Observational Study

Rapid Test (Immunochromatography) *Versus* Real Time RTPCR in diagnosis of Influenza-A

Mohd Raheem Hussain[1], S V Prasad[2], P N S Reddy[3], A Sai Kumar[4], M Vijay Kumar[5]

This is a Prospective study comparing the Efficacy of Rapid Test (Immunochromatography/OSOM Influenza-A & B) *Versus* Real Time RTPCR in diagnosis of Influenza-A. In 50 Influenza suspects were identified using CDC definition of Influenza suspect & their nasal swabs were subjected to Rapid test for Influenza-A virus (Immunochromatography/ OSOM Influenza A & B) on the spot at Government General & Chest Hospital, Hyderabad, The Nasal & Throat swabs of these suspects were simultaneously subjected to Real time –RTPCR for Influenza-A Virus, H1N1A (Pandemic Influenza-A) virus at IPM, Hyderabad during the period from October 2009 to June 2010. In 16 patients were positive for Influenza-A by Real Time RTPCR & out of them 13 patients are Positive for H1N1 Influenza-A (& only 2 of these 13 patients were positive with Rapid test) & 3 patients are Positive for seasonal Influenza-A (None of them were Positive with rapid Test). Sensitivity of Rapid Test for screening of Influenza-A appears to be very low in this small study.

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***Key words* : Rapid Test (Immunochromatography), Real Time RTPCR, Influenza-A.**

In late March and early April 2009, an outbreak of H1N1 influenza A virus infection was detected in Mexico, with subsequent cases observed in many other countries including the United States[1,2]. On June 11, 2009, the World Health Organization raised its pandemic alert level to the highest level, phase 6, indicating widespread community transmission on at least two continents[3].

On August 10 2010 WHO Director General declared end of H1N1 Pandemic and announced that the H1N1 Influenza event has moved into post pandemic phase.The pandemic that began in March 2009 was caused by an H1N1 influenza A virus that represents a quadruple reassortment of two swine strains, one human strain, and one avian strain of influenza.

Aims & Objectives :

There is a paucity of literature regarding the efficacy of Rapid tests (Immunochromatography /OSOM Influenza-A& B) in the screening of Influenza-A especially in our Indian population. Presently Influenza A is diagnosed by Real Time Reverse Transcriptase Polymerase Chain Reaction (RRT-PCR) which is more sensitive & specific. RRT-PCR cannot be used for Screening because it is very costly, requires sophisticated lab with Biosafety Level 2 or more & it is available at very few places in India hence this study was conducted .

Rapid Tests can be done at the point of care & do not require sophisticated Laboratory, Result is available with in 10 minutes, less cumbersome, do not require Laboratory Personnel.

Study Design :

This is a Prospective comparative study.

This study was considered as a minimum risk in human research and was approved by the ethics committee of the Institute

Materials and Methods

Procedure :

Inclusion Criteria :

(1) Patients with symptoms of Influenza like illness ranging from 0 to 10 days duration.

(2) Symptomatic contacts of H1N1A positive patients.

Exclusion Criteria :

(1) Patients with symptoms of Influenza like illness of >10 days duration.

(2) Asymptomatic contacts of H1N1A positive patients.

Results and Analysis of Data :

Fifty Influenza suspects were identified using CDC

[1]MD, Department of Pulmonary Medicine, Osmania Medical College, Government General & Chest Hospital, Hyderabad 500038. Presently : Junior Consultant, Department of Pulmonary Medicine, Care Hospital, Hyderabad 500020 and Corresponding author

[2]MD, DTCD, FCCP, Professor, Department of Pulmonary Medicine, MNR Medical College, Sanga Reddy, MEDAK District, Andhra Pradesh 502294

[3]MD, DTCD, MNAMS, FCCP, Senior Pulmonologist Yashoda Hospital, Hyderabad 500082

[4]MD, Professor & Head, Osmania Medical College, Government General & Chest Hospital, Hyderabad 500038

[5]MD, Assocaite Professor, Gandhi Medical College, Government General & Chest Hospital, Hyderabad 500038

definition of Influenza suspect & their nasal swabs were subjected to Rapid test for Influenza virus (Immunochromatography/ OSOM Influenza A & B) which was done on the spot at Government General & Chest Hospital, Hyderabad which gives results with in 10 minutes & also their Nasal & Throat swabs were simultaneously subjected to RRT-PCR for Influenza virus at Institute of Preventive Medicine, Hyderabad which gives result with in 1day.The study was conducted during the period from October 2009 to June 2010 (Flu Pandemic period) .

These patients were divided into 2 groups basing on the result of RRT-PCR.

Group A : In 34 patients, Influenza A was negative by RRT-PCR & also by rapid test .They were considered as control group.

Group B : In 16 patients, Influenza A was positive by RRT-PCR. Out of 16 patients, 13 patients were H1N1A positive by RRT-PCR (out of them 2 patients are Positive with even Rapid test) & 3 patients were Positive for Seasonal Influenza-A (Rapid test was negative in all 3 of them). In Group B only 2 patients were Positive with rapid test & 14 patients were negative with rapid test. These 16 patients were considered as cases.

Fourteen variables as mentioned below were taken into consideration (including 6 comorbidities). Between the 2 groups only history of traveling abroad is statistically significant as a risk factor for H1N1 Influenza-A during the pandemic period. Results were analysed by Windostat Version[8.6].

Demographic Profile of patients

(1) Age :

- Upto 10 years
  (a) GroupA: 0 , (b) Group B:1
- 11 to 20 years
  (a) GroupA: 9, (b) Group B:1
- 21 to 40 years
  (a) GroupA: 19, (b) Group B:12 (rapid test was positive for Influenza-A in 2 patients)
- 40 to 60 years
  (a) GroupA: 6, (b) Group B:2

(2) Sex Distribution:

- Males
  (a) GroupA:20, (b) Group B:11(rapid test was positive for Influenza-A in 1 patient)
- Females
  (a) GroupA:14, (b) Group B:5(rapid test was positive for Influenza-A in 1 patient)

(3) Duration of Symptoms :

- Upto 3 days
  (a)GroupA:11, (b)Group B:4
- 4 to 7 days
  (a) GroupA:21, (b) Group B:12 (rapid test was positive for Influenza-A in 1 patient)
- 8 to 10days
  (a) GroupA:2, (b) Group B:0

(4) H/O travel abroad :

(a)GroupA:0, (b)Group B:3(rapid test was positive for Influenza-A in 1 patient)

(5) H/O contact with Influenza A positive patient :

(a)GroupA:2, (b) Group B:2 (rapid test was positive for Influenza-A in 1 patient)

Clinical Features:

(6) Running Nose :

(a) GroupA:21, (b) Group B:11 (rapid test was positive for Influenza-A in 2 patients)

(7) Sore Throat :

(a) GroupA:11, (b) Group B:6 (rapid test was positive for Influenza-A in 2 patients)

(8) Chest X ray, suggestive of consolidation :

(a) GroupA:11, out of them 2 patients Sputum for Gram stain & Culture was positive for Klebsiella pneumoniae

(b) Group B:6

Comorbities :

(9) Diabetes Mellitus:

(a) GroupA:1, (b) Group B:2

(10) Pregnancy :

(a) GroupA:0, (b)Group B:2

(11) Obstructive sleep apnoea :

(a) GroupA:0, (b) Group B:1

(12) Epilepsy :

(a) GroupA:0, (b) Group B:1

(13) COPD :

(a) GroupA:0, (b) Group B:1

(14) Hypertension :

(a) GroupA:1, (b) Group B:1

Analysis of Data :

Analysis of Data in terms of Sensitivity, Specificity, PPV, NPV of Rapid test in Comparision with RRT-PCR:

The Sensitivity, Specificity, PPV, NPV of Rapid test in comparision with RRT-PCR were 12.5%, 100%, 100% & 70.83% respectively.

Discussion

50 Influenza suspects were identified using CDC definition of Influenza suspect & their nasal swabs were subjected to Rapid test for Influenza virus (Immunochromatography/ OSOM Inf A & B) on the spot at Government General & Chest Hospital, Hyderabad which gives results with in 5 to 10 minutes & also their Nasal & Throat swabs were simultaneously subjected to RRT-PCR for Influenza virus at IPM, Hyderabad which gives result with in 1day. The study was conducted during the period from October 2009 to June 2010.

These patients were divided into 2 groups basing on the result RRT-PCR.

Observed Frequencies

	1 Col	2 Col
1 Row	**2.** 0.64	. 1.36
2 Row	**14.** 15.36	**34.** 32.64

χ^2 Pearson	4.42668	Two sided	0.03538
χ^2 Yates Corrected	1.77005	Two sided	0.18338
χ^2 Mantel-Haenszel	4.33814	Two sided	0.03727
Fisher's Exact Test			
Odd's Ratio: significantly different from 1		Two sided	0.09796
Odd's Ratio: significantly < 1		Left sided	1.00000
Odd's Ratio: significantly > 1		Right sided	0.09796
Rosner: 2 * minimum(0.5, Left-Tail, Right-Tail)		Two sided	0.19592

Test		95% Confience Interval	
Statistics	**Value**	**Lower**	**Upper**
Odds Ratio	48571.4300	0.5257	
Relative Risk	3.4284	0.6215	3.4286
Kappa	0.1627	-0.0282	0.1627
Sensitivity	0.1250	0.0252	0.1250
Specificity	1.0000	0.9531	1.0000
+ve Predictive Value	1.0000	0.2019	1.0000
-ve Predictive Value	0.7083	0.6751	0.7083
Difference in Proportion	0.7083	-0.1230	0.7083
Nos Needed to Treat	1.4119	1.4118	infinite
Overall Acuuracy	0.2800		
Prevalence Rate	0.6800		
Youden's J Index	0.1250	-0.0217	0.1250
Phi Coefficient	0.2975	-0.0517	0.2976
Yule's Q	1.0000	-0.3108	1.0000
Contingency Coefficient	0.2852	0.2852	0.2852
Adj. Contingency Coefficient	0.4033	0.4033	0.4033
Diagnostic Odd's Ratio	48913.0200	0.5257	
Error Odd's Ratio	0.0000	0.0013	
Forbes NMI Index	0.0755	0.0023	0.0756

Group A : In 34 patients, Influenza A was negative by RRT-PCR & also by rapid test. They were considered as control group. Out of them 2 patients Sputum for Gram stain & Culture was positive Klebsiella pneumoniae.

Group B : In 16 patients, Influenza A was positive by RRT-PCR. Out of 16 patients, 13 patients were H1N1A positive by RRT-PCR (out of them 2 patients are Positive with even Rapid test) & 3 patients were Positive for Seasonal Influenza-A (Rapid test was negative in all 3 of them). In Group B only 2 patients were Positive with rapid test & 14 patients were negative with rapid test. These 16 patients were considered as cases.

14 variables were taken into consideration (including 6 comorbidities) Between the 2 groups only history of traveling abroad is statistically significant as a risk factor H1N1 influenza-A virus infection during the pandemic period . Results were analysed by Windostat Version 8.6.

31 Suspects (62% of Suspects) were in the age group of 21 to 40 yrs & out of them, 12 patients in Group B (75% of Group B) were positive with real time RTPCR for Influenza-A & 2 patients were positive with Rapid test for Influenza-A.

8 suspects (16%) were in the age group of 40 to 60 yrs & out of them, 2 patients in Group B (12.5% of Group B) were positive with real time RTPCR for Influenza-A.The p value for ANOVA for age between Group A and Group B is 0.78275 & is hence statistically not significant.

31 Suspects (62% of suspects) were males & out of them, 11 patients in Group B (75% of Group B) were positive with real time RTPCR for Influenza-A.The p value for ANOVA for sex differentiation between Group A and Group B is 0.50989 & is hence statistically not significant.

15 suspects (30%) included in the study presented with duration of onset of symptoms of upto 3 days & out of them,4 patients in Group B (25% of Group B) were positive with real time RTPCR for Influenza-A.

33 suspects (66%) included in the study presented with duration of onset of symptoms from 4 to 7 days & out of them,11 patients in Group B (75% of Group B) were positive with Real time RTPCR for Influenza-A & 2 patients were positive with rapid test for Influenza-A.

2 suspects included in the study presented with duration of onset of symptoms >7days & None of the were positive with Real Time RTPCR for influenza-A.

The p value for ANOVA for duration of onset of symptoms between Group A and Group B is 0.81412 & is hence statistically not significant.

History of Travelling abroad was found in 3 suspects (6% of suspects) & all 3 of them in Group B (18.75% of group B) were positive with Real time RTPCR for Influenza-A & only 1 patient was positive with rapid test for Influenza-A.

The p value for ANOVA for History of Travelling abroad between Group A and Group B is 0.00850 & is hence statistically significant as a risk factor H1N1 influenza-A virus infection during the pandemic period.

History of Contact with H1N1 Influenza-A positive

patient was found in 4 (8%) of suspects & out of them, 2 patients in Group B (12.5% of Group B) were positive with Real time RTPCR for Influenza-A & only 1 was positive with rapid test for Influenza-A. The p value for ANOVA for History of Contact with H1N1 Influenza-A positive patient between Group A and Group B is 0.43139 & is hence statistically not significant.

Clinical features such as Running nose was found in 32 (64%) suspects & out of them 11 patients in Group B (8.7% of Group B) patients were positive with Real time RTPCR for Influenza-A & 2 patients were positive with rapid test for Influenza-A.

Sore Throat was found in was found in 17 (64%) suspects & out of them, 6 patients in GroupB(37.5% of Group B) were positive with Real time RTPCR for Influenza-A & 2 patients were positive with rapid test for Influenza-A.

Chest X Ray, suggestive of Consolidation was found in was found in 17 (34%) suspects & out of them,6 patients in Group B (37.5% of Group B) were positive with Real time RTPCR for Influenza-A.

Comorbities such as Diabetes Mellitus was found in 3 (6%) suspects & out of them 2 patients in Group B (12.5% of Group B) were positive with Real time RTPCR for Influenza-A.

2 (4%) suspects were pregnant & all of them in Group B (12.5% of Group B) were positive with Real time RTPCR for Influenza-A.

Obstructive sleep apnoea was found in 1 (2%) suspect & same patient in Group B (6.5% of Group B) was positive with Real time RTPCR for Influenza-A.

1 (2%) suspect was epileptic & same patient in GroupB was positive (6.5% of Group B) with Real time RTPCR for Influenza-A.

1 (2%) suspect was suffering from COPD & same patient in Group B (6.5% of Group B) was positive with Real time RTPCR for Influenza-A.

2 suspects were Hypertensive & out of the them, 1 patient in Group B (6.5% of Group B) was positive with Real time RTPCR.

The Sensitivity, Specificity, PPV, NPV of Rapid test (Immunochromatography/ OSOM Influenza A & B) in comparision with RTPCR were 12.5%, 100%, 100% & 70.83% respectively.

Hence this rapid test for Influenza-A virus (Immunochromatography/ OSOM Influenza A & B) has very low sensitivity.

The higher sensitivity of rapid tests are reported from USA and other western countries,probable reason could be that they use nasopharyngeal aspirates for testing not the usual Nasal or throat swabs.

Comparision with other studies :

(1) Ghebremendhin *et al* Actim Inf A & B Sensitivity was 65.2% & Specificity was 100%

(2) E.R. Barriera *et al* Quick vue Inf A+B Sensitivity was 40.4% & specificity 96.2%

(3) S.M.AlJohani *et al*

BD Directigen EZ Sensitivity was 20.6% & Specificity 99%

Tru Flu Sensitivity-9.7% & Specificity-98.2%

(4) Z.R. Zetti *et al*

Binax Now Sensitivity-4.4%, Specificity-100%

Quick Vue Sensitivity-4.4%, Specificity-100%

Rockeby Sensitivity-12.5%, Specificity-90.9%

BD Directigen EZ Sensitivity-37%, Specificity-98.1%

Results of Rapid test of Current study resembles results of other Rapid tests as mentioned below.

(1) Binax Now, Quick vue, Rocke by conducted by Z.R. Zetti *et al*

(2) BD DirectigenEZ,Truflu conducted by SMAl Johani *et al.*

Conclusions :

(1) Sensitivity of Rapid Test (Immunochromatography/ OSOM Inf. A&B) for Influenza-A is very low when compared with Real time Reverse Transcriptase Polymerase Chain Reaction.

(2) Rapid Test does not appear to be useful as a screening test in this small study. If we look at the expenditure incurred on both tests, Rapid test appears to be less costly but as sensitivity is very low, lot of positive cases (in this study 14 out of 16 cases) are missed.

(3) If Cost of Real Time Reverse Transcriptase Polymerase Chain Reaction is lowered and is made available at the point of care in the form of rapid test, then Real Time Reverse Transcriptase Polymerase chain Reaction will be more useful.

(4) As per WHO guidelines for management of H1N1 Influenza-A infection, patients can be started on antiviral therapy awaiting test results.

Limitations of Study :

(1) Sample size of this study was small with only 50 Influenza suspects.

(2) Equal number of Healthy controls were not included which will provide better information.

(3) The cause of Infection was not known in 32 out of 34 suspects in whom Real Time Reverse Transcriptase Polymerase Chain Reaction for Influenza-A was negative.

References

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