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Isolation and Typing of the Influenza Viruses in the Caspian Littoral of Iran

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Present study introduces results of common influenza virus strains in the north of Iran. Samples collected from 65 patients with acute respiratory illness by throat washing and swabs, randomly from north of Iran (Mazandaran, Golestan and Guilan provinces). The patients suffered from fever (high), cough, sore throat, general malaise, chill and myalgia. Viruses were isolated by cell culture and confirmed with HA (Hemagglutination) test and then typed by and HI (Hemagglutination Inhibition) test. Out of 65 throat samples, 12 influenza viruses were isolated and typed. Isolated viruses belonged to A (H1N1), A (H3N2) and B influenza viruses. This study showed that Influenza viruses displayed identical pattern to other provinces in Iran and to other countries. To fight against epidemics and pandemics, we should collect enough data about status of influenza each year and data of exact vaccine formulation application for use in different areas of the world. Acquired data has shown that the vaccine for above viruses that confirmed by WHO can result in decreased risks of influenza in at risk cases in provinces of north Iran.

Key words: Influenza virus A, influenza virus B, North of Iran

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INTRODUCTION

Influenza virus infection is a broad spectrum clinical response from an asymptomatic infection to fatal viral pneumonia. Influenza viruses are transmitted person-to-person through respiratory secretions (David *et al.*, 2001). High percent of the world population is infected with the virus annually. In Iran large populations acquire the infection in the epidemic months and most of them present with systemic and local clinical features like: high grade fever, chills, dry cough, headache, agitation and generalized muscular pain (White and Fenner, 1994; Maurice, 2002).

Influenza is noted as an important, acute and common infection of the respiratory tract. Causal influenza viruses have 3 types: A, B and C (David *et al.*, 2001, White and Fenner, 1994). Type A, the most important type, causes annual epidemics in the cold seasons. Type B and C are less important (Maurice, 2002).

Type A can cause epidemics and pandemics in the world, that is the result of antigenic drift and antigenic shifts of the surface antigens (H = Hemagglutinin and N = Neuraminidase) (David *et al.*, 2001; Peter *et al.*, 2001). Influenza epidemics occur in the cold months, naturally. It causes at list 3000-4000 deaths in England in annually and death rate is higher in the old ages (Health Organizaition, 2005).

As a network of national laboratories is created by WHO to monitor Influenza and isolate the common strains and it presents new vaccines due to the reports of such laboratories; so it is wise to evaluate the common viruses in all local provinces. Iran national Influenza center is located in the Institute of Hygienic Researches in Tehran University of Medical Sciences that isolates and types the viruses to identify new strains.

Present study introduces results of common influenza virus strains in the north of Iran (Mazanderan, Golestan and Guilan) in, 2005.

MATERIALS AND METHODS

Sampling: Samples of pharyngeal washing and throat swab were taken from 65 suspected patients with symptoms of acute respiratory infection in December, January and February (2005-2006).

Samples were taken randomly from clinics and primary schools. All cases had fever (high), chill, cough, sore throat, agitation and muscular pain. All age groups were included. Sampling continued to the first influenza viruses A and B and no special sample size is noted. They were transported to the national influenza laboratory at Division of Virology, Department of Pathobiology, School

of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences within 5 days, in cold string (4°C) in transport buffer (PBS with penicillin, streptomycin and amphotricin B and 0.5% glycerol).

Virus isolation: Samples were inoculated with MDCK (Madin Darby Canine Kidney) and monitored as the cellular changes in a week. If a live virus presents cenotaphic effects could be seen in 2-3 days but cellular cultures were evaluated to one week for more accuracy. Method culture had showed this the best growth in RPMI (Roswell Park Memorial Institute, Gibco, UK). Serum free cultures with 2 mg mL⁻¹ TPCK were used in the inoculation time for easy isolation and kept in 33-34°C.

Confirmation of virus presentation with HA: In samples with CPE due to virus growth, hemagglutination with chicken or hamster RBCs was done after getting the surface product.

Globules were agglutinated if virus was present. Negative samples or lower titers were inoculated to the cellular cultures again and HA was done on the final products.

Typing of the isolated viruses with HI: Samples with antigenic titer of 4 or higher units of hemagglutinin, were typed and samples with titers less than 4 units were added to the cells to increase the titer.

Typing was done with the reference anti-sera [A/H3N2/Fujian/411/2002-like, A/H1N1/New Caledonia/20/99-like, A/H3N2/Moscow/10/99-like, B/Hong Kong/330/2001-like, B/Sichuan/379/99-like (WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia)] and hemagglutination inhibition method. Chicken or hamsters RBCs were used.

RESULTS

Thirty eight samples from female (58.5%) and 27 from male (41.5%) were taken as shown in Table 1. The youngest was a 2-years-old boy and the oldest was a 75-years-old male. The mean age was 33 years. Twelve influenza viruses were isolated and typed from 65 suspected cases. Both influenza virus type A and B were present in north of Iran.

Table 1: Types of common Influenza viruses in North of Iran (2005-2006)

Cases	Type of virus
2	A/New/Caledonia/20/99-like(H1N1)
4	A/Moscow/10/99-like (H3N2)
3	B/Sichuan/379/99-like
3	B/Hong Kong/330-2001-like
12	Total

DISCUSSION

A natural epidemic of influenza contaminates 5-15% of the population (Bridges *et al.*, 2000). It can cause serious disease and high rate of death in children and old ages (80-90% of deaths in the epidemics belong to the persons more than 65-years-old), immunocompromised patients or chronic heart diseases, diabetes mellitus, chronic gastrointestinal diseases with difficult defecation, cancer patients, alcoholics and asthmatic patients (Nobert *et al.*, 2002; Gurfinkel *et al.*, 2002; Anonymous, 2003).

The Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP) and the American Academy of family physicians now recommend that all adults above age 50 be routinely offered the vaccine, in addition to younger people with chronic medical conditions including those with heart disease (Peter *et al.*, 2001).

ACIP have proposed that the age of vaccination should be decreased from 65 years to 50-years-old, because 24-32% of persons over 50-years-old suffer from chronic diseases, their hospitalization due to influenza infection is long lasting and sometimes results to death (Bridges *et al.*, 2000).

Some practices have been done in countries like America, European country, in Asia prepared valuable practical programs to be used in other that countries initiating the program. Coming pandemics are expected, despite these progressions (Nancy *et al.*, 2003).

Accurate monitoring of the virus changes is the best method to fight it. Virus antigenic changes should be noted annually with isolation and typing of the suitable vaccines and medicines should be prepared (David *et al.*, 2001).

As shown in the Table 1, in 2005-2006 in the north of Iran, influenza virus type A (H1N1) was circulated in a low level, like the other parts of the world and it was similar to A/new caledonia/20/99 virus.

Influenza virus type A (H3N2) isolated in this study was similar to A/Moscow/10/99 virus. Presentation of this subtype is not reported from the other sites of the country.

Influenza virus type B was more prevalent in the area. In the other sites of the country, influenza virus type B were seen in eastern Azarbaijan, Hamedan, Yazd, Hormozgan, Boushehr, Isfahan and Khorasan. But in other countries, except for Japan, it has a lower level (Weekly Epidemiological Record, 2005). No prevalence of this type was reported in September 2005 and January 2006 (Weekly Epidemiological Record, 2006).

Influenza virus type A (H3N2, H1N1) is reported in other provinces (unpublished data). In 1997 influenza virus type A and B were isolated in national influenza center (Mokhtari-Azad *et al.*, 1999).

Seroepidemiologic studies in Iran showed Influenza virus type A (H3N2) in 1991-92, 1997-98 and 1999-2000, then virus type A (H1N1) and type B were reported in 2000-2001 (Mokhtari-Azad *et al.*, 2004).

Four epidemics of influenza virus type A (H3N2, H1N1) are reported from 50 countries that reported their influenza activities in September 2005 to January 2006. A (H3N2) is reported in Canada, Japan and the United States. A (H1N1) is reported in Tunes. But Type B is not reported (Weekly Epidemiological Record, 2006).

Wet and moderate weather in the north of Iran may plays a role in the incidence and severity of clinical presentation of influenza. A relationship between influenza and temperature has been suggested before (Kyriazopolou *et al.*, 1995).

We should have enough data about the influenza states in the region with examination on the samples especially in high risk persons to prepare suitable protocols for the community.

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