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Original Article

Seasonal Outbreak of Influenza A virus Infection in Pediatric Age Groups During 2004-2005 in South of Iran

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Abstract

Background: The pandemic and regional influenza outbreaks resulting from antigenic variation of influenza viruses have been the subject of numerous studies which are crucial to the preparation of the vaccine. Frequent global winter outbreaks of influenza viruses require a constant surveillance of emerging influenza variants in order to develop efficient influenza vaccine.

Methods: This study was conducted from December 2004 to March 2005, during an outbreak of influenza in 300 children of one to 15-year-old. MDCK cells grown in microwell plates inoculated with pharyngeal swabs. Hemagglutination (HA) of guinea pig erythrocytes by viral isolates was used for initial screening of influenza viruses. The final viral identification was performed by HA inhibition test using reference antisera prepared against WHO reference antigens.

Results: Based on the results of HA tests with culture fluid of MDCK inoculated with 300 pharyngeal swabs, 31 (10.3%) of the specimens were suspected to contain influenza viruses of which 10 proved to be either A/H_1N_1 or A/H_3N_2 subtypes. by reacting against aforementioned antisera in HA inhibition test. H_3N_2 subtype prevailed until January 2005 and mostly affected children of younger age, whereas viruses emerging subsequently were only of H_1N_1 subtype and predominantly isolated from children of older age.

Conclusion: A/H_3N_2 isolate appears to be antigenically different from corresponding reference antigen. This is evident by a 16-fold reduction in antibody titer by using reference antiserum against A/H_3N_2 Isolate (1:8), as compared to that of reference antigen antibody (1:128).

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Keywords ● Human influenza ● vaccine ● H3N2 ● H1N1 ● pediatric

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Introduction

Periodical influenza outbreaks prevail during winter months, mostly in January to March each year and simultaneous with other respiratory infections of viral origin.[1] Epidemiological and virological surveillance of influenza are reported through numerous studies carried out across

diverse geographic regions.[2-5] Influenza illness is characterized by fever, chills, headache, muscle aches, cough, congestion, sore throat and malaise with bronchitis which commonly occur in small children who are potential sources of infecting adults. The mutability of influenza A and B viruses stipulates the need for a constant surveillance of emergent strains, a process fundamental to the development and reappraisal of current influenza vaccines.[6] The present study describes the first attempt to isolate and identify influenza viruses from children during December 2004 to March 2005 in Shiraz, south Iran.

Material and Methods

Specimen collection and processing

This study was conducted from December 2004 to March 2005. Pharyngeal swabs were collected from 300 patients of 1-15-years old by sterile cotton-tipped sticks. They were then placed in sterile screw-capped containers with Eagle's minimum essential medium and transported, under refrigeration, to the laboratory where they were gently agitated and extracted for further processing. The extracts were kept at -70°C until used.

Cell culture

Madin-Darby canine kidney (MDCK) cells were supplied by central influenza reference laboratory in Tehran affiliated to the World Health Organization (WHO). These cells were used after three passages in Dulbecco's minimum essential medium (DMEM, Sigma; USA). The medium was supplemented with 7% (V/V) fetal bovine serum (GIBCO; USA), 100 unit/ml penicillin G and 100µg streptomycin /ml.

Viral isolation

Cells grown for 48h in 24 microwell plates were used for inoculation of clinical specimens. This was achieved by discarding the growth medium and rinsing the monolayers with 2 ml phosphate buffered saline (PBS). Each monolayer was then inoculated with 200µl of the sample and incubated at 34°C for 60 min, followed by addition of 1 ml serum-free DMEM containing 2 µg/ml of trypsin (DIFCO). The plates were incubated at 34°C in 5% CO_2 for 96 hr for cytopathic effect (CPE) to develop, if cell monolayers had been inoculated with virus positive specimens. The ability of influenza viruses to hemagglutinate was determined by treating monolayers with 0.5% suspension of guinea pig erythrocytes in PBS at 4°C for 2 hr. This tentative viral identification was subsequently confirmed by hemagglutination inhibition (HI) test with reference antisera.

HI Test

A modification of HI test described elsewhere,[7] was used in the present study. Briefly, serial two-fold dilutions of each reference antiserum in 25µl of PBS were prepared in 96 microwell plates. An aliquot of 25µl containing four HA units of the antigen under study was then added to each microwell. The contents of microwells were mixed by gentle agitation and held at room temperature for one hr, when 50µl of a 0.3% suspension of guinea pig erythrocytes was added to each microwell. The plates then shaken gently and the results recorded two hrs after of incubation at 4°C.

Serotyping

The identification of the types and subtypes of viral isolates was achieved using HI test and reference antisera. These comprised antisera against A/NewCaledonia/20/99(H_1N_1)-Like, A/Moscow/10/99(H_3N_2)-Like, B/Sichuan/330/2001/-Like and B/Hong Kong/330/200/-Like Antigens.[7]

Results

This study was conducted from December 2004 to March 2005. Of 300 pharyngeal specimens inoculated into MDCK monolayers, 31 (10.3%) agglutinated guinea pig erythrocytes and were suspected of containing influenza viruses. Table 1 shows reciprocal hemagglutination-inhibition antibody titers against four reference influenza virus antigens using homologous and heterologous reference antisera. Initially, 14 of 31 tentative influenza virus isolates were examined by HI test for their inhibition by 4 reference typing antisera. As a result, 10 isolates proved to be influenza viruses of which 4 were H_1N_1 subtype and 6 H_3N_2.

Table 1: Reciprocal Hemagglutination-inhibition antibody titers of four reference influenza virus antigens against homologous and heterologous reference antisera

Reference Antigens	Reference Antisera			
	A	B	C	D
A	128	8	<1	<1
B	<1	128	<1	<1
C	<1	<1	128	8
D	<1	<1	4	128

A= A/NewCaledonia/20/99 (H_1N_1)-like;
B= A/Moscow 10/99 (H_3N_2);
C= B/Sichuan 330/2001-like;
D= B/Hong Kong 330/2001-like

H_3N_2 subtype prevailed until January 2005 and mostly affected children of younger age. The subtype emerging from January 2005 until February 2005 was only H_1N_1 and predominantly

Table 2: Reciprocal Hemagglutination-inhibition antibody titers of A/ H_1N_1 A/ H_3N_2 subtypes.

No	Field Isolates (Shiraz)	Age (yr)	Reciprocal antibody titer			
			A	B	C	D
1	A/Shiraz/1/2005 (H_1N_1)	5	64	<1	<1	<1
2	A/Shiraz/2/2005 (H_1N_1)	6	64	<1	<1	<1
3	A/Shiraz/3/2005 (H_1N_1)	5	64	<1	<1	<1
4	A/Shiraz/4/2005 (H_1N_1)	10	64	<1	<1	<1
5	A/Shiraz/5/2005 (H_3N_2)	2	<1	4	<1	<1
6	A/Shiraz/6/2005 (H_3N_2)	3	<1	8	<1	<1
7	A/Shiraz/7/2005 (H_3N_2)	5	<1	8	<1	<1
8	A/Shiraz/8/2005 (H_3N_2)	11	<1	4	<1	<1
9	A/Shiraz/9/2005 (H_3N_2)	2	<1	4	<1	<1
10	A/Shiraz/10/2005 (H_3N_2)	3	<1	8	<1	<1

A= A/NewCaledonia/20/99 (H_1N_1)-like; B= A/Moscow 10/99 (H_3N_2); C= B/Sichuan 330/2001-like; D= B/Hong Kong 330/2001-like

isolated from children of older age (Table 2). A/H_3N_2 isolate appears to be antigenically different from corresponding reference antigen. This is evident by a 16-fold reduction in antibody titer against A/H_3N_2 Isolate (1:8), as compared to that of reference antigen (1:128).

Discussion

The present study describes the first attempt to isolate influenza viruses from pharyngeal specimens during winter outbreak in Fars province south of Iran. Serotyping by standard reference antisera showed that at least 10 of 31 hemagglutinating viruses tested, were either H_1N_1 or H_3N_2 influenza subtypes. Undoubtedly, the remaining isolates were likely other respiratory viruses. In connection with the results obtained, H_3N_2 strain was more frequently isolated from younger children, aged from 18 months to 5 years, whereas H_1N_1 subtype was mostly isolated from those aged from 5 to 10 years.

A review of influenza infection in the Netherlands showed a varying seasonal incidence of 46% among children from few months zero to 19 years with an overall incidence of 5% to 9.5% per yr.[8] In this context, more than 50% of H_1N_1 infection was reported to occur among subjects aged 10-34 years.[9] This was in accord with the results of the present investigation in which H_1N_1 subtype prevailed among children of older age (Table 2).

According to an update of influenza virus activity during the 2004-5 influenza seasons, influenza A/H_3N_2, but not A/ H_1N_1 subtype, was isolated from Iran and several Asian countries.[3] It is noteworthy that the present study reported the isolation of influenza (H_1N_1) subtype from southern Iran. On the basis of the results obtained, the outbreak due to H_1N_1 subtype preceded the emergence of H_3N_2 outbreak and was of short duration, a finding consistent with low level circulation of H_1N_1 in most parts of the world.[4]

Interestingly, the reciprocal antibody titer of reference antiserum against A/H_3N_2 isolate was 16-fold lower than the homologous titer (Table 2). In this regard, the antigenic drift in HA molecule of A/H_3N_2 isolated may account for the low titer of reference antibody against this subtype.[10-14] This was supported by a continuous drift of A/H_3N_2 strain reported during 2004 outbreak in Nepal.[15] This finding might warrant the reappraisal of current influenza vaccine. This was consistent with the study performed in New Caledonia, where identification of circulating viral types was carried out to gather information for the local vaccination program.[16] In this connection, the isolation of H_3N_2 subtypes in the present study may suggest that the current influenza vaccine may not be sufficiently protective against influenza outbreaks caused by this subtypes.[9] but the final identification awaits further antigenic analysis and molecular studies.

Conclusion

In the light of antigenic difference of A/H_3N_2 shown in the present investigation, currently used influenza vaccine is not suitable for vaccination in southern Iran and needs to be reappraised.

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