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The Prevalence of Respiratory Viruses Among Patients with Influenza-Like Illness in Zahedan, Southeastern Iran

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Abstract

Background: Acute viral respiratory diseases are among the most prevalent diseases in humans. Viral respiratory infections are the main reasons for hospitalization and death in developing countries.
Objectives: The aim of this study is to determine the prevalence and clinical symptoms of the respiratory viruses including influenza viruses A and B, respiratory syncytial virus type A and B (RSV-A and RSV-B), human parainfluenza virus type 1 to 4 (HPIV-1-4) among patients with Influenza-like illness (ILI) in Zahedan City, Southeastern Iran from October 2015 to March 2016.
Methods: Clinical and epidemiological data from patients who presented to outpatient clinics with ILI from October 2015 to March 2016 in Zahedan were collected. A total of 240 throat swabs were tested for Influenza virus by RT-PCR, and then those with negative results were tested for RSV-A, RSV-B, and HPIV type 1 - 4 using Multiplex-PCR.
Results: Among 240 patients, 115 (47.9%) were male and 125 (52.1%) were female. Influenza A virus was detected in 196 (81.7%) patients, out of them 157 (65.4%) had H1N1 subtype and the remaining patients had H3N2 subtype. Influenza B virus was observed in 9 (3.8%) patients. Respiratory syncytial virus type A and B, and human parainfluenza virus type 1 to 4 were not detected in this study. The highest rate of influenza A infection was in the age range of 16 - 45 years old and for influenza B was in the age group of more than 46 years. The most common clinical manifestations in both influenzas A and B were fever, cough, and myalgia. Over half of the patients with influenza B had dyspnea compared to 30% of ILI patients with influenza A virus infection.
Conclusions: The results of the study revealed the highest rate of Influenza A infection with H1N1 subtype among patients presented to the outpatient clinics with the clinical manifestations of influenza-like illness. This study suggests continuing surveillance, infection control, and annual vaccination for Influenza.

Keywords: Influenza-Like Illness, Influenza Virus, Human Parainfluenza Virus, Respiratory Syncytial Virus

1. Background

Viral infections commonly affect the upper or lower respiratory tracts with different clinical syndromes such as common cold, croup, and bronchiolitis (1). Influenza-like illness (ILI), also known as an acute respiratory infection is a medical diagnosis of possible influenza or other illness causing a set of common symptoms such as fever > 38 °C or at least two acute respiratory symptoms, cough and/or sore throat, nasal obstruction/rhinorrhea (2). Viral agents cause these infections, including influenza virus, human parainfluenza virus (HPIV), respiratory syncytial virus (RSV), rhinovirus, coronavirus, adenovirus, and metapneumovirus.

Acute viral respiratory tract infection is a major cause of mortality in developing countries. Death can result either from a viral infection or a bacterial superinfection, especially in high-risk patients. Even though these viral diseases are self-limited, they can cause a variety of complications in children, the elderly, pregnant women, immunocompromised patients and those with chronic renal disease, heart failure, or asthma (1-4).

The Center for Disease Control and Prevention confirmed the definition of influenza-like illness, so the patients in accordance with this definition are infected with influenza. However, several studies showed that respiratory viruses other than influenza virus might be responsible for similar condition (5).

The influenza virus is responsible for annual epidemics, which is characterized by a sudden increase in

febrile respiratory illness and the absence from schools and workplaces. Seasonal influenza viruses with variable clinical pictures are common during the fall and winter. Its differentiation from other agents is important in clinical practice because it has specific antiviral treatment. Also, the identification of influenza virus is crucial as appropriate infection control practices play a major role in outbreak prevention. Influenza virus outbreaks in hospitals can be devastating, especially for immunocompromised patients who are highly susceptible to life-threatening influenza virus infection.

Human parainfluenza viruses include four serotypes that infect humans. Their route of transmission and pathogenesis are similar to that of the influenza viruses. Human parainfluenza viruses can cause mild upper respiratory tract infections (URTIs), pharyngitis, and tracheobronchitis (5, 6). However, HPIV1 and HPIV3 are associated with mild URTI, and croup in children and infrequently, lower respiratory tract infections (LRTI), which occur predominantly in the fall. Human parainfluenza virus type 2 is generally associated with LRTI. Human parainfluenza virus type 4, which is generally associated with mild URTI, is comprised of the least common virus of this group. Overall, HPIVs cause 15 to 30% of non-bacterial respiratory disease in children requiring hospitalization (6).

Respiratory syncytial virus (RSV) subtypes A and B are major causes of bronchiolitis and pneumonia in infants. Most infections occur between late fall and early spring, with a peak prevalence in the winter. The initiation of the disease is often with rhinitis, which develops severe respiratory symptoms. However, RSV-A is more severe than RSV-B (7, 8).

Respiratory infections caused by the mentioned viruses usually present with clinical features that are really difficult to distinguish (8). The diagnosis is made by the detection of pathogens by polymerase chain reaction (PCR), cell culture, or serologic tests. More rapid diagnostic tests should be reserved when the pathogen-specific diagnosis is important for clinical management. The increased sensitivity of PCR over conventional methods like cell culture and serology makes it more practical for the diagnosis of respiratory viral infections (9). However, organism-specific RT-PCR assays, which require separate amplification of every single virus, are resource- and labor intensive as well as time-consuming (10).

The Multiplex RT-PCR method is rapid, sensitive, specific, and more cost-effective than alternative methods such as culture (11). The Multiplex RT-PCR, which is both highly sensitive and specific for each virus and detection of co-infection in clinical samples, can be used in studies of viral respiratory infections in both surveillance and laboratory diagnostic tests (12).

2. Objectives

A lot of studies have reported respiratory viral infections to characterize the prevalence, demographic, and clinical manifestations of ILI in their local regions (1, 2, 5, 6, 8). In this study, we investigated the occurrence of some of the respiratory viruses responsible for ILI, including Influenza Viruses (A and B), RSV-A and B, HPIV1-4 among patients who referred to outpatient clinics related to Zahedan University of Medical Sciences from October 2015 to March 2016.

3. Methods

The study population included patients who presented with ILI to the outpatient clinics of Zahedan University of Medical Sciences, Southeastern Iran, from October 2015 to March 2016. It should be mentioned that most patients attending to these clinics were adults as the majority of children with ILI referred to children clinics. The inclusion criteria were sudden onset of illness and the presence of at least two of the following conditions: fever > 38°C, cough, sore throat, Coryza, dyspnea, and shortness of breath with or without headache, myalgia and gastrointestinal symptoms within the past 1-3 days. Participants who agreed to participate in this study were given a questionnaire, asking information about the clinical course of their illness as mentioned above. Demographic data such as age and sex were also recorded. Throat swab was obtained from each patient for PCR detection of respiratory pathogens. The samples were placed in a transportation medium, maintained at 4°C and sent within 72 h to the laboratory of Infectious Diseases and Tropical Medicine Research Center, Zahedan where they were stored at -70°C.

First, all samples were tested for influenza A and B by RT-PCR. The total RNA was extracted according to the manufacturer's protocol using RNX plus kit High Pure Viral RNA Kit, Roche, Germany. Finally, the PCR products were loaded on 2% agarose gel, containing ethidium bromide and the results were observed under gel documentation apparatus. Furthermore, those who were negative for influenza virus were tested for other viruses, including respiratory syncytial virus A and B, and Parainfluenza virus type 1 to 4 using Multiplex PCR assay.

Primers used in this study were designed using dual priming oligonucleotide (DPO) technology, which allowed specific detection of viruses without any non-specific amplification. Multiplex RT-PCR was performed and a set of primers, including RSV-A (248 bps), RSV-B (316 bps), PIV-1 (400 bps), PIV-2 (636 bps), PIV-3 (192 bps), and PIV-4 (539 bps) were designed and used in a single set of multiplex RT-PCR (13).

4. Results

Overall, 240 patients with ILI, including 115 (47.9%) male and 125 (52.1%) female were tested for the influenza A and B viruses. One hundred and ninety-six out of the patients had a positive test for influenza A that 101 (51.5 %) were female and the remaining patients were male. Among the patients with influenza A infection, 157 (65.4%) had H1N1 and 39 (34.6%) H3N2 subtypes. Only nine (5 males and 4 females) out of 240 patients with ILI had an influenza B infection (Table 1).

The highest number of the patients with influenza A was in the age range of 16 - 45 years and for influenza B was more than 45 years old. The age distribution of patients with ILI was shown in Table 2. Multiplex RT-PCR on 35 samples, which were negative for influenza virus, was also negative for parainfluenza 1 to 4, and respiratory syncytial virus A and B. Individual symptoms with ILI are summarized in Table 3. Among 240 patients with ILI 222 (92.5%) presented with fever and 195 (81.3%) with cough. Subsequently, myalgia, headache, gastrointestinal symptoms, and dyspnea were reported in 184 (76.7%), 135 (65.3%), 75 (32.9%), and 73 (30.4%) of the patients, respectively.

The most common symptom was fever in 187 (95.4%) of influenza A and 9 (100%) of influenza B patients. Cough with 162 (82.6%) for influenza A and 9 (100%) for influenza B was the next most common clinical manifestations of the patients. The following symptoms in both influenzas A and B were myalgia, headache, gastrointestinal symptoms, and dyspnea (Table 3). Dyspnea was more prominent in influenza B. Over half of the patients with influenza B had dyspnea compared with 30% of the patients with influenza A virus infection.

Table 1. The Number and Percentage of the Two Subtypes of Influenza A and B in Patients with Influenza-Like Illness

Influenza Viruses	No. (%)
Influenza A	196 (95.6)
H1N1	157 (65.4)
H3N2	39 (34.6)
Influenza B	9 (4.4)
Total	205 (100.0)

Table 2. The Age Distribution of Influenza A and B Virus Infection Among Patients with Influenza-Like Illness[a]

Age Group, y	Influenza A	Influenza B	Total
< 15	17 (8.6)	1 (11.1)	18 (8.8)
16 - 30	59 (30.1)	0	59 (28.8)
31 - 45	64 (32.7)	2 (22.3)	66 (32.2)
46 - 60	37 (18.9)	3 (33.3)	40 (19.55)
> 60	19 (9.7)	3 (33.3)	22 (10.7)
Total	196 (81.6)	9 (3.75)	205 (100)

[a]Values are expressed as No. (%).

Table 3. The Clinical Manifestations in Patients with Influenza-Like Illness, and Influenza A and B[a]

Symptoms	Patients with ILI (240 Cases)	Influenza A (196 Cases)	Influenza B (9 Cases)
Fever	222 (92.5)	187 (95.4)	9 (100)
Cough	195 (81.3)	162 (82.6)	9 (100)
Myalgia	184 (76.7)	169 (86.2)	7 (77.7)
Headache	135 (65.3)	119 (60.7)	5 (55.5)
GI symptoms	75 (32.9)	71 (36.2)	1 (11.1)
Dyspnea	73 (30.4)	59 (30.1)	5 (55.5)

[a]Values are expressed as No. (%).

5. Discussion

Influenza-like illness (ILI), also known as an acute respiratory infection, is a contagious respiratory illness caused by a variety of flu viruses. The importance of these viral infections is their ability to spread quickly from one person to another. Fortunately, there is an effective vaccine to prevent influenza virus infections, which are the most common viruses responsible for ILI. This study evaluated viruses causing ILI in patients who referred to outpatient clinics affiliated with Zahedan University of Medical Sciences during autumn and winter of 2015 - 2016. Influenza A viruses were the most common viruses observed in this study.

Research at 26 intensive care units in northern Italy and among adults hospitalized patients in France revealed influenza, respiratory syncytial virus, adenovirus, parainfluenza, and picornavirus as the most commonly detected viruses in patients with ILI (14, 15). Influenza is one of the most important causes of morbidity and mortality worldwide with an estimated 3 to 5 million severe cases of ILI and 250000 to 500000 deaths annually (16). Clinical specimens from 843 pediatric patients with respiratory symptoms in India from July 2009 to August 2011 showed Rhinovirus as the most frequently detected virus (17.2%) followed by respiratory syncytial virus B (15.4%), H1N1pdm09 (8.54%), parainfluenza virus-3 (5.8%) and metapneumovirus (5.2%) (13).

Respiratory viral pathogens were presented in 85.5% of our samples that is higher than other studies in which from 37% to 75.1% of the samples were positive, similar to many studies influenza virus A was the most common virus detected in our patients with ILI (17-19). From June 1st

to 11th November 2009, there were 2662 confirmed cases of pandemic influenza A (H1N1) by real-time PCR in Iran. The results of this study showed that the death prevalence had no significant correlation with sex and age. The geographic distribution of the reported cases showed the highest rates were in the Central and Eastern provinces of Iran with the highest prevalence in October 2009 (2).

Data from the 53526 registered cases of influenza-like illnesses from sentinel sites of healthcare centers in Iran from 2010 to 2015 revealed 7684 (14%) positive cases of influenza with 71% type A and 28% type B virus. The majority of the outbreaks occurred in winter and autumn (20). A wave of illness in the winter of 2015 was reported in Iran, which was more extensive than the one in 2009 with the highest rates and mortality in Southern and Southeastern provinces of Iran (21).

One of the strengths of this study is that all samples were investigated in a sub-national influenza laboratory qualified by the Iranian Ministry of Health and all of the tests were carried out by a trained laboratory expert that reduces the technical diagnostic errors. This study has also some limitations. First, we only examined samples from patients who attended the outpatient clinics and hospitals affiliated with Zahedan University of Medical Sciences. The samples from patients admitted to the non-educational hospitals were not included in this study. These outpatient clinics are mostly for adults, thus not too many children were included in this study. Therefore, caution should be taken for interpretation of the findings and generalization of the results. Second, in case of negative results for any of the tested pathogens, no further tests were performed to identify other etiological agents, including bacteria or other viral pathogens responsible for ILI. Third, it is possible that these observations were limited by the particular strains of respiratory viruses circulating in the study region. Therefore, larger studies involving specimens from different health settings would be helpful to assess the reliability and validity of the study findings.

In conclusion, the results revealed the higher prevalence of influenza viruses, especially type A in patients with ILI in our local area, which can suggest continuing the surveillance, infection control and annual vaccination for Influenza virus infection.

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Footnotes

Authors' Contribution: Study concept and design: Maliheh Metanat and Seyed Mehdi Tabatabaei; acquisition of data and performing tests: Nargess Arbabi, Farnoosh Sharify Mood, and Tahereh Khalili; statistical analysis and interpretation of data: Farnoosh Sharify Mood and Batool Sharifi-Mood; editing and revising the article: Roya Alavi Naini.

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