Epidemiology and Molecular Characterization of Seasonal Influenza Viruses in Iraq

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Abstract

The importance of influenza viruses in respiratory infections in The Middle East has been historically overlooked, including in Iraq. Now days with the OCVID 19 pandemic the importance of prevention of other respiratory disease such as seasonal influenza could be a critical step in the health management system. This study therefore aimed at evaluating the prevalence and seasonal occurrence of influenza viruses in Iraqi population presenting with influenza-like illness (ILI) or severe acute respiratory infection (SARI) from 2015 to 2017. The study also aimed at identifying the periods with increased influenza transmission for vaccination recommendations in Iraq. In this study, we present the cases of infection by influenza A or B viruses. For testing influenza virus types A (H1N1 and H3N2) and B, 1359 throat and nasal swabs were taken from patients with influenza-like illness (ILI) or severe acute respiratory infection (SARI). RNA was extracted and amplified using a set of primers and probes. Out of the total number of patients, females frequency was 1616 (45%) and 1974 (55%) was for males. The mean age of the patients who took part in the research was 31.71±22.68 with minimum age of 1 month and maximum of 96 years. Influenza virus type A was the most predominant with incidence of 16.2% followed by Type B with 0.33% incidence. December was the most prevalence month of getting infection by the two types of influenza viruses detected with 30.02%, 0.48% for type A and B, respectively. Vaccination in September would likely protect the highest number of patients. It was clear that influenza A virus is predominate on Type B.
In Iraq, influenza A and B viruses were found in a large percentage of ILI and SARI cases. Males are more likely to become infected than females.

**Keywords:** Real-Time RT-PCR, Influenza type A, Influenza type B

**1. Introduction**

Influenza A and B are the most common influenza viruses that cause epidemic human disease, and they are further divided into subtypes (for A viruses) and lineages (for B viruses) based on antigenic differences. Subtypes of influenza A viruses have been identified. Because point mutations and recombination events may occur during viral replication, resulting in frequent antigenic change (i.e., antigenic drift), new influenza viruses may emerge [1].

Influenza viruses are envelope viruses with a segmented RNA genome. They are part of the Orthomyxoviridae family. Influenza is a respiratory illness caused by a virus. The following signs and symptoms, or at least part of them, are the most common signs and symptoms of influenza: Fever, headache, myalgia, prostration, coryza, sore throat, and cough are all symptoms of the flu. [1]. The influenza virus is divided into three types: A, B, and C. (been considered different genera). In humans, the sickness is known as "flu," and type A is the most frequent, resulting in Influenza disease, followed by type B, which is spread through airdrops from infected people or intimate contact with infected animals. [2]. Influenza A is the more frequent strain, and it kills more people than influenza B [3]. The surface antigens hemagglutinin (H) and neuraminidase (N) determine the subtypes of influenza type A. [4]. During typical influenza seasons, when influenza viruses are transmitted, there is an increase in mortality.

Despite the fact that not all additional occurrences occurring during these times can be traced directly to influenza, these assessments are valuable for tracking influenza-related outcomes from season to season.

Estimates that only include outcomes contribute to pneumonia and influenza (P&I) are likely to overlook the number of serious diseases that are at least partially attributed to influenza, because deaths caused by the worsening of dependent cardiac and pulmonary diseases that are linked to influenza infection are not included in this grade [5]. There isn't a specific diagnostic test available.
Although anecdotal reports work out on false-negative test results, rapid antigen-based tests for influenza appear to be suitable for pandemic H1N1 influenza and others (6).

In developing countries, acute viral respiratory tract infection is the leading cause of hospitalization for newborns and young children, and in developing countries, it is a leading cause of death (7, 8). Because of quicker reversal times and greater sensitivity, nucleic acid testing by RT-PCR has essentially replaced classical virus culture in the clinical diagnosis of influenza. 9). Since influenza activity and influenza-like illness (ILI) are widespread across the country. It is critical to recognize the differences between these conditions and the most appropriate treatment. In addition, you should know what pathogen is the most common cause of influenza.

2. Materials and Methods

From 2015 to 2017, influenza surveillance was carried out at the Central Public Health Laboratory. A total of 3561 specimens were obtained from 3561 patients who had developed respiratory symptoms.

Patients' symptoms ranged from mild upper respiratory tract infections (URTI) such as fever above 38 degrees Celsius, common cold, cough, coryza, sore throat, and shortness of breath to lower respiratory tract infections (LRTI) such as laryngitis, bronchiolitis, and pneumonia. All information concerning these patients, including their medical history, was included in the report. Throat swab or nasopharyngeal swab specimens were collected and directly immersed into a sterile tube, and for maintaining viral viability during transportation and until its arrival to laboratory, Viral Transport Media (VTM, Copan, USA) were used. Specimens were transported by a cool box to Virology department, National Influenza Center at the Central Public Health Laboratory, and the specimens were stored at -80 °C till the time of analysis.

2.1. RNA extraction

For all 3561 respiratory specimens, viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions, and the specimens were then kept at -80 C until use.
By using rRT-PCR CDC Influenza Virus Real-Time RT-PCR A/B Typing Panel kit (Atlanta, USA), all the clinical specimens were tested for influenza A and B. Master mix was prepared using SuperScript III platinum one step RT-PCR kit (Ivitrogen, USA). A 25 µl master mix contained 12.5 µl reaction buffer (5x), 0.5 µl Super Script TM III RT/Platinum TM Taq mix, 0.5 µl of each primer and probe (40 µM conc. for each primer and 10 µM conc. for probe), 5.5 µl PCR water, 0.5 µl Rox dye (1/10 dilution) and 5 µl of specimen RNA template. The amplification and detection were done with Fast 7500 Real-Time PCR system (Applied Biosystems) as follows: RT step activation at 50 °C for 5 min, Initial denaturation at 95 °C for 2 min, followed 45 cycles: 95 °C for 3 sec and 55 °C for 30 sec.

Specimens positive for influenza A were subjected to subtyping with CDC Influenza Virus Real-Time RT-PCR Subtyping Infuenza A(H3/H1pdm09) Panel kit (Atlanta, USA). The master mix and RT-PCR thermal profile as described above.

2.2. Statistical analysis

The statistical analysis system was analyzed by IBM SPSS Statistics version 25. All values, proportions and their frequencies were checked by applying the Pearson chi – square (X²) and cross tab test to investigate significant comparison between viral infection percentages in different studying markers of population study.

3. Results

3.1. Demographic and clinical characteristics of enrolled patients

Starting in January 2015 till December 2017, an active surveillance of patients presenting with ILI was implemented in Central public health laboratory as a total of 3561 specimens were collected during that period.

Out of the total number of patients, 1616 (45%) were females and 1974 (55%) were males. The mean age of patients enrolled was 31.71±22.68 with minimum age of one year and maximum of 96 years.
3.2. Etiological characteristics

Out of the total (3950) cases surveyed, 582 (14.73%) were positive for influenza A virus, 12 (0.3%) were positive for influenza B virus, the total negative cases were 2996 (75.84%) (Figure 1).

Further detection for subtyping of influenza A was done and result showed the predominance of H1N1 (14.60%), H3N2 (5.26%) and (0%) for each of H5N1 and H7N9 (Figure 2).

![Graph showing the percentage of influenza A subtypes](attachment:image.png)

Figure 2. Rate of influenza A subtypes

When comparing age groups with infection, results showed that younger people were more in getting infection 1-10 years with influenza A than older cohorts; and group 11-20 years was the lower 4 (1.1%) in getting infection than others (Figure3).
Figure 3. Rate of infection according to age groups

Figure 4. Rate of infection according to months

Figure 4 shows that when the month is used as a parameter for case distribution, December is the month with the largest prevalence of infection by all types A, with 30.02 percent, while type B has the highest frequency in October, with 0.48 percent.
Table 1. Comparison between males and females in ILI infection in three years of study

<table>
<thead>
<tr>
<th>Gender</th>
<th>Year</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Female</td>
<td></td>
<td>938</td>
<td>442</td>
<td>236</td>
<td>1616</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>47.3%</td>
<td>41.6%</td>
<td>43.5%</td>
<td>45.0%</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>1047</td>
<td>620</td>
<td>307</td>
<td>1974</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>52.7%</td>
<td>58.4%</td>
<td>56.5%</td>
<td>55.0%</td>
</tr>
</tbody>
</table>

Pearson Chi-Square Tests

<table>
<thead>
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<th>Chi-square</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.497</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Bivariate analysis by sex and year indicated that a higher proportion of men 1974 (55%) were infected than were women 1616 (45%). High significance is clearly appear in these results (Table 1).

Table 2. Comparison between significancies

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Chi-square</th>
<th>df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95.319</td>
<td>18</td>
<td>.000*</td>
</tr>
<tr>
<td>FLUA</td>
<td>107.339</td>
<td>2</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>109.672</td>
<td>2</td>
<td>.000*</td>
</tr>
</tbody>
</table>
4. Discussion

The first step in controlling transmissible diseases is to guarantee precise and reliable diagnosis. The fact that several distinct organisms can cause respiratory illness with identical clinical signs makes a physician's diagnosis of influenza problematic. In the diagnosis and surveillance of influenza viruses, molecular approaches applied directly on clinical materials play an essential role. According to the Centers for Disease Control and Prevention's annual vital statistics report, between 12 and 32 million occurrences occur each year. Due to reduced turnaround times and higher sensitivity, RT-PCR nucleic acid testing has largely supplanted classical virus culture in the clinical diagnosis of influenza [7]. Some results (for example, influenza illness validated by viral culture or polymerase chain reaction [PCR] are more specific than others (e.g., influenza-like illness defined by a clinical case definition, without definite diagnostic testing). Clinical mortality rates have been found to be high in several studies. (10)

Specimens were collected throughout the course of three years, in all of their seasons, to provide an impression of infection in all seasons, as well as a broader concept of the distribution of infection and its types throughout the year. As influenza is a highly contagious sickness. According to infection rate statistics over the three-year study period, negative cases account for 82.65% of total cases, which could be a good result due to increased influenza vaccination use in Iraq, as well as greater awareness of prevention and transmission.

The exact dates of the onset, peak, and end of influenza activity vary from season to season and cannot be predicted with certainty. Annual influenza epidemics, however, are more common in the autumn and winter in various nations. Influenza frequently begins to spread. (11). This is supported by our findings, which demonstrate that, based on the seasonal distribution of infection, the winter
months have the highest rates, peaking in December, November, January, and February in that order. This outcome is consistent with the findings of another investigation (12).

Influenza is contagious in people of all ages. Influenza is difficult to assess precisely because many, if not the majority, of those afflicted do not require medical attention and hence are not diagnosed (13). Of the total (3950) cases surveyed, 582 (16.2) were positive for influenza A virus by RT-PCR, 12 (0.3) were positive for influenza B virus, and 29 (0.8) were positive. In a comparison between males and females in getting influenza infection, results showed that males were higher in getting the infection than females with frequencies of 55% and 45% respectively. The mechanisms that determine the differences between the sexes are complex and can include hormonal, immunological, behavioral, and genetic factors. When compared with males, females generate higher adaptive and innate immune responses. The uneven susceptibility of females and males to infectious disease has been linked to mating competition and diet as behavioral and environmental factors (14). Further research that are consistent with this study and explain why males get less infection than females. Our data also show that after vaccination, females are better protected against lethal challenge with new influenza virus strains than males (15). The death and alive outcome of infection was recorded and gave the highest result with 7.7% in 2015 and 2017, compared to 1.1 percent in 2016, which may be explained by influenza being regarded a non-lethal infection except for specific types that gave those results which coincided with this.16) When it came to the most common forms of influenza virus, type A was the most common, accounting for 16.2 percent, followed by Type B (0.33 percent). With 82.65%, the remaining percent was for negative cases which was left out of the typing. Influenza is contagious, according to a study (17) when it came to the most common forms of influenza virus, type A was the most common, accounting for 16.2 percent, followed by MERS virus (0.81 percent), and Type B (0.33 percent). The remaining 25% was for negative situations, with 82.65% being eliminated from typing.
References


