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Original Article

Reverse Transcription Polymerase Chain Reaction (RT-PCR) as an influenza diagnostic test among children in Yogyakarta

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Abstract

Background Influenza virus type A, especially H5N1 subtype or avian influenza, is a highly pathogenic agent that causes epidemic in the world with high mortality. Most cases are preschool and school children. Anti-viral drug is effective when given at early phase. The gold standard for the diagnosis of influenza is viral culture, which takes 2 to 10 days. A rapid and accurate diagnostic test is needed to control further viral infection.

Objective To determine the accuracy of RT-PCR as a diagnostic test for children with influenza compared with viral culture.

Methods A cross-sectional study was conducted in primary health cares of Jetis I, Godean I, II and Dr Sardjito Hospital Yogyakarta between January 2005 and May 2007. The specimens, taken by trained health personnel, were collected from both anterior nares and throat of children aged from birth to 14 years who met the eligibility criteria, then were stored in a frozen extraction tube and sent to Jakarta for RT-PCR and viral culture as the gold standard.

Results There were 347 children enrolled in this study. Influenza infection was confirmed in 63 children (18.2%). There were 24 children with H3N2 subtype of influenza virus, 13 children with HINI subtype, and one child with H5N1 subtype. The sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio of RT-PCR test were 89%, 90%, 67%, 97%, 9, 3, 0, and 12 respectively.

Conclusions RT-PCR is accurate enough as influenza diagnostic test in children. **[Paediatr Indones 2008;48:288-91]**.

Keywords: *influenza, PCR, diagnostic test*

Influenza virus can cause respiratory disease in all age groups. Preschool and school age children are the most affected age groups.[1-3] Out of three types of influenza viruses (A, B, and C), influenza virus type A is the most pathogenic one. Subtypes of influenza virus are determined by hemagglutinin (H) and neuraminidase (N) tests.[4-6] These viruses are reported to cause outbreak worldwide both pandemic and endemic. A pandemic of influenza occurred in Hong Kong in 1977 caused by H5N1 known as Avian Influenza or bird influenza.[3,7,8]

Until April 2007 there had been 291 cases confirmed bird influenza around the world with the mortality rate of 60%. The total cases in Indonesia until April 2007 were 99 cases, with fatal cases reported by 77 or about 79%. This condition ranked Indonesia as the second highest place after Vietnam.[8] Mortality caused by this disease is commonly related to complications such as pneumonia, encephalitis, and ARDS (*acute respiratory distress syndrome)*. In

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America, death caused by influenza in children less than five years of age is estimated to be 0.4/100,000 children.[9]

The diagnosis of acute respiratory infection based on only clinical findings is difficult,[10] while antivirus is more effective if it is given within the first 48 hours since the symptoms appear.[11,12] The 'gold standard' diagnostic tool is viral culture that takes 2 to 10 days for the result. The culture result also depends on time, specimen taking method, and specimen storage; good result if it is done in the early of disease development.[8,13-15]

Reverse Transcription Polymerase Chain Reaction (RT-PCR) test is a molecular level diagnostic test that can detect viruses by replicating DNA accurately and rapidly. Another advantage of PCR is that the reaction can be done by using components in a small number and it does not need live viruses.[16, 17] RT-PCR test takes about two to four hours with high sensitivity and specificity as the diagnostic test tool.[16,18-20] However, this technique is costly. High sensitivity and specificity was found in Indonesian children four years of age and adults,[19] but the study exclude subtype H5N1 which is actually the major cause of death.[6] This study was conducted to investigate the value of RT-PCR in diagnosing influenza viruses using viral culture as the gold standard. Besides, the prevalence of influenza and the pattern of influenza virus subtypes among children in Yogyakarta can be established which will be beneficial as the material for policy making regarding healthcare field in Indonesia.

Methods

We conducted a diagnostic test study on the value of RT-PCR diagnostic tool of influenza among children in Yogyakarta. Subjects were children aged 0 to 14 years old suspected to have respiratory infection treated in Dr. Sardjito Hospital and primary health centers of Jetis and Godean, Yogyakarta, started from 1st January 2005 to 30th May 2007. We included subjects with fever ≥ 37.5°C accompanied by one or more of the underlying symptoms such as cough, runny nose, difficult or painful to swallow, or pain at the throat with or without difficulty to breath, and the parents agreed to participate in the study, and excluded patients had got antiviral previously.

Sample size was calculated with sensitivity and specificity of 90%, significance value of 0.1 and the prevalence of 20% to obtain the minimal sample size of 180. Informed consent was obtained from the parents.

We performed history taking and physical examination to all study subjects. The trained health personnel took the specimen from nasal and throat swabs by inserting a sterile cotton bud to the anterior part of the nose and the lateral cartilage, spinning the cotton bud gently and placing the cotton bud with the nasal swab into the specimen tube. The throat swab was taken by inserting a cotton bud into the inner side of the mouth until it touched the pharynx, right and left sides of the tonsil, by avoiding the tool touching the tongue. Specimens were placed in closed tube and each tube was labeled and put in a zip lock (plastic bag). Next, the bags were sent to Jakarta by placing each bag in *biobottle* that contained absorbent. *Biobottle* was then placed in fibreboard box with 4-5 bars of ice in it. Specimens were sent to virology laboratory NAMRU Jakarta every week for RT-PCR test and viral culture. Data collected and the values of diagnostic test were analyzed using computer.

Results

During the study period 347 children were studied. **Table 1** shows the characteristics of the study subjects; out of 347 children, 155 (45.4%) were 1 to 5 years of age. Males (52.5%) slightly outnumbered females. On physical examination, fever, cough, and runny nose were the most common symptoms found reaching 42.4%, followed by other symptoms i.e., fever, cough, and dysphagia (13.8%), fever, cough, and difficult to breathe (3.7%), fever and cough (9.8%) (**Table 1**).

Out of 347 subjects, 63 children were positive to have influenza confirmed by culture findings. Thirty eight children presented with influenza type A (13 children classified as H1N1, 24 as H3N2, and 1 as H5N1 subtypes) and 25 children presented with influenza type B.

RT-PCR test showed that 83 children were positive to have influenza type A (46) and type B (37). Out of 46 children with influenza type A, 22 had H1N1 subtype, 23 had H3N2, and 1 had H5N1 subtype.

The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of RT-PCR test were 89%, 90%, 67%, 97%, 9.3, and 0.12 respectively **(Table 3)**.

Discussion

The results of this study showed that influenza infection was found to be greater in male (52.5%) than in female and it is in line with a study by Atmar *et al*[19] and Beckett *et al.*[19] According to age distribution, age group of 1-5 years old was found to get the disease in a greater number (54.4%) than did children under one year and above five years old. This was not in accordance with a study by Beckett *et al*[19] which only included children above four years old and adults. But, it was constituent with the study done by WHO[8] and ACIP[9,12,15] that found that the highest influenza incidence was at the age of six to 24 months old so that immunization is strongly suggested.

From the clinical symptoms in this study, fever, cough, and runny nose were the most common symptoms found (30.3%) and it is similar with the previous study results.[6,19]

This study found that 63 patients (18.2%) had positive culture while a study by Beckett *et al*[19] found around 11.1%. Influenza virus type A was greater in number than type B and H3N2 was the greatest among the subtypes that is in accordance with WHO result.

The sensitivity of RT-PCR test in this study was 89% (95% CI 81% to 97%), which is similar to a study by Atmar *et al,*[18] *i.e.,* 98% (95% CI 75% to 100%); on contrast, similar study by Beckett *et al*[19] had the sensitivity of 78%. Many factors affected the results, such as storage and time of specimen collection. In this study, the specimen delivery was done weekly. Specimen collection in day 1-3 after the onset would give a higher number of viruses.

The specificity of this RT-PCR test was 90% (95% CI 87% to 94%) while in a study by Atmar *et al*[18] and Beckett *et al*[19] was 98% and 97%, respectively.

A sensitivity of 89% was good enough as diagnostic test, meaning that only 11% detected to be false negative. The sensitivity value had to be good enough to establish diagnostic test especially for H5N1 subtype considering that this subtype would be lethal if the best therapy was not given soon. The specificity of this study was good; only 10% were detected to be diagnosed incorrectly because of false positive and it was not too dangerous because it would deal with the giving of antiviral therapy that had no fatal side effects. Besides, the patient isolation would be beneficial for the patients themselves though they were not proven to be infected by avian influenza.

Despite the good results of RT-PCR test, the feasibility of this test as diagnostic test for public still needs further consideration because of its high cost.

Table 1. Characteristics of subjects

Variable	Subjects	
	n	%
Sex		
Male	182	52.4
Female	165	47.5
Age (years)		
<1	35	10.0
1-5	157	45.4
6-10	118	34.0
>10	37	10.6
Time for nasal swab and throat swab		
Fever ≤ 3 days	268	77.2
Symptoms		
Fever ,cough	34	9,8
Fever, cough and runny nose	105	30.3
Fever, cough and sore throat	48	13,8
Fever, cough and difficult to breath	13	3,7

Table 2. The result of RT-PCR test

RT-PCR (+)	n (%)	Culture (+)	n (%)
Influenza virus type A	46 (55.4)	Influenza virus type A	38 (60.3)
HINI	22 (47.8)	H1N1	13 (34.2)
H3N2	23 (50.0)	H3N2	24 (63.4)
H5N1	1 (2.1)	H5N1	1 (7.8)
Influenza virus type B	37 (44.6)	Influenza virus type B	25 (39.7)
Total	83 (23.9)	Total	63 (18.2)

Table 3. The result of diagnostic test value of RT-PCR test.

	Value	95% confidence interval
Sensitivity	89%	81% to 97%
Specificity	90%	87% to 94%
PPV	67%	57% to 78%
NPV	97%	95% to 99%
PLR	9.3	6.46 to13.53
NLR	0.12	0.06 to 0.25

Note : PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio

The limitation of this study was that sample size was estimated not based on the prevalence of influenza in Indonesia because the data of influenza prevalence in Indonesia was not available. It is then probable that the sample size did not represent the population in Indonesia. Besides, considering that this study was conducted not only by us but also by Indonesian Influenza Surveillance Team with NAMRU where RT-PCR test and viral culture were done in Jakarta so that many things affected the results of this study such as quality control that could not be done by us or we could not determine the inclusion criteria from the study subjects that would, of course, influence the results. Therefore, further study with the correct sample size and procedures is urgently needed.

We have shown that the diagnostic values of RT-PCR for influenza were good. However, the feasibility of this test as the influenza diagnostic test for public still needs further consideration because of its high cost.

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