CHAPTER III

EVALUATION OF RAPID INFLUENZA VIRUS TESTS IN PATIENTS WITH INFLUENZA-LIKE ILLNESS IN THAILAND

(Status: Published on Clinical Laboratory)

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Abstract

Influenza virus is responsible for causing major respiratory tract symptoms. The fast, accurate diagnosis will be essential in efficient treatment, especially in patients with complications. The Rapid Influenza Diagnostic Tests (RIDTs) for influenza detection have been developed to subtype influenza virus. The re-evaluation of rapid test is needed in terms of specificity, sensitivity and accuracy. From August 13, 2010 to September 22, 2011, 1,076 nasal aspirates were obtained from patients, age ranging from 15 days to 98 years, with symptoms of Influenza-like illness (ILI) and evaluated by 2 kinds of RIDTs, Standard Diagnosis (SD) and QuickVue (QV) Rapid test followed by real-time RT-PCR. The results from the rapid tests diagnosis were compared to those from real-time RT-PCR. During 2010 to 2011, the estimated sensitivity of the SD rapid test for seasonal H3, human pandemic H1N1 and influenza B infection was 49.4%, specificity was 84.1%, positive predictive value was 47.6% and negative predictive value was 85% while those in QV rapid test were 63.4%, 96.7%, 94.8% and 80.3% respectively.Infant patients ($\leq$5 years) yielded less false negatives while adolescents and adults (older than 5 years) showed more false negatives 8.8% and 15.2% respectively. Using rapid test diagnosis, H3N2 influenza virus was founded with more false negative results (11.1%) than the other viruses(1.1-3.5%). The SD rapid test appeared to be more sensitive than the QV test during high season activity while the QV test was more sensitive during the period of low influenza virus activity. Due to persistent genetic drift of influenza virus, the available RIDTs should be continuously re-evaluated each year. During 2010-2011, QV rapid test showed more reliable results than those in SD rapid test. However, the false negative results of H3N2 influenza virus detection during its peak should be concerned and some of the results, e.g. patients with complications should be compared with real-time RT-PCR as gold standard method to detect influenza virus infection.

Introduction

Influenza is an infectious disease caused by influenza virus, one member of the family *Orthomyxoviridae*. Influenza virus infection can elicit various symptoms such as fever, cough, runny nose, sore throat, headache and fatigue, even vomiting and diarrhea. These symptoms can be mild to severe and in case of host complications cause death [115]. Influenza virus has been classified into influenza A, B and C. Influenza A represents the main influenza virus spreading continuously and causing most serious respiratory illness. It is classified into 16 HA subtypes and 9 NA subtypes based on the variations in Hemagglutinin (HA) and Neuraminidase (NA) antigenic proteins. The virus can infect many species ranging from aquatic birds, which serve as reservoir hosts to mammals such as felines, pigs and humans [21]. Recently, the U.S. Centers for Disease Control and Prevention have reported that influenza viruses circulating in human populations in recent years are influenza A subtypes H3N2, pandemic H1N1 and influenza B virus [116]. Due to continuous circulation, improved detection methods have been devised to diagnose seasonal influenza virus infection. Of those, a fast and simple diagnostic method is the rapid strip test which has become available to detect the viral protein of influenza virus and also discriminate between types and subtypes of seasonal influenza virus in the samples. Early diagnosis can assist in selecting the most effective anti-viral treatment and thus, decrease morbidity particularly, with pH1N1 infection which can cause severe complications especially in infants, obesity, pregnant women, diabetics, and immuno-compromised patients [102]. However, the efficiency of RIDTs should be continuously assessed in terms of sensitivity, specificity and accuracy as the influenza virus genome is constantly subject to gradual mutation. The tests should be evaluated in comparison with real-time RT-PCR (Reverse Transcription Polymerase Chain Reaction), which represents the gold standard for

influenza virus detection and diagnosis. Yet, its use is limited due to the high cost and the requirement for sophisticated facilities. Hence, a simpler method that can provide reliable results is essential to efficiently interpret the surveillance of epidemic influenza virus. This study has been aimed at estimating the efficiency of rapid test diagnosis of influenza virus in terms of specificity, sensitivity and accuracy in ILI patients in Thailand compared with standard real time RT-PCR.

Materials and methods

Ethical Consideration

The protocol 392/52 "The surveillance and characterization of pandemic H1N1 in Thailand" was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University. The specimens were collected as anonymous with the permission by the director of the Bankpakok International Hospital and the Director of King Chulalongkorn Memorial Hospital.

Subjects

From August 13, 2010 to September 22, 2011, nasal swabs were obtained from 1,076 patients, their age ranging from 15 days to 98 years, with symptoms of Influenza-like illness (ILI), comprising fever ($\geq$ 38°C), cough and sore throat. The collected specimens were divided into 2 groups: specimens collected during high activity of influenza virus, with more than 10% positive for influenza virus infection and specimens collected during low activity of influenza virus, which displayed less than 10% positive results. The specimens were provided by Bangpakok 9 International Hospital, Thailand after usual rapid test diagnosis. The specimens were sent in viral transport media (VTM)

to the Center of Excellence in Clinical Virology, Chulalongkorn Memorial Hospital to confirm the laboratory results by using real-time RT-PCR and stored at -70° C until used.

Rapid test assay

From August 13, 2010 to March 31, 2011 all samples had been performed for influenza diagnosis by the SD rapid test kit (Standard Diagnostic Corporation, Pusan, Korea). From April 1, 2011 to September 22, 2011, the samples were subjected to the QuickVue influenza A+B test (Quidel Corporation, CA). The tests were performed according to the manufacturer's instructions. There are 2 different interpretations of 2 types of RIDTs. Each RIDT provides a different extent of information on influenza virus. The QV test can only differentiate the influenza A virus from influenza B virus but it is unable to distinguish pH1N1 virus from the other influenza A virus. Contrary, the SD rapid test can identify the pH1N1 influenza virus, seasonal H1N1, H3N2 and influenza B virus.

Real-time RT-PCR assay

After RNA extraction by a commercially available viral nucleic acid extraction kit (RBC BioscienceCo, Taiwan), one step real-time RT-PCR was performed using Taqman probes as previously described [79,93,117]. Briefly, each sample was subjected to the seven-reaction investigation system to detect the GAPDH gene (as internal control), M gene of influenza A and B, and further classified into H1, H3 and H5 subtypes of the influenza A HA (hemagglutinin) gene.

Statistical evaluation

Sensitivity and specificity of the SD and QuickVue (QV) rapid test were compared with real-time PCR as the gold standard associated with patient age and sex.

Statistical analysis was performed by Chi-square test at alpha = 0.05. The SPSS statistical software program version 17.0 (IBM, NY) was used for statistical evaluation.

Results

Patients

One thousand and seventy-six specimens were collected from ILI patients from August 13, 2010 to August 24, 2011. This group comprised 501 males and 575 females, their age ranging from 15 days to 98 years with a mean age of 24.84 and a median age of 25 years. Male to female ratio was 1:1.14. Using real-time RT-PCR, 67/1076 samples (6.23%) were identified as positive for human pandemic H1N1 (+ve pH1N1), 248/1076 samples (23.05%) were positive for seasonal influenza subtype H3N2 (+ve H3N2), 39/1076 samples (3.62%) were positive for influenza B virus and 722/1076 samples (67.10%) were negative for influenza virus infection (-ve).

During the outbreak of influenza virus in Thailand (Fig13), all samples collected from August 2, 2010 (week 31of 2010) to October 31, 2010 (week 43 of 2010) and from June 6, 2011 (week 26 of 2011) to August 24, 2011(week 34 of 2011) were identified as samples collected during the influenza peak (representing more than 10% positive for influenza virus infection). There were 719 samples collected during high peaks and 357 samples collected during the low season for influenza virus infection.

Rapid Test Results

Two RIDTs were used separately in different influenza seasons based on the hospital's decision. All data were analyzed in terms of sensitivity, specificity, false positive predictive value, false negative predictive value and accuracy correlated with types of influenza virus, sex and age range of patients, collection period (divided

between high season and low season of prevalence). In total, the estimated sensitivity of the SD rapid test for seasonal H3, pH1N1 and influenza B infection was 49.4% specificity was 84.1while the estimated sensitivity and specificity of the QV rapid test were 63.4% and 96.7% respectively. The SD rapid test yielded a false positive percentage of 12.3 and a false negative percentage of 11.4 compared to 1.4 and 14.7, respectively with the QV rapid test. The percentages of accuracy of the SD and QV tests were 76 and 84%, respectively (data not shown). With infant patients ($\leq$ 5 years old), sensitivity and specificity were appraised as 46.7 and 87.8% respectively for the SD rapid test while sensitivity and specificity were estimated at 64.2 and 98.3%, respectively for the QV rapid test. In adolescents and adults (> 5 years old) the SD rapid test showed a sensitivity of 52.2%, specificity of 82.8whereas the those of the QV test in adolescents and adults were 63.6, 95.2%, respectively (Table7). Percentages of false positives with the SD and QV rapid tests in infants were 8 and 1.2% while percentages of false negatives were 8.8 and 11.3%, respectively. With adolescents and adults, percentages of false positives for the SD and QV rapid tests were 14 and 1.5% while percentages of false negatives were 8.8 and 15.2%, respectively (Table 7).

Table 7. Sensitivity and specificity of SD and QV rapid test based on age groups

Age (years)	SD								QV							
	Sensitivity	95% CI	Specificity	95% CI	PPV	NPV	False Positive	False Negative	Sensitivity	95%CI	Specificity	95% CI	PPV	NPV	False Positive	False Negative
≤ 5	46.7	30.2-63.9	87.8	78.5-93.5	60.9	80.2	8	8.8	64.2	50.7-75.7	98.3	93.9-99.5	94.4	84.9	1.2	11.3
6-20	50.0	23.7-76.3	86.5	72.0-94.1	50.0	86.5	10.6	10.6	75.4	62.9-84.8	97.1	90.2-99.2	95.6	82.9	1.5	11.0
21-40	51.9	34.0-69.3	78.9	70.6-85.4	36.8	87.4	17.0	9.2	58.9	48.6-68.5	97.8	93.7-99.2	94.6	78.1	1.6	16.4
41-60	55.6	26.7-81.1	90.2	77.5-96.1	55.6	90.2	8.0	18.0	61.5	45.9-75.1	96.9	89.3-99.1	92.3	80.5	1.8	14.7
>60	50.0	5.5-94.5	81.8	52.3-94.9	20.0	94.7	18.0	0.0	52.6	31.7-72.7	95.2	77.3-99.2	90.9	69.0	2.5	22.5
total	49.4	38.8-60.0	84.1	79.3-87.9	47.6	85.0	12.3	11.4	63.4	57.4-69.1	96.7	95.7-98.8	94.8	80.3	1.4	14.7

For H3N2 influenza virus infection, the estimated sensitivity of the SD rapid test was 60.9% and specificity was 98.7% while the estimated sensitivity and specificity of the QV rapid test were 65.6 and 96.7% respectively. For influenza B virus infection, the estimated sensitivity of the SD rapid test was 72.7% and specificity was 94.0% while those of the QV rapid test were 80.8% and 99.1%, respectively. The appraised sensitivity of the SD rapid test for human pandemic H1N1 influenza virus infection was 38.3%, specificity was 90% respectively (Table8).

During the low activity season of influenza virus, the sensitivity and specificity of the SD rapid test was 43.8% and 80.6% in comparison to 30.8 and 97.9% when using the QV rapid test. False positives and false negatives of the SD test were 11.6 and 22.5% while the QV rapid test produced 1.1 and 16.1% of false positives and false negatives, respectively. During the high activity periods of influenza virus, the estimated false positives and false negatives of the SD rapid test amounted to 12.9 and 13.9% and of the QV rapid test to 2.1 and 9.7%, respectively (Table9).

Table 8. Sensitivity and specificity of SD and QV rapid test based on types of influenza virus

Influenza virus	SD						QV					
	Sensitivity	95% CI	Specificity	95% CI	PPV	NPV	Sensitivity	95% CI	Specificity	95% CI	PPV	NPV
pH1N1 2009	38.3	25.8-52.6	90	85.7-93.1	40.9	88.9	53.3	30.1-75.2	98.9	97.5-99.5	61.5	98.4
H3N2	60.9	40.8-77.8	98.7	96.3-99.6	82.4	96.3	65.6	59.0-71.6	96.7	94.6-98.0	90.4	85.5
Influenza B	72.7	43.4-90.3	94.0	90.3-96.3	34.8	98.7	80.8	62.1-91.5	99.1	97.7-99.6	84	98.9

Table 9. Sensitivity and specificity of SD and QV rapid test during high and low season of influenza activity

	High Season		Low Season	
	SD	QV	SD	QV
Sensitivity	63.2	65.0	43.8	30.8
95%CI	47.3-76.6	58.8-70.7	30.7-57.7	16.5-50.0
Specificity	79.4	97.5	80.6	97.9
95%CI	67.8-87.5	93.7-99.0	70.0-88.0	95.5-99.0
PPV	64.9	96.3	60.0	66.7
NPV	78.1	76.6	68.2	89.6
% False Positive	12.9	2.1	11.6	1.1
% False Negative	13.9	9.7	22.5	16.1

Discussion

Evaluating the efficiency of Rapid Influenza Diagnostic Tests (RIDTs) is essential for influenza virus spreads which tend to co-circulate. In Thailand, co-circulation of influenza B, influenza A subtypes H3N2 and pandemic H1N1 virus has been reported. We analyzed the efficiency of 2 types of RIDTs used in Thailand for detecting influenza virus infection from nasal aspirates, the SD and QuickVue rapid tests. The results obtained from the QV test can be interpreted as negative, influenza A or B infection with moderate sensitivity and high specificity [118-122] while SD rapid test can specify pH1N1 virus infection separated from seasonal influenza A virus. Also, the SD test yielded s more false positives. Significant differences in sensitivity and specificity between the SD and QV test with respect to age group were not established in this study. Still, infant patients ($\leq$5 years) yielded less false negatives while adolescents and adults (older than 5 years) showed more false negatives, which might be due to

increased viral shedding in children [123,124]. Significant differences between virus strains were found in this study (Table8). As for virus types detected by RIDTs, H3N2 influenza virus more frequently causes false negatives (11.1%) than the other viruses (1.1-3.5, data not shown). Although the QV test can detect pH1N1 virus but pH1N1 infection would not be identified by the test. A previous study reported a low-moderate sensitivity of the QV test ranging from 53-69% with a high specificity of 91-99% for the pH1N1 virus [119-122], which was correlated to the findings of this study. The pH1N1 infection can be detected by the SD rapid test. However, both sensitivity and specificity of the SD test seemed to be below than those of the QV rapid test based on the results of this study. Due to the difference in the extent of the information provided by each RIDT, the results of QV test should not be compared with those of SD test apparently.

Thus, the test results should be interpreted with utmost care especially, during the peak of H3N2 influenza virus infection. From August 2010 to September 2011, there were 2 seasons of influenza virus infection (Fig13). Primarily, the first wave between the 31^{st} – 42^{nd} weeks was dominated by pH1N1 infection while the second wave from the 26^{th} to 36^{th} week was caused by influenza A subtype H3N2. Specimens were collected during the high season period, defined as more than 10% of samples positive for influenza virus infection. According to this definition, the high season for influenza infection lasted from the first week of August to the last week of October, 2010 and from the second week of June to the third week of August, 2011. During the high season, the SD rapid test appeared to be more sensitive than the QV test while the QV test was more sensitive during the period of low influenza virus activity (Table9). Yet, the QV test yielded less false negatives or positives during both high and low activity of influenza virus infection. In addition, the results suggested that more false positives would appear during the high season of influenza virus while during the low season, clinicians should be aware of

potentially more false negatives having escaped detection by the rapid test. Although the predominant strains of influenza virus can change during each year, but the pH1N1, H3N2 and influenza B still continue to circulate in human population which cause usual flu-like symptoms or develop a severe illness in people with underlying complications. Applying a sensitive, specific and accurate rapid diagnostic test would facilitate more efficient treatment. Due to persistent genetic drift of influenza virus, the available RIDTs should be continuously re-assessed.

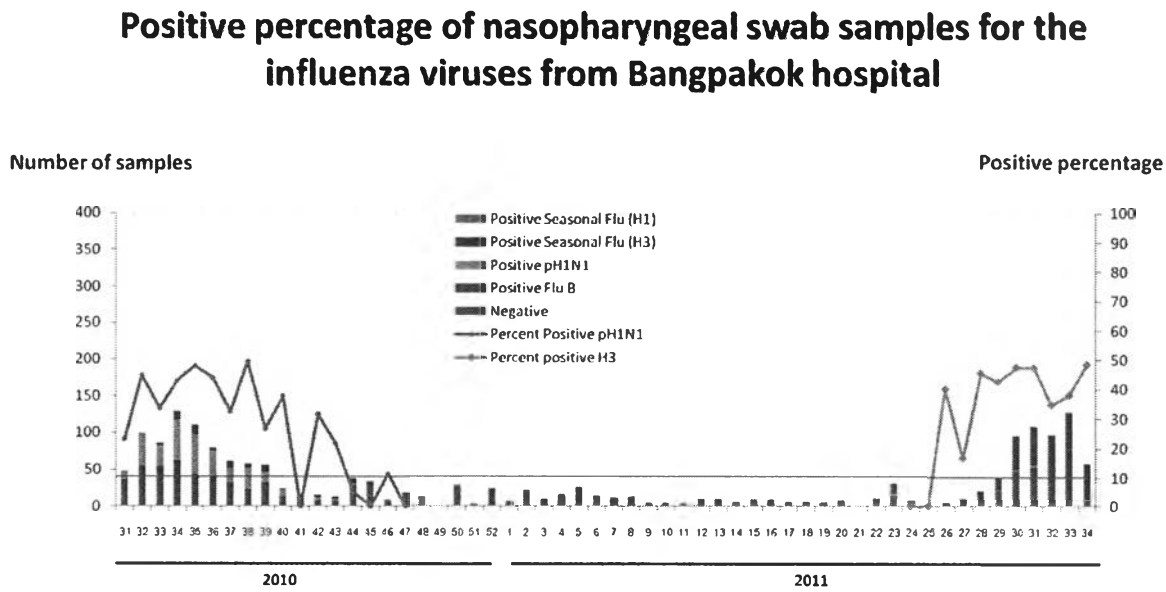

Figure13. Percentage of NP Swab Samples Positive for Influenza Viruses in Thailand during 2010-2011 (Prachayangpreecha S, 2011)