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A five-year study of *Influenza A (H1N1) pandemic 09* from Central India

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ABSTRACT

Introduction : Influenza virus is a typical human pathogen causing serious respiratory infection. While declaring the last pandemic to be over in August 2010, World Health Organisation (WHO) conveyed that Pandemic Influenza A (HINI) virus would circulate as Seasonal Influenza virus for years to come. This study was undertaken to observe trends of Influenza A (H1N1) pandemic 09 over last five years in Vidarbha region of Maharashtra.
Material and Methods: Throat/nasal/nasopharyngeal swabs/aspirates from clinically suspected cases of influenza like illness in category C received in the Viral Research and Diagnostic Lab (VRDL), Indira Gandhi Govt Medical College (IGGMC), Nagpur from October 2015 to September 2019 were included in the study. The samples were tested by reverse transcriptase real time polymerase chain reaction (RT-PCR). Clinical and epidemiological parameters were also noted.
Results : Influenza A(H1N1)pandemic 09 positivity ranged from 03% to 21% in last five years. Males and females were almost equally affected except in 2019. Maximum positive cases were seen in age group of 41-60 years in 2015 (24%), 0-10 years in 2017(24%), 21-40 years in 2018 (9%) and 11-20 years (26%) in 2019 . Dual peaks of infection were seen in 2017, 2018 and 2019. Bhandara, Gondia and Akola districts had highest positivity .
Discussion : Influenza A(H1N1)pandemic 09 is showing shift towards younger age groups and females slightly, both of which needs attention. Waning and waxing trend in last five years indicate unpredictable and changing epidemiology of the virus which should be studied further in details with respect to time, place, person and molecular characterisation of the virus.

1. Introduction

Respiratory infections due to influenza virus are a major cause of morbidity and mortality all over the world. The first case of influenza A H1N1 was reported from Mexico in April, 2009.[1] Subsequently, the infection spread to across 74 countries with 30, 000 confirmed cases on June 11, 2009. This prompted the World Health Organization to raise the warning from phase 5 to phase 6.[2] This was a new strain of the virus which was never seen before. Total 214 countries were affected by the pandemic worldwide. In India, the first case of influenza A H1N1 was reported on May 16, 2009 from Hyderabad.[3] The World Health Organization declared the post pandemic phase on August 10, 2010.[4] Subsequently, the influenza activity in the six regions of the world is on decline.[5] This pandemic A(H1N1)2009 virus has been widely circulating across the globe since 2009, and is now established in human populations as a seasonal influenza virus. Currently there is no longer a pandemic virus circulating in the world. Yet, in India, the past decade saw as many as 1.58 lakh persons being infected by the virus and over 10,000 succumbing to it. IDSP data show that the epidemiology of influenza A (H1N1) is changing in terms of geographical distribution, age and seasonality.[6] In view of lack of epidemiological information on trends

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of pandemic influenza A H1N1 from India, the present study was conducted at Viral Research and Diagnostic Lab (VRDL), Indira Gandhi Govt. Medical College (IGGMC), Nagpur which is one among five identified Govt. labs involved for testing of Influenza A (H1N1)pandemic 09 in Maharashtra State and one and only lab in Vidarbha region of Maharashtra.

2. Materials and Methods

Clinically, suspected cases belonging to Category C as per the guidelines on the categorisation of H1N1 cases[7] only were included in the study. Throat/nasal /nasopharyngeal swabs/aspirates were collected by the treating physician in the viral transport medium and transported to Viral Research and Diagnostic Lab (VRDL), Indira Gandhi Govt. Medical College (IGGMC), Nagpur at 4°C. Till further processing, samples were stored at 4°C. Samples were received from all govt. institutions/ medical colleges from Vidarbha region of Maharashtra. Patients were divided into five age groups for the analysis of the results. Age Group I, Group II, Group III , Group IV and Group IV comprising 0-10, 11–20, 21–40, 41-60 and 61 years and above, respectively. Samples received from Oct-2015 (lab started functioning in oct-15) to Sep-2019 were included in the study. Different clinical and epidemiological parameters were noted in all positive cases.

The study was approved by Institute's

2.1. Ethical committee

Samples were processed as under –

a) Ribonucleic acid (RNA) extraction – Coloumn based extraction was performed by QIAmp® viral RNA Mini Kit from Qiagen, USA.

b) Mastermix preparation – Mastermix was prepared by using following components and concentration and Extracted RNAs added last into the mix–

Table 1:

S. No.	Component	Volume per reaction
1	Nuclease free water	6 μl
2	2X SuperScript III Master mix	12.5 μl
3	Primer probe mix	0.5 μl
4	Taqman Polymerase Enzyme	1 μl
5	Extracted RNA	5 μl
	Total Volume	25 μl

c) Positive and Negative template control (PTC & NTC) were provided by National Institute of Virology (NIV), Pune.

Primer probe sequence's that were used in the study were as follows –

d) RT-PCR : Samples were tested by reverse transcriptase realtime PCR (RT-PCR) for influenza type A using Step one and Step one plus Applied Biosystems Real time PCR machine by Thermofischer Scientific using cycling conditions of 50°C for 30 min of reverse transcription followed by Taq inhibitor inactivation at 95°C for 10 min and PCR amplification (45 cycles) at 95°C for 15 seconds and at 55°C for 30 seconds.

Table 2:

Primers and probes	Sequence (5' > 3')
Inf A Forward	GAC CRA TCC TGT CAC CTC TGA C
Inf A Reverse	AGG GCA TTY TGG ACA AAK CGT CTA
Inf A Probe	FAM – TGC AGT CCT CGC TCA CTG GGC ACG
Pdm H1 Forward	GTG CTA TAA ACA CCA GCC TCC CAT T
Pdm H1 Reverse	AGA YGG GAC ATT CCT CAA TCC TG
Pdm H1 Probe	FAM – ATA CAT CCR ATC ACA ATT GGR AAA TGT CCA AA
Rnase P Forward	AGA TTT GGA CCT GCG AGC G
Rnase P Reverse	GAG CGG CTG TCT CCA CAA GT
Rnase P Probe	FAM – TTC TGA CCT GAA GGC TCT GCG CG

e) Results were interpreted depending upon the Cycle Threshold (C_T) values. Curve for the given target was considered as positive if it crossed the threshold on or before 35 cycles. All samples indicated a positive curve for RNase P (crossed the threshold on or before 35 cycles, indicating the presence of sufficient human RNase P gene). Positivity for RNase P gene is a marker to assess the quality of the specimen.

f) Interpretation –

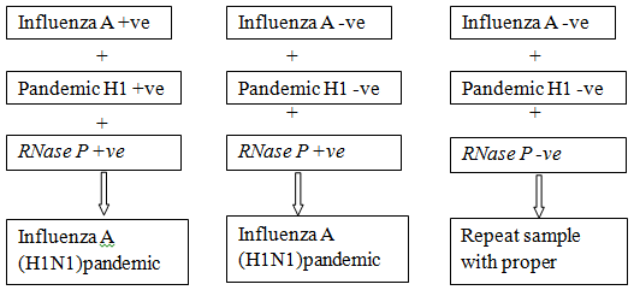

Fig. 1:

During the study period, a total of 3690 samples were received for testing. Of these 3690 samples, 553 (15%) samples tested positive for influenza A H1N1 pandemic 09. Year wise distribution of cases is shown in Figure 2 . From 2015 – 2019, no. of females patients suspected to be having Influenza A (H1N1)pandemic 09 infection were more than male patient but positivity was almost equal throughout these years except in 2019 (M/F positivity was 7/12 %) as

Table 3: Year and gender-wise distribution of sample

Year	Total tested	No. of Positive	Positivity in males	Positivity in females
2015	387	74 (19%)	35/179 (19%)	39/208 (19%)
2016	310	1 (0.3%)	1/310 (0.3%)	0
2017	1765	377 (21%)	177/774 (23%)	200/991 (20%)
2018	922	70 (8%)	27/368 (7%)	43/554 (7%)
2019 (till date)	473	47 (10%)	12/173 (7%)	35/300 (12%)

shown in Table 3. In 2015, maximum affectivity was seen in age groups 21-40 and 41- 60 years, which changed to 0-10 years in 2017 followed by again 21-40 years in 2018 and 11-20 years in 2019 as shown in Table 4. Looking at the districts most affected, in 2015 , Bhandara , Gondia and Wardha were most affected. In 2017, again Bhandara district was hit the most by the virus followed by Chandrapur district. In 2018, again Gondia had highest positivity and in the current year, Akola is the worstly affected district which was never in top three hit list districts from 2015 to 2017. From 2018, Akola came into top three districts with highest H1N1 positivity. Table 5 shows districtwise distribution of cases. Influenza A (H1N1) pandemic 09 is known to circulate in between Aug-Nov months. In last five years, we have seen peaks of infection in this same time period but peaks have also been noted in summer months (Figure 3). Clinical features associated with the infection are mentioned in Table 6.

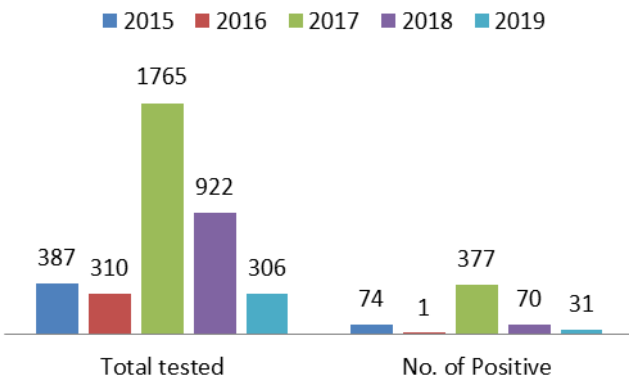

Fig. 2: Yearwise distribution of cases

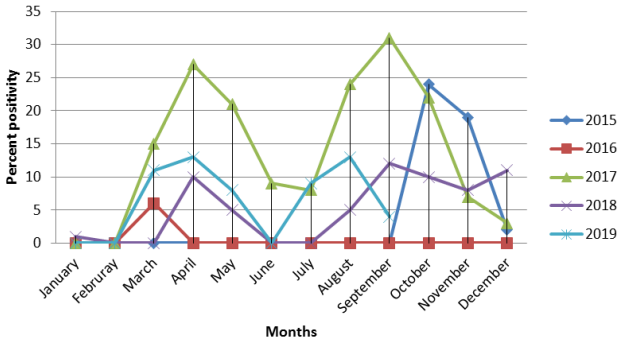

Fig. 3: Seasonal variation in circulation of H1N1

3. Discussion

India witnessed one of the large Influenza A (H1N1) pandemic 09 outbreak in 2009 and had 27,236 confirmed cases and 981 deaths. During post pandemic period in 2010, 20,604 confirmed cases with more deaths (1763) were reported. Severely affected states were Maharashtra, Delhi , Rajasthan, Gujarat , Madhya Pradesh, Karnataka , Haryana, Kerala, Tamil Nadu and Andhra Pradesh with high fatality in Rajasthan. Another influenza outbreak hit in India in 2015, during which most affected states were Rajasthan, Gujarat , Delhi Maharashtra, Karnataka and Madhya Pradesh with more number of deaths in Rajasthan and Gujarat.[8] Integrated Disease Surveillance Programme (IDSP) data show that the epidemiology of influenza A (H1N1) is changing in terms of geographical distribution, age and seasonality but studies related to this are scarce. In our study, we tried to focus on changing trends of influenza activity in central India. Influenza activity continue to be reported every year, more during winter. However, as per IDSP data, 2017 was an unusual year, with the virus spreading rapidly throughout the country, and showing different epidemiological parameters as compared to previous years in terms of period (two peaks observed), place (infection reported for the first time from some of the north-eastern states) and person (comparatively more cases among children).[9] In our study , we found maximum suspected and positive cases in the year 2017 in last five years in spite of year 2017 being hotter than normal in last 15 years. Our study showed and support similar change in time periods of peaks of circulation of H1N1 in 2017, 2018 and 2019 where we got two peaks, one in Mar-May and other during Aug-Nov. Similar findings of two peaks in a year is also seen in an outbreak reported by Mudhigeti N et al. in Andra Pradesh.[10] But some studies showed winter prevalence of H1N1[11,12] and some showed post monsoon peaks.[13,14] In one more study by Broor et al., maximum cases were reported in rainy season.[15] Dual peaks have also been reported from other Indian author's[16] and other parts of the globe.[17,18]

Although these years, age group 21-40 and 41-60 years were mostly affected in our study. Since 2017, we noted shift in age group towards 0-10 and 11-20 years. In 2018

Table 4: Age-wise distribution and positivity ofsamples n = no. of cases tested in that age group

Age group	2015		2016		2017		2018		2019	
	n	Positive	n	Positive	n	Positive	n	Positive	n	Positive
0-10 yrs	103	16 (15%)	51	1 (2%)	283	68 (24%)	119	6 (5%)	29	1 (3%)
11- 20 yrs	36	3 (8%)	31	0	140	31 (22%)	119	9 (7%)	41	11 (26%)
21-40 yrs	129	31 (24%)	129	0	688	153 (22%)	329	30 (9%)	153	17 (11%)
41-60 yrs	78	20 (25%)	72	0	418	87 (21%)	227	19 (8%)	117	8 (7%)
61 and above	33	4 (12%)	20	0	191	31 (16%)	116	5 (4%)	60	6 (10%)

Table 5: District wise distribution of cases n = no. of cases tested from that district

Year	2015		2016		2017		2018		2019	
District	n	Positive	n	Positive	n	Positive	n	Positive	n	Positive
Nagpur	292	47 (16%)	243	1 (0.4%)	986	188 (19%)	555	31 (5%)	308	27(9%)
Amravati	16	4 (25%)	0	0	359	86 (24%)	126	11 (8%)	26	2(8%)
Bhandara	14	5 (35%)	1	0	37	14 (38%)	1	0	3	0
Gondia	22	7 (31%)	25	0	9	1 (11%)	5	2 (40%)	0	0
Wardha	23	8 (34%)	4	0	113	26 (23%)	35	1 (3%)	11	1(9%)
Yavatmal	18	3 (16%)	19	0	51	12 (23%)	44	5 (11%)	4	0
Washim	2	0	0	0	3	0	1	0	0	0
Akola	0	0	18	0	147	31 (21%)	137	20 (14%)	106	18 (17%)
Chandrapur	0	0	0	0	54	19 (35%)	9	0	15	0
Gadchiroli	0	0	0	0	4	0	9	0	0	0

Table 6: Clinical features of positive cases

Symptoms	2015	2016	2017	2018	2019
	n=74 (n%)	n=1 (n%)	n=377 (n%)	n=70 (n%)	n=31 (n%)
Fever	74 (100)	1 (100)	377 (100)	70 (100)	31 (100)
Breathlessness	53 (72)	1 (100)	313 (83)	53 (76)	21 (69)
Cough	61 (83)	1 (100)	264 (70)	56 (81)	23 (76)
Sore throat	19 (26)		79 (21)	13(18)	5 (16)
Myalgia	46 (62)		109 (29)	48 (69)	18 (58)
Arthralgia	15 (21)		72 (19)	13 (19)	6 (21)
Rhinorrhoea	10 (14)		45 (12)	11 (16)	5 (18)
Nausea	21 (28)		19 (5)	18 (26)	9 (31)
Haemorrhagic manifestations	0	0	19 (5)	1 (1.4)	1 (3.2)
Conjunctivitis	0	0	0	1 (1.4)	1 (3.2)
Diarrhoea	0	1 (100)	4 (1.2)	1 (1.4)	2 (6.4)
Encephalitis	0	0	0	1 (1.4)	1 (3.2)

and 2019, again younger age groups found to be mostly affected in our study. In a study by Ganesh Nandhini et al., maximum positivity was seen in age group 20-49 years[19] and in 51-60 years by Prasad S et al.[20] While Siddharth et al.[12] stated that influenza A H1N1 pandemic 09 relatively spared the older population and had stricken the younger population. The age shift in our study shows that virus has now started establishing in all age groups more towards younger population. This needs high alert suspicion of the infection and demands increased attention. Majority of the positive cases had the clinical manifestations of fever, breathlessness, cough, followed by sore throat and myalgia. Apart from respiratory symptoms, cases also presented with diarrhea, nausea, haemmorhagic manifestations and conjunctivitis. In a study, the main presenting symptom was fever that is 83.3%, followed by dyspnea (76.7%), cough (70%), throat pain and least common was chest pain.[21] While KN Bhatt et al. reported , nausea/ vomitting along with respiratory symptoms similar to our study.[16] Many other studies have reported similar clinical findings with changing percentage.[22,23] In our study, female patients were more with Influenza like illness (ILI) than male patients; But no significant difference in positivity among males and females was noted. Similar findings are also given by KN Bhatt et al. and Mudhigeti N et al and Amaravathi KS et al.[24] In a study by Arvind Chandora et al. where they compared six year's data from 2011-2015 as in our study and[25] they found that female population was more (>50%) than male from 2010 to 2014 but in year 2015 male population was more (>50%) than female. But in our study, in current year 2019, females are more affected (12%) than males (7%) which is statistically significant. Different set of years of study period could be a reason for this opposite findings. Similar to our study, Prasad S et al. also showed slight female preponderance. As pregnancy is a known risk factor among females to acquire more complications due to H1N1,[26] slight shift of infection towards females demands keen vigilance and rapid management. The geographic region is also widened by the virus due to increased travel. Almost all districts of Vidarbha region are having peaks of infection in one or the other year. More population is now at high risk of getting infection by H1N1 making it a big public health challenger. Though percent positivity is decreasing down these years, changing epidemiology of the virus needs attention. Based on molecular analysis of isolates from Chennai and Pune, the dominant flu strain in India during 2017 was A/ Michigan/7/2009 (H1N1) pandemic 09 virus, replacing A/California/7/ 2009 (H1N1) pandemic 09 seen during 2016.[27] This is not an antigenic shift but a minor drift, but this change in circulating strain should be a focus of future research. WHO listed the virus in 'Top Ten Threat's' world can face in 2019. Who says- 'The world will face another influenza pandemic – the only thing we don't know is when it will hit and how severe it will be. Global defences are only as effective as the weakest link in any country's health emergency preparedness and response system.'[28]

4. Conclusion

Although the pandemic era of H1N1 has settled, epidemics are a constant reminder of the hidden danger. Silent spread of the virus has to be kept an eye on to see - What is changing/ changed/ expected to change ?

1. Epidemiology
2. Agent
3. Clinical Features
4. Diagnostics
5. Treatment
6. Prevention

5. Acknowledgment

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6. Source of funding

None.

7. Conflict of interest

None.

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