMPV and RSV was large shared similarity.[9] Thus, antibody of hMPV could cross-react to RSV. From our laboratory data, these two cases were negative for RSV by real time PCR. The cross reactivity between hMPV and RSV in this study might not be concerned. Moreover, one PCR positive and IFA negative sample was considered as false negative. The false negative result might be caused by inadequate cells in smear and sampling period. PCR can detect hMPV even a few cells. The collection of hMPV specimens during 8-14 days after onset demonstrated negative for IFA but positive for PCR.[8,10] Our previous data was supported the IFA sensitivity of this study which showed that the sensitivity of IFA was not 100% comparing to the real time PCR (unpublished data).

The application of IFA to detect hMPV infection during 2012-2014 was performed and demonstrated that the prevalence of hMPV was 3.8-5.7% in children aged ≤ 5 years. This was concordance to the other countries such as Cambodia (1.7%), England (2.2%), the Netherlands (7.5%), Canada (14.8%), and China (6.8%).[3,11,12] Moreover, the peak of hMPV infection was found in August to November, the late rainy to early winter season in Thailand. This study presents data during the rainy season in the same pattern as other tropical countries.[3,11,13]

Conclusion

In this study, the development of IFA to detect hMPV infection can be used as alternative method for rapid detection. The sensitivity, specificity, PPV and NPV was 95.5%, 94.7%, 91.3% and 97.3%, respectively. Moreover, we apply IFA for epidemiological study of hMPV.

Acknowledgements

We are grateful to Assist. Prof. Dr. Popchai Ngamskulrungroj, Department of Microbiology, Faculty of Medicine Siriraj Hospital for his comment on the manuscript.

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