Laboratory surveillance of H1N1 swine influenza A virus infection in patients with severe acute respiratory illness (SARI)-an institutional study

Ravi Prakash G.N[1], Chakrapani K[2], Swarna Latha G[3], Surekha A[4], Sailaja[5], Sudhakar P[6]

[1]Dr. G N Ravi Prakash, Assistant Professor, Departmrnt of Microbiology, [2]Dr. K. Chakrapani, Post Graduate in MD Microbiology, [3]Dr. G. Swarna Latha, Professor & HOD, Department of Microbiology, [4]Dr. A. Surekha Professor, Department of Microbiology, [5]Dr. Sailaja Professor & HOD, Department of Pulmonology, [6]Dr. P. Sudhakar Professor & HOD, Department of General Medicine; All are affiliated with Kurnool Medical College, Kurnool.

Address for correspondence: Dr. G N Ravi Prakash, Email: gnrprakash2004@yahoo.co.in
……

Abstract

Introduction: Lower respiratory tract infections (LRTI) are a leading cause of death in developing countries and are also third leading cause of death worldwide. Influenza is an acute viral and highly contagious respiratory infection causing significant morbidity and mortality. The 2009, swine flu pandemic is caused by a novel reassorted H1N1 subtype of H1N1 influenza A virus. **Objectives:** The present laboratory study was conducted for the active surveillance of H1N1 swine influenza A virus infection in type C category of patients with Severe acute respiratory illnesses (SARI). **Materials and Methods:** In clinically suspected cases of swine flu, a total of 130 deep nasal / throat swabs were collected and inoculated into viral transport medium (Himedia labs) and In the reference lab, samples were tested with Real time reverse transcriptase PCR for universal influenza A (inf A), swine influenza A (SWinfA) and swine H1 (SW H1) probes. **Results:** Among the total 130 samples, 32 are showing positive by Real time RT-PCR with a prevalence of 24.61%. In 0-5 age group, 10 were positive with prevalence of 62.5%. In total 66 were males with 12 positives (18.18 %), 64 were females with 20 positives (31.25%). **Conclusion:** H1N1 influenza A is a potentially infectious disease, which spreads by droplet infection, so laboratory based surveillance is necessary to know the intensity of infection , seasonal trends and epidemiological factors like variations according to gender, age and region; which is also helpful for local health authorities to take the appropriate control measures.

Keywords: H1N1 swine influenza A virus, severe acute respiratory illnesses (SARI), Swine flu, RT- PCR, H1N1 Pdm virus.
……..

Introduction

Lower respiratory tract infections (LRTI) are a leading cause of death in developing countries and are also third leading cause of death worldwide [1]. Severe acute respiratory illnesses (SARI) due to influenza virus infection are a major cause of morbidity and mortality worldwide [1]. Influenza is an acute viral and highly contagious respiratory infection causing significant morbidity and mortality worldwide with public health implications. Two main types of A and B of Influenza virus are responsible for recurrent epidemics in humans. As compared to Influenza B, Influenza A has more

significant impact on public health due to its faster evolution and diverse host range. Seasonal influenza viruses continually circulate in yearly epidemics mainly during the rainy season in tropical region and winter months in temperate climates, while antigenically novel strains emerge sporadically as pandemic viruses [2].

Influenza viruses are a member of family Orthomyxoviridiae. Based on the antigenicity virus may be classified into 16 Haemagglutinins (H1-H16) and 9 Neuraminidase (N1-N9) subtypes. Influenza A virus genome is composed of eight segments of single stranded, negative sense RNA, and each of which encodes ten proteins, the HA protein is critical for

binding to cellular receptors and fusion of the viral and endosomal membrane. The 2009, swine flu pandemic is caused by a novel reassorted H1N1 subtype of H1N1 influenza A virus that had not been recognized previously in swine or humans. This newly emerged strain represents a quadruple reassortment of two swine strains, one human strain and one avian strain of influenza [3].

WHO, declared on June 11, 2009, that H1N1 swine influenza A virus is pandemic ,there have been nearly 30,000 confirmed H1N1 cases across 74 countries. [4] In April 2009, the first case of H1N1 influenza A was reported from Mexico [5]. In India, the first case of H1N1 influenza A was reported on May 16, 2009 from Hyderabad. The central and state government in India had taken the pandemic very seriously and made several unprecedented and innovative interventions including pandemic preparedness plan [1,4,5]. The present laboratory study was conducted for the active surveillance of H1N1 swine influenza A virus infection in type C category of patients with Severe acute respiratory illnesses (SARI) as per the guidelines given by ministry of health and family welfare.(MOHFW). The prevalence rate, risk factors will be analyzed according to age, gender and seasonality etc and implications will be discussed.

Aims and Objectives

(1) Present study was conducted to confirm the clinically suspected cases of H1N1 swine influenza A virus infection. (2) Active surveillance done to know the prevalence of H1N1 swine influenza A virus infection in type C category of patients admitting in govt. General hospital, Kurnool. (Sep2013-15) (3) To study the risk factors of infection as per the epidemiological parameters. (4) Helpful to formulate the necessary measures for pandemic preparedness by local health authorities.

Materials and Methods

The present laboratory study was started with following case definitions: *Suspected case* defined as a patient who are having type C category of severe acute respiratory illnesses (SARI) with high grade fever, severe sore throat, breathlessness, chest pain, drowsiness, low BP, sputum mixed with blood ,bluish discoloration etc. as per the guidelines given by ministry of health and family welfare. [5]. *Confirmed case* is considered as whenever a suspected case

positive for H1N1 swine influenza A virus infection by Real time reverse transcriptase -polymerase chain reaction (RT-PCR) [6].

Under the guidelines given by MOHFW, India, in 2010, pandemic preparedness for H1N1 swine influenza A virus infection was started with by formulating a rapid response team in GGH, Kurnool. All the *Suspected* type C category patients, admitted under various departments i.e. general medicine, TBCD, pediatrics etc; were shifted to the isolation ward with all necessary facilities. From about 130 patients, deep nasal / throat swabs samples were collected for confirmation test. The method of collection of samples, types of swab used, time of collection after the onset of illness, and cold chain maintenance precautions also important for recovery of viral nucleic acid. The ideal time of collection is within 7 days of onset of fever and sore throat; because the viral shedding is more during this period in upper respiratory secretions. General biosafety measure for sample collection was followed by using of complete complement of personal protective equipment (PPE) i.e. N95 mask, latex disposable gloves ,disposable apron ,cover hair with head cover ,used protective eye wear .e.g. goggles/face shields [7,8] Dacron or polyester tipped swabs with plastic shaft were used. For nasal swab, the swab is inserted deeply into the nostril parallel to the palate and left in place for few seconds; specimens from both nostrils obtained with the same swab. For throat swab, both nostrils and posterior pharynx are swabbed vigorously and immediately after collection, the applicator stick is broken off and the tip of the swab placed in to a vial containing viral transport medium (Himedia labs India) ,later transported to Microbiology with all cold chain maintenance precautions [,6,7,8] after that the shipment of the samples done to *institute of preventive medicine*, Hyderabad with trilayered packing, biohazardous symbol and cold chain maintenance by person. The particulars of patient along with case history also enclosed with samples. In the reference lab, samples were tested with Real time RT-PCR. Viral RNA was extracted from 140μl of clinical sample by using Q1Amp viral RNA mini kit (Qiagen, Germany). The CDC real time RT-PCR assay was used for novel swine flu virus identification in Mx 3005P quantitative PCR system (stratagene, USA). The assay is based on taqman *chemistry* including a panel of oligonucleitide primers and dual labeled hydrolysis probe sets [universal influenza A(inf A); swine influenza A (SW inf A); swine H1(SW H1) and RNase P (RP) employing invitrogen superscript III platinum, one step

quantitative kit (Invitrogen , USA). [3,8] The samples positive for all the 3 probes were considered as novel strain of swine influenza A H1N1 Pdm virus.

Results

In present laboratory study, a total of 130 deep nasal / throat swabs were collected and tested by Real time RT-PCR. Among 130 samples, 32 were showing positive by RT-PCR universal influenza A (inf A), swine influenza A (SWinfA) and swine H1 (SW H1) with a prevalence of 24.61% (Table 1). Out of 130 patients; 16 were in 0-5 age group, among them 10 was positive with prevalence of 62.5%; 10 were within 6-14 age group with 0 % positivity observed. ; 64 were in 15-40 age group, among them 18 was positive with prevalence of 28.12%; .40 were in 41 - 65 age group, among them 4 were positive with prevalence of 10%. (Table 1)

In total, 66 were males, among them 12 were showing positive with prevalence of 18.18 %. And 64 were females, 20 were positive with prevalence of 31.25%. (Table2). Spreading pattern of virus during an epidemic in a year monthly wise was shown in (Table3).

Table 1: Distribution of samples according to age group as follows:

Age group	Total	Positives
0-5	16	10 (62.5%)
6-14	10	0
15-40	64	18(28.12%)
41- 65	40	4(10%)
Total	**130**	**32(24.61%)**

Table 2: Distribution of samples according to gender

Age group	Total		H1N1 Positives	
	Males	**Females**	**Males**	**Females**
0-5	14	2	8	2
6-14	8	2	-	-
15-40	20	44	2	16
41- 65	24	16	2	2
Total	**66**	**64**	**12(18.18%)**	**20(31.25%)**

Table 3: Month wise distribution

Month	Total	Positives for H1N1 (N=32)
January	2	0
February	20	6 (18.75%)
March	44	12(37.5%)
April	24	8(25%)
May	12	4(12.5%)
June	02	-
July	-	-
August	6	-
September	4	-
October	4	2(6.25%)
November	10	-
December	2	-

Table 4: Distribution according to living conditions

Region	Total	Positives (N=32)
Rural	76	20 (62.5%)
Urban	34	10(31.25%)
Semi urban	20	2(6.25%)

Out of the 32 H1N1 positives cases (n=32) 6 patients were positive in the month of February with incidence of (18.75%)., 12 patients were positive in March with incidence of 37.5%, 8 patients were positive in April with incidence of 25%, 4 patients were positive in the May with incidence of 12.5%. A sporadic of 2 positive cases occurred in October month with incidence of 6.25% also observed. (Table 3) samples were categorized according to living conditions as follows; rural area consists of 76, urban areas consists of 34 and semi- urban includes 20 patients, among them 2 patients were positive in rural areas with incidence of 62.5%; 10 patients were positive in urban areas with incidence of 31.25%; 2 were positive in the semi urban areas with incidence of 6.25%. (Table 4)

Discussion

Influenza recognized as one of the re-emerging viral disease, due to appearance of a novel and potentially pandemic strains of Avian and Swine origin. Pandemic influenza caused by H1N1 influenza A virus significantly associated with high morbidity, mortality, economic loss and impact on social life [9]. In view of the seasonal differences and different climatic changes in our region, laboratory based surveillance is essential to know the spreading pattern of H1N1 virus, its epidemiology and disease burden on community. It is also helpful for local health authorities towards the pandemic preparedness.

In light of above, the present study was showing a prevalence of 24.61% during a period of Sep 2013 to Oct 2015. A total of 32 positive cases of H1N1 influenza A in type C category patients by Real time RT-PCR were noted. Among them, higher prevalence was noted in females of 31.25%. In the age group 0-5yrs were showing high prevalence of 62.5% followed by 15-40yrs age group of 28.2%. During a year, even though the positive cases started to appear in winter, but the highest rate of positivity observed in between February and May; which following the spreading pattern of virus in tropical countries like south-east Asian countries i.e. Bangladesh, China etc; while in temperate countries i.e., UK, USA , where the highest spreading of virus occur in winter months. When compared to living conditions, the rural people were more affected than the urban population, least in semi-urban areas, which attributes to more exposure to out-side environment. History of travel to endemic areas is also one of the risk factor in urban areas. *Mahendra Singh et al* revealed that during outbreak of a total of 1372 suspected patients tested for influenza A H1N1 out of which 24.6 %(157) were found to be positive for the disease.27.2% of all suspected and 33.2% of all positive cases were seen during the month of Jan 2013 which are correlating our study.70.1 % were seen amongst the age group of 15-45 years .67.4% cases were seen in females .most cases (215) and deaths (28) were seen in jodhpur district; in comparision to above study, present study was showing high positivity in females ,but age group affected is 0-5 years which differs from above [**10**]. *Archana choudary and supriya Singh et..al* conducted a study at division of Microbiology ,IDSP ,NCDC ,New Delhi ,India 2011 results as follows ,a total of 33,751 samples ,both throat and nasal swab samples from each patient were tested for H1N1 influenza virus ,of which 7943 (23.5%) were positive for pandemic influenza A H1N1 and 3759 (11.1%) were positive for influenza A (seasonal flu) .maximum number of positive cases (N=2792 ,35.1%) were from 20-39 yrs age group ,comprising 1790 (22.5%) males and 1182 (14.8%) females. (**1,11**) **,** the total prevalence was correlating with our study.

Conclusion

H1N1 influenza A is a potentially infectious disease, which spreads by droplet infection. So, it is commonly associated with occupational health hazard. Prevention of infection by taking appropriate precautions like wearing personal protective equipment, (PPE), oseltamavir (tamiflu) prophylaxis and vaccination is essential during the management of cases in isolation ward. For confirmation of the case with Real time RT-PCR collection of appropriate sample like deep nasal /throat swab, time of the collection of sample, technique of collection, skilled personnel, type of swab used,

suitable viral transport media and cold chain maintenance is essential for recovery of virus. It is a disease of high morbidity and mortality, so laboratory based surveillance is essential to know the intensity of infection, seasonal trends, and epidemiological factors like variations of disease according to the gender, age and region is helpful for local health authorities to take the appropriate control measures and pandemic preparedness against the swine flu.

References

1. Choudhry A, Singh S, Khare S, Rai A, Rawat DS, Aggarwal RK, Chauhan LS. Emergence of pandemic 2009 influenza A H1N1, India. Indian J Med Res. 2012 Apr;135(4):534-7.

2. Badar N, Bashir Aamir U, Mehmood MR, Nisar N, Alam MM, Kazi BM, Zaidi SS. Influenza virus surveillance in Pakistan during 2008-2011. PLoS One. 2013 Nov 8;8(11):e79959. doi: 10.1371/journal.pone.0079959. eCollection 2013.

3. Shashi S, Gaurav J, Paban Dash K, Parida MM (2015) Laboratory Investigation and Molecular Epidemiology of H1N1pdm Virus 2012-2013 from India, J Phylogen Evolution Biol 3: 139. doi:10.4172/2329-9002.1000139

4. Dandagi GL, Byahatti SM. An insight into the swine-influenza A (H1N1) virus infection in humans. Lung India. 2011 Jan;28(1):34-8. doi: 10.4103/0970-2113.76299.

5. Centers for Disease Control and Prevention (CDC). Update: novel influenza A (H1N1) virus infection - Mexico, March-May, 2009. MMWR Morb Mortal Wkly Rep. 2009 Jun 5;58(21):585-9.

6. Ministry of health and family welfare, India .information of swine flu ,new Delhi MOHFW,available from http:// www.mohfw.nic.in /swine flu .htm accessed on November 18,2011 .

7. World health organization .world now at the starting of 2009 influenza pandemic .statement to the press by WHO, director general.Dr.margaret chan,available from :http:/who.int/media centre/news/statements /2009/h1n1_ pandemic _phase 6 _ 2009 011/en/index .html,accessed on November 18,2011.

8. World health organization. H1N1in post pandemic period .available from: http:/who.int/media centre/news/statements /2010, H1N1_VPC_20100810/en/index .html, accessed on Nov, 18, 2011.

9. Influenza (seasonal) , World health organization. April 2009, available from http:/who.int/media centre/fact sheets /fs211/en/accessed on April 16, 2012.

10. Singh M and SharmaS, An epidemiological study of recent outbreak of influenza A H1N1 (swine flu) ,in western Rajasthan region of India .JMed Allied Sci.2013;3(2):48-52.

11. Garten RJ, Davis CT, Russell CA et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science. 2009 Jul 10;325(5937):197-201. doi: 10.1126/science.1176225. Epub 2009 May 22.

....
How to cite this article?

Ravi Prakash G.N, Chakrapani K, Swarna Latha G, Surekha A, Sailaja, Sudhakar P. Laboratory surveillance of H1N1 swine influenza A virus infection in patients with severe acute respiratory illness (SARI)-an institutional study. *Int J Med Res Rev* 2016;4(2):169-173. doi: 10.17511/ijmrr.2016.i02.008.
……