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Original Article

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, including Iraq, has been historically overlooked. Nowadays, with the pandemic of corona virus disease 2019, the importance of prevention from other respiratory diseases, such as seasonal influenza, can be a critical step in the health management system. Therefore, this study aimed to evaluate the prevalence and seasonal occurrence of influenza viruses in the Iraqi population presented with influenza- like illness (ILI) or severe acute respiratory infection (SARI)within2015-2017. Moreover, this study was conducted to identify the periods with increased influenza transmission for vaccination recommendations in Iraq. In the present study, we presented the cases of infection by influenza A or B viruses. To test influenza virus types A (H1N1 and H3N2) and B, 1,359 throat and nasal swabs were collected from patients with ILI or SARI. Ribonucleic acid was extracted and amplified using a set of primers and probes. The frequency rates of infection were obtained at 1,616 (45%) and 1974 (55%) in females and males, respectively. The mean age of the participants was estimated at 31.71±22.68 with a minimum and maximum ages of 1 month and 96 years, respectively. It was revealed that influenza virus type A was the most predominant with an incidence of 16.2%, followed by type B with 0.33% incidence. It was also found that December was the most prevalent month of being infected by influenza viruses types A and B (30.02% and 0.48%, respectively). Vaccination in September would likely protect the highest number of patients. It was clear that the influenza A virus was predominant over type B. In Iraq, influenza A and B viruses were found in a large percentage of ILI and SARI cases. Additionally, males were reported to be 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 are further divided into subtypes (for A viruses) and lineages (for B viruses) based on antigenic differences. Subtypes of influenza A viruses have already been identified. Since 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 enveloped viruses with a segmented ribonucleic acid (RNA) genome and are members of the Orthomyxoviridae family. Influenza is a respiratory illness that is caused by a virus. The most common signs and symptoms related to influenza, which can include all or some of them, are fever,

headache, myalgia, prostration, coryza, sore throat, and cough (1). The influenza virus is divided into three types, namely A, B, and C, which are 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 most frequent strain and kills more people than influenza B (3). The surface antigens hemagglutinin and neuraminidase determine the subtypes of influenza A (4).

An increase in mortality is observed during typical influenza seasons when influenza viruses are transmitted. Despite the fact that not all additional occurrences occurring during these times can be traced directly to influenza, the assessments of mortality incidence during influenza seasons are valuable for tracking influenza-related outcomes from season to season. The estimates that only include outcomes contribute to pneumonia and influenza and are likely to overlook a number of serious diseases that are at least partially attributed to influenza since neither the deaths caused by the aggravation of dependent cardiac nor pulmonary diseases 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 the primary reason for death (7, 8). Because of quicker reversal times and greater sensitivity, nucleic acid testing by reverse transcription polymerase chain reaction (RT-PCR) has 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 both the differences between these conditions and the most appropriate treatment. In addition, it is necessary to diagnose the pathogen that is the most common cause of influenza.

2. Material and Methods

Influenza surveillance was carried out at the Central Public Health Laboratory from 2015 to 2017. A total of 3,561 specimens were obtained from 3,561 patients who had developed respiratory symptoms. Patients' symptoms ranged from mild upper respiratory tract infections, such as fever above 38°C, common cold, cough, coryza, sore throat, and shortness of breath, to lower respiratory tract infections, such as laryngitis, bronchiolitis, and pneumonia. All information concerning these patients, including their medical history, was considered in the report. Throat swab or nasopharyngeal swab specimens were collected and directly immersed into a sterile tube, and Viral Transport Media (VTM, Copan, USA) were used for maintaining viral viability during transportation and until its arrival to the laboratory. Specimens were transported to the Virology Department, National Influenza Center at the Central Public Health Laboratory, in a cool box and stored at -80°Ctill the analysis time.

2.1. Ribonucleic Acid Extraction

For all 3,561 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.

All the clinical specimens were tested for influenza A and B using real-time RT-PCR CDC Influenza Virus Real-Time RT-PCR A/B Typing Panel kit (Atlanta, USA). The master mix was prepared using a Super Script III platinum one-step RT-PCR kit (Invitrogen, USA). A 25-µl master mix contained 12.5 µl reaction buffer (5x), 0.5 µl SuperScript TM III RT/Platinum TM Taq mix, 0.5 µl of each primer and probe (40 µM concentrationfor each primer and 10 µM concentration for probe), 5.5 µl PCR water, 0.5 µl Rox dye (1/10 dilution), and 5 µl of specimen RNA template. Subsequently, amplification and

detection were performed 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 by 45 cycles: 95°C for 3sec and 55°C for 30 sec.

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

2.2. Statistical Analysis

The statistical analysis system was analyzed in IBM SPSS Statistics version 25. All values and proportions and their frequencies were checked by applying the Pearson Chi-square (X^2) and cross tab test to investigate the significant comparison between viral infection percentages in different investigating markers of the study population.

3. Results

3.1. Demographic and Clinical Characteristics of Enrolled Patients

Active surveillance was conducted on patients presenting with ILI referring to the Central Public Health Laboratory within January 2015-December 2017, which rendered for the collection of 3,561 specimens. Out of the total number of patients, 1,616 (45%) and 1,974 (55%) cases were female and male, respectively. The mean age of the enrolled patients was obtained at 31.71±22.68 with a minimum and maximum ages of 1and 96 years, respectively.

3.2. Etiological Characteristics

Out of the total surveyed population (n=3,950), 582 (14.73%) cases were positive for influenza A virus, 12 (0.3%) subjects were positive for influenza B virus, and 2,996 (75.84%) patients were negative.

Further detection for subtyping of influenza A was conducted; the results of which showed the predominance of H1N1(14.60%), H3N2 (5.26%), and (0%) for each of H5N1 and H7N9 (Figure 1).

The comparison of age groups with infection revealed that younger people were more susceptible in getting infection. 1-10 years old individuals were significantly infected with influenza A compared with older cohorts (Figure 2).

Figure 3 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%, while type B has the highest frequency in October, with 0.48%.

The results of bivariate analysis by gender and year indicated that a higher proportion of men (n=1,974, 55%) were infected than women (n=1616, 45%). High significance is apparent in these results (Table 1 and 2).

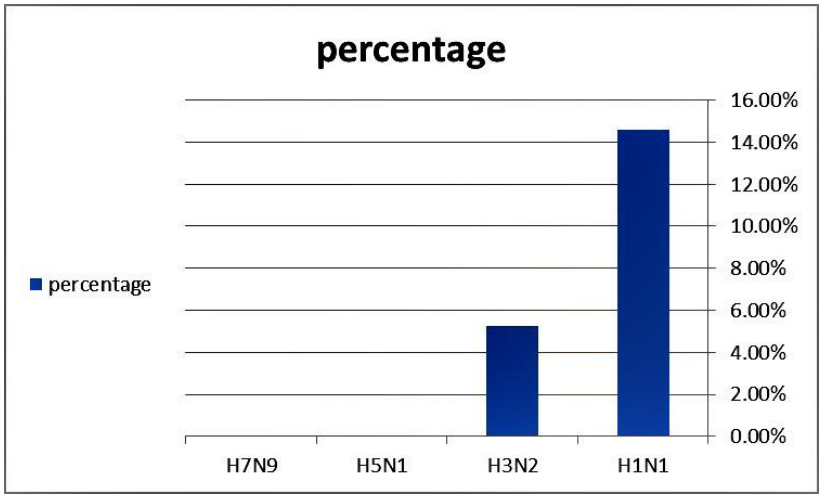

Figure 1. Percentage of influenza A subtypes

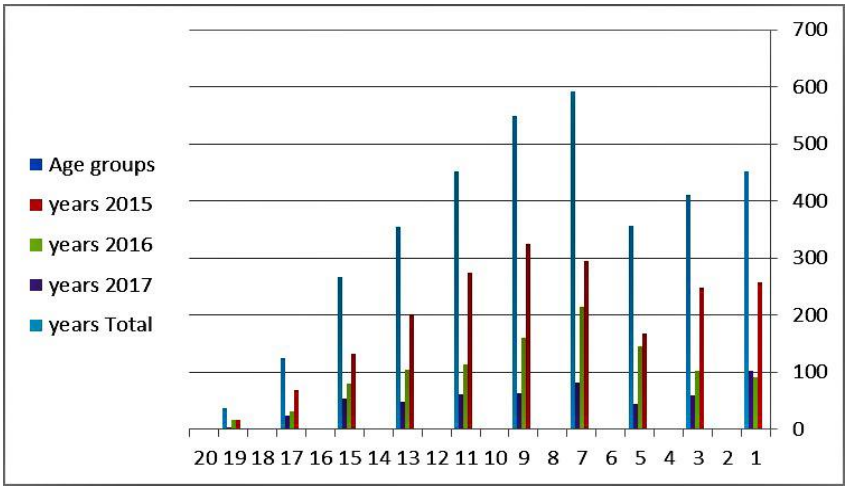

Figure 2. Rate of infection according to age groups

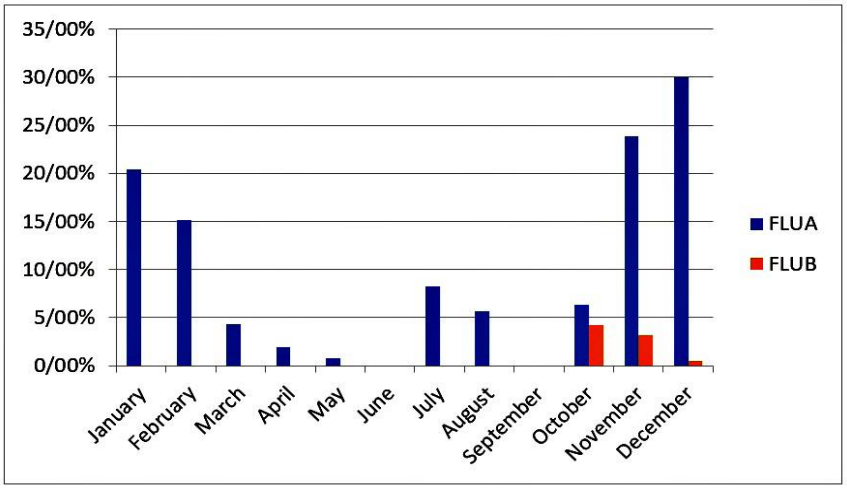

Figure 3. Rate of infection according to months

Table 1. Comparison between males and females in influenza-like illness infection in three years of study

		Year			
		2015	**2016**	**2017**	**Total**
Gender	Female	938	442	236	1,616
		47.3%	41.6%	43.5%	45.0%
	Male	1,047	620	307	1,974
		52.7%	58.4%	56.5%	55.0%
Pearson Chi-square Tests	Chi-square9.497	Sig.0.009			

Chi-square=9.497 Sig.=0.009

Table 2. Comparison between significances

Age groups	Chi-square	95.319
	df	18
	Sig.	0.000*
FLUA	Chi-square	107.339
	df	2
	Sig.	0.000*
FLUB	Chi-square	109.672
	df	2
	Sig.	0.000*
Outcome	Chi-square	1.989
	df	2
	Sig.	0.370

FLUA: Influenza A virus; FLUB: Influenza B virus

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 illnesses with identical clinical signs makes a physician's diagnosis of influenza problematic. Molecular approaches applied directly to clinical materials play an essential role in the diagnosis and surveillance of influenza viruses. 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 (e.g., influenza illness validated by viral culture or PCR) are more specific than others (e.g., ILI 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 in all seasons ofa3-year investigation to provide an impression of infection in all seasons and a broader concept of the distribution of infection and its types throughout the year. As influenza is considered a highly contagious sickness. According to the infection rate statistics over the three-year study period, negative cases accounted for 82.65% of total cases, which could be a good result due to the 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), which is supported by our findings, demonstrating that, based on the seasonal distribution of infection, the winter months had the highest rates, peaking in December, November,

January, and February in descending order. This outcome is consistent with the findings of another investigation (12).

Influenza is contagious in people of all ages and is difficult to be assessed precisely since numerous, if not the majority, of those afflicted, do not require medical attention, and therefore, are not diagnosed (13). Among the population of this study (n=3,950), 582 (16.2%) cases were positive for influenza A virus by RT-PCR, 12 (0.3%) subjects were positive for influenza B virus, and 29 (0.8%) were positive. The results of comparing males and females regarding the development of influenza infection showed that males were more affected than females (55% vs.45%, respectively). The mechanisms that determine the differences between genders are complex and can include hormonal, immunological, behavioral, and genetic factors. It has been revealed that females generate higher adaptive and innate immune responses, compared to males. The uneven susceptibility of females and males to infectious diseases has been attributed to mating competition and diet as behavioral and environmental factors (14). Based on the findings of the present study, after vaccination, females were better protected against lethal challenges with new influenza virus strains than males. The death and alive outcomes of infection were recorded as the highest result with 7.7% in 2015 and 2017, compared to 1.1% in 2016. The reason for this difference might be explained by influenza being regarded as a non-lethal infection, except for specific types (15). In the current study, the most common form of influenza virus was reported to be type A, accounting for 16.2%, followed by type B with 0.33% frequency. The remaining percent (82.65%) was related to negative cases, which was left out of the typing. According to the results of a study (16), considering the most common forms of influenza virus, type A was the most prevalent (16.2%), followed by the Middle East Respiratory Syndrome virus (0.81%) and type B (0.33%). The remaining 25% was for negative situations, with 82.65% being eliminated from typing.

Authors' Contribution

Study concept and design: I. M. A.
Acquisition of data: A. M. K.
Analysis and interpretation of data: L. G. A.
Drafting of the manuscript: M. A. A. A.
Critical revision of the manuscript for important intellectual content: F. M. M. S.
Statistical analysis: I. M. A.
Administrative, technical, and material support: I. M. A.

Ethics

All the procedures were approved by the Ethics Committee at the Public Health Directorate, Baghdad, Iraq. Under the project number 2021-54789-78411.

Conflict of Interest

The authors declare that they have no conflict of interest.

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