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Comparison of Real-Time Polymerase Chain Reaction and Conventional Cell Culture for Detection of Influenza A in Tabriz, Iran

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Background: Influenza type A (H1N1) causes an epidemic disease, resulting in thousands of deaths throughout the world.
Objectives: Our aim was comparing the efficacy of two different methods in isolating the Influenza (H1N1) virus including cell culture and real-Time PCR in Tabriz and suburbs.
Patients and Methods: Of throat swab samples, 220 were collected in viral transport medium (VTM) from patients with a suspected influenza virus infection referred to hospitals and clinics of Tabriz University of Medical Sciences. All the samples were examined through virus culturing in Madin-Darby canine kidney epithelial cells (MDCK line) and also real-time polymerase chain reaction (RT-PCR). The collected data were analyzed by SPSS version 16.
Results: RT-PCR detected 41 cases of influenza A (H1N1), compared with the virus isolation methods that detected 18 cases.
Conclusions: RT-PCR provided a sensitive and specific route compared with virus isolation. In addition, urgent planning for the vaccination program of influenza A is suggested, which can definitely prevent the spread of virus in this part of Iran.

Keywords: Influenza a Virus, H1N1 Subtype; Real-Time Polymerase Chain Reaction; epidemics; Pandemics

1. Background

Human influenza viruses continue to cause occasional epidemics and pandemics yearly. In recent years, a possible influenza pandemic arising from the A (H1N1) virus has been the subject of many reports. The high predominance rate of influenza A among adolescence stresses the significance of preventative policies among this age group (1). Any reporting data on comparative epidemiology and viral nature of the pandemic influenza A (H1N1) virus can help the sanitation authorities to enhance the capability of general practice and help the community and public health actions for the influenza pandemic (2). For the world travelers who may encounter a different antigenic drift or shift of influenza, unique situations should be considered. A number of important sufficient details in influenza prevention as well as control guidelines should be included in travel medicine. Travelers might bring in the novel strains into domestic populations (3). Entry screening may result in finding a novel strain of influenza virus; so, early detection and isolation can slow the spread of infection in communities. There is still worldwide fear from the possible pandemic wave of the new swine influenza virus (4) Using the latest and most adequate data can fill the gap in the essential information gathering for opposing the pandemics (5). Moreover, programming on controlling the influenza infection may lower its morbidity and mortality. It will also provide methods for decreasing the prevalence of influenza infection worldwide. The international de-novo incidence of emerging H1N1 infection made the pandemic threat alert as the level 6 by the World health Organization (WHO) (5). The 2009 H1N1 pandemic highlighted the significance of the unpredictable nature of influenza virus. Therefore, the risk of 2009 H1N1 influenza strain or other future strains causing severe diseases remains an open question (6). More attempts will be needed during an influenza pandemic to clarify the capacity of healthcare systems for optimizing the ability of general practice in supporting the population. This should include revising the public health communication strategies and prepandemic evaluation of practice ability (2).

Tabriz city is located near Aras river in northwest Iran, a free trade zone to three countries including Azerbaijan, Nakhchivan and Armenia; thus, Tabriz has an essential role in influenza transmission to and from the neighboring countries. There is a hypothesis that introduces Asia as an epicenter for influenza (7); thus, illustration of the epidemiological features of influenza seems to be important to people living in Tabriz and its suburbs. In addition, we tried to evaluate the relationship between educational level and influenza infection in this part of

Iran. Competent and precise detection was considered as a priority for effective infection detection and treatment.

2. Objectives

Our aim was comparing the efficacy of two different methods including cell culture and real-time polymerase chain reaction (RT-PCR) for isolating the Influenza (H1N1) virus in Tabriz and suburbs. RT-PCR assessment for diagnosis of pandemic viruses seemed essential prior to further pandemics (8). This study tried to answer the questions about the significance of influenza virus early detection using highly sensitive methods, which may result in less admission rate, shorter hospitalization and early treatment (9).

3. Patients and Methods

We collected samples of patients suspected to human influenza viruses in Tabriz during influenza seasons from October 2009 to March 2010. The samples were tested in the virology lab, Faculty of Medicine, Tabriz University of Medical Sciences. Data of all the patients infected with influenza and admitted to hospitals, including signs and symptoms and level of education, were collected and analyzed by SPSS version 16.

3.1. Cell Culture

To isolate the influenza virus by cell culture, the Dulbecco's modified eagle medium (DMEM) (Gibco-BRL) cell line was used. The Madin-Darby canine kidney epithelial (MDCK) cell line was provided by Dr. Mokhtari-Azad, Department of Virology, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran, which was kindly denoted by WHO. The MDCK cells were incubated under a humid atmosphere with 5% CO_2 at 37°C. To keep sterility in our procedure, passage of the cells was performed on a class II laminar flow cabinet. The MDCK cells were supplemented with streptomycin (100 μg/mL) and penicillin (100 IU/mL), Glutamine (2 mM), 1% nonessential amino acids and 10% fetal calf serum. T25 flasks were used to culture the cells, till confluent within three to four days. The cells were trypsinized using 0.5% trypsin and incubated at 37°C for 3-5 minutes. The trypsin was inactivated by adding DMEM. A volume of 5×10^3 MDCK cells was transferred to a 96 well plate. After one day, 100 μL of each patient's sample was inoculated into wells containing MDCK cells and kept in incubator at 34°C for 3-7 days. The culture medium was then tested for hemagglutinin activity, using a 0.5% suspension of hen erythrocytes.

3.2. Polymerase Chain Reaction Amplification

Viral RNA was extracted from 150 μL of each sample, using PureLink® viral RNA/DNA mini kit (Invitrogen, USA). To perform RT-PCR using Rotor-Gene Q RT-PCR cycler (QIAGEN, USA), amplification of each RNA segment was carried out by reverse transcriptase PCR according to the manufacturer's instruction. Specific oligonucleotides were designed to obtain specific products from the positive samples by PCR. The cycling procedure included two minutes at 94°C followed by 45 cycles of 30 seconds at 94°C, 30 seconds at 53°C for annealing, one minute at 72°C for polymerization, and finally 10 minutes polymerization at 72°C.

4. Results

Clinical manifestations of patients were myalgia, fever, cough, headache, and sore throat with frequencies of 100%, 91.6%, 91.1%, 4.5%, and 1.5%, respectively (Table 1). Regarding the education level of patients, 18.8%, 48%, 32.2%, and 1% were illiterates or had primary, secondary, or postgraduate education, respectively (Table 2). The comparison of influenza detection based on RT-PCR and cell culture showed that 41 (20.3%) of 202 patients were positive using PCR, in comparison with 18 (8.9%) positive results detected by cell culture (Table 3).

Table 1. Signs and Symptom of Influenza in The Infected Population

Signs and symptoms	Frequency	Percentage	Valid Percentage	Cumulative Percentage
Myalgia	202	100.0	100.0	-
Fever	185	91.6	91.6	100.0
Cough	184	91.1	91.1	100.0
Headache	9	4.5	4.5	100.0
Sore Throat	3	1.5	1.5	100.0

Table 2. The Level of Education in The Population Infected with Influenza

Level of education	Frequency	Percentage	Valid Percentage	Cumulative Percentage
Illiterate	38	18.8	18.8	18.8
Primary education	97	48.0	48.0	66.8
Secondary education	65	32.2	32.2	99.0
Postgraduate education	2	1.0	1.0	100.0
Total	202	100.0	100.0	-

Table 3. Comparison of Sensitivity of Cell Culture and Real-Time Polymerase Chain Reaction in Influenza Detection [a]

Cell Culture	Frequency	Percentage	Valid Percentage	Cumulative Percentage
Positive	18	8.9	8.9	8.9
Negative	184	91.1	91.1	100.0
Total	202	100.0	100.0	-
RT-PCR				
Positive	41	20.3	20.3	20.3
Negative	161	79.7	79.7	100.0
Total	202	100.0	100.0	-

[a] Abbreviation: RT-PCR, real-time polymerase chain reaction.

5. Discussion

The significance of influenza as a major cause of morbidity and mortality in traveling populations needs further investigations by health care authorities. A research in Iran indicated that chronic respiratory diseases as well as hypertension, diabetes mellitus and pregnancy were the most common risk factors (10). Moreover, the most common clinical causes of mortality were acute respiratory distress syndrome and viral pneumonia (10). In the current research, myalgia (100%), fever (91.6%), and cough (91.1%) were the most common symptoms among infected patients to influenza, but headache and sore throat were present in only 5% and 1.5% of the patients, respectively (Table 1). This observation was in accordance with the results obtained in another research, declaring that myalgia, fever, and cough were the best prognostic models for H1N1 infection (11). The educational level of infected people to influenza showed that with the exception of illiterates, the number of infected patients declined as the educational level increased, which seems logical (Table 2). This was in agreement with a research performed in Iran, confirming the role of education as a significant predictor of peoples' knowledge about influenza (12). In the current research, we detected influenza A (H1N1), which was similar to the results of study performed by Gooya et al. reporting detection of 2662 cases of pandemic influenza A (H1N1) in Iran by RT-PCR, from June 1st to November 11th 2009 (13). Another study performed in Tehran, the capital of Iran, isolated A/H1N1, A/H3N2 and B viruses (14).

To assess the sensitivities of cell culture and RT-PCR, we performed both methods to detect the type and subtype of human influenza virus in the obtained samples. We concluded that RT-PCR was significantly more sensitive than cell culture ($P < 005$) (Table 3). This was in agreement with results of numerous studies confirming that RT-PCR is a trustable detection method for quantification of influenza A virus (14-17). However, we faced restrictions using this method; they can be influenced by sequence variations of the influenza A hemagglutinin (HA) and neuraminidase (NA) genes. This may cause obstacles for technologists in clinical laboratories who are using primers and probe targets to plan for potential pandemics, which need to be controlled regularly (18).

To protect people, we need widespread and continuous efforts to increase the public knowledge about the efficacy of influenza vaccine, which is particularly important in developing countries. People should know that washing mouth and throat with salted water can be the most efficient protection of influenza epidemics and pandemics (19). Moreover, increasing the seasonal vaccine production will enlarge the pandemic vaccine capacity (20).

This part of Iran, as a free trade zone to three countries including Azerbaijan, Nakhchivan and Armenia, may have an essential role in transmission of influenza to and from this area. This zone is a distribution and storage center for convenience of neighboring countries. However, evolvement of flu pandemic and continuous close contacts can seriously raise the risk of respiratory infections spreading in these areas. It may also affect this free trade zone. In addition to the over-expanded health resources, it may have a greater impact on travel and tourism industry by decreasing the number of visitors in this area.

The epidemiological study of the disease in Tabriz may assist for efficient action and better planning to control the influenza in this zone. Further preparations for the role of general practice in pandemics should contain review of the public health intercourse strategies, prepandemic evaluation of practice capacity, and enhanced integration of public health interaction (2). This would provide a golden opportunity to prevent the widespread transmission of the disease in a considerable proportion of people in this area. Our results can help the sanitation authorities in Tabriz to prioritize and provide further efforts and policies for controlling the travelers. Incoming travelers have to be subjected to health declarations regarding influenza-like illness signs and symptoms during the pandemic to slow the extension of the virus. Border screening may prevent a pandemic influenza virus from entering to this part of Asia as well as its spread to other regions. Long-term travelers are more prone to afflict the disease, so they have to learn the pretravel advices carefully, such as using preventive travel medicines. In some cases, quarantine conditions for the travelers should be individualized according to susceptibility to influenza.

In conclusion, prevalence of avian flu and swine flu viruses in recent years has caused financial losses as well as mortalities around the world. This shows the importance of rapid diagnosis of common serotypes in our society. Detection of antigenic types of circulating viruses has a great importance in vaccine preparation for high-risk individuals. Therefore, using RT-PCR is recommended for early identification and rapid detection of individuals infected with influenza virus. In addition, it can accelerate vaccine production. Molecular methods should be applied for vaccine production in cases of acute infections. Moreover, planning the urgent influenza A vaccination program is suggested, which can definitely prevent the spread of virus in this part of Iran.

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