The dominant genotypes of influenza virus and their correspondence to the vaccine strain used in the year 2019-2020

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Abstract: Respiratory tract infection is the most common cause of illness, especially in developing countries. Influenza remains a major public health problem and an endemic respiratory disease in Iraq, therefore this study was conducted to identify the influenza virus (IV) Types and subtype existed. 2515 nasopharyngeal swaps taken from a hospitalized patients suffering from acute respiratory tract infection a cross sectional study was conducted in Iraq from January 2019 to February 2020. The RT-qPCR was used to detect human IV, and IV genotypes among the study population. The (IV) surveillance showed (33.44%) of tested samples were found positive to the influenza virus including two types A 25.45% , and B11.96%. influenza A subtype which contains H1N1 17.41%, and H3N2 4.05%, and Type B Victoria was represented of positive infection. These findings indicated that subtype H1N1 is the dominant strain in Iraq. The age group under 15 years old is considered the most vulnerable to contracting Influenza type A H1N1 25% and type B viruses (40%) followed by the age group 30-45 years old. On the other hand, the age group 15-30 recorded the highest infection rate 25% out of the total H3N2. The non-significant percentage related to the age of over 75 years to all types and subtypes. Baghdad has the high significances incidence of influenza Type A, and B with the percentage 65.92%, and 52.16% respectively of the total tested cases, followed by Dohuk Governorate by percentage 7.2%, 11.96% respectively. Infections of influenza A subtypes H1N1, and H3N2 were distributed differently among governorates, as the subtype prevailed H1N1 in the governorates Baghdad, Dohuk 5.7%, Diyala 6.84%, Salahaddin 2.05%, The Qar 1.83% respectively, while the subtype H3N2 prevailed in the governorates Anbar 30.4%, Baghdad 17.2%, Erbil 14.70%, Dohuk 13.72%, Basra 9.85/5, Diwaniya 2.94% respectively. The epidemic spread of the virus showed earlier primary peak began from October until the end of February, an increase in infection rates rising in the winter months in both the types Influenza type A with the highest peak in a December, and a decline in the summer of type B and a decrease in the subtype H2N3.

Key words: Influenza viruses, H1N1,H3N2,Influenza B, Dominant strain, Genotype

Introduction:

Influenza is a viral disease belonging to orthomyxoviridea which is communicable and affects the upper and lower respiratory tract, can infect humans through inhalation the respiratory droplet of infected person during coughing, talking, and sneezing, or touching objects or devices soiled with the virus and touching the nose or eye, the viruses can be transmitted. In early stage when the patient is a symptomatic and up to 5 to 7 days after infection. Influenza viruses including four types: A, B, C, and D. During the epidemic season, Type A and B of influenza cause humans every year. Influenza A has multiple subtypes expressed on the surface of viruses, according to the combination of hemagglutinin (H) and neuraminidase (N) proteins. Throughout history, pandemics have happened. In the Center Age, The 20th century saw three outbreaks; Spanish flu, 1918. Cases of death, approx. 50 million Asian flu, 1957. Cases of death: approx. 2 million, Hong Kong flu, 1968. Cases of death: approx. 1 million. Infectious disease outbreaks can easily cross borders to threaten economic and regional stability, as has been demonstrated historically by the HIV, 2009 H1N1 influenza, H5N1, and SARS epidemics and pandemics. As governments choose policies to follow in response to the pandemic, it is imperative that we understand precisely how both the pandemic and potential government interventions will impact us. Greatest burden of illness usually occurs among children, whereas the greatest burden of severe illness (in terms of hospitalization and death) occurs in those with underlying medical conditions, infants and young children, and the elderly. Current circulating influenza strains in humans include influenza A(H1N1)pdm09, influenza A(H3N2), and both influenza B viruses (B/Victoria and B/Yamagata). In seasonal epidemics, influenza spreads rapidly and efficiently. Every fall and winter in temperate regions, influenza epidemics occur and affect a significant proportion of adults and children, after infection, the healthiest patients will fully recover within a few days, but in high-risk groups the complications such as pneumonia with mortality are common. These including high-risk groups young kids, senior citizens, women and pregnant women with immune-affection. The World Health Organization (WHO) recommends annual vaccination, prioritizing high risk groups including pregnant women, children under 5 years of age, the elderly, and those with underlying health conditions. Therefore, this study was conducted to identify the influenza virus (IV) subtype existed, and the extent of its conformity with the vaccine strain.

Material and method

Samples collection: A cross sectional study was conducted in Iraq from January 2019 to February 2020. Iraq data and samples were collected from the national influenza center (NIC)/ central public health (CPHL). The study population consists of 2515 nasopharyngeal swaps taken from a hospitalized patients suffering from acute respiratory tract infection, stored as frozen at -70 °C until used for the experiments.

Viral genomic RNA extraction: RNA extraction by using Qiamp viral RNA extraction kit (Qigen / Germany) according to the manufacturing instruction. The quantification and qualification of extracted RNA was conducted using Nano drop.
Molecular genotyping of Influenza virus

The viral genome extracts submitted for reverse transcription Real-time polymerase chain reaction (RT-qPCR) using Super Script™ III RT/Platinum™/USA, to detect human IV, and IV genotypes among the study population. The positive samples were submitted to conventional PCR for Hemagglutinin gene flanking region amplification using design primers which was design in this study, Type A influenza matrix gene; Hemagglutinin gene specific for influenza A subtypes: A (H1N1) pdm09 virus, A (H3N2), former seasonal A(H1N1), and Hemagglutinin gene specific for influenza B virus. The data was statistically analyzed by SAS (2012) program.

Result

The Molecular study was carried out to identify the (IV) surveillance showed (33.44%) of tested samples were found positive to the influenza virus including two types A 25.45%, and B 11.96%. influenza A subtype which contains H1N1 17.41%, and H3N2 4.05%, and Type B was represented of positive infection. These findings indicated that subtype H1N1 is the dominant strain in Iraq.

![Figure (1) the infections rate in different genotypes of influenza virus](image)

Also a high rate of infection 25% with the virus Influenza type A subtype H1N1 in the age group below 15 years old, followed by 23% in the age group between 30-45 years old, then the 21% in the age group 45-60 years old. On the other hand the Infections with Influenza type A subtype H3N2 virus followed another pattern in its relationship with age groups, as the age group 15-30 recorded the highest infection rate 25% out of the total H3N2 cases, and followed by 21% in the age group 60-75, and less infection rate 19% in the younger age below 15 years old while an increase in the rate of infection with the Influenza type B was recorded, and the age group below 15 years old was more vulnerable to infection with a highly rate 40%, followed by 30.9 percentage in the age group 15-30 years old. Iraqi province and demographic factor showed that Baghdad has the highly significances incidence of influenza Type A with the percentage 65.92% of the total tested cases, noting that it is the most densely populated among the governorates, followed by Dohuk Governorate by percentage 7.2%, Diyala 6.481%, and Anbar governorates by percentage 6.11%. Infections of influenza A subtypes H1N1, and H3N2 were distributed differently among governorates, as the subtype prevailed H1N1 in the governorates Baghdad, Dohuk.
5.7%, Diyala 6.84%, Salahaddin 2.05% ,TheQar 1.83% respectively ,while the sub type H3N2 prevailed in the governorates Anbar 30.4%, Baghdad 17.2%, Erbil 14.70%, Dohuk 13.72%, Basra 9.8/5, Diwaniya 2.94% respectively. Non-significant incidences related to the no cases of the Influenza A were recorded in the governorates Kirkuk, Nineveh, and Muthanna. On the other hand, influenza type B infections prevailed in the Baghdad 52.16%, Duhok11.96%, Dayala 11.3%and Wasit 9.96% provinces respectively.

The gender relationship with infection, the study approved Influenza infection rates converged between males and females, and the results of the statistics showed that the relationship between infection and the sex of the infected person was not significant. The results showed a significant relationship when p=0 .02, between the sex of the deceased and infection with the influenza virus, as the mortality rate was higher in males than females especially in the infection with H1N1 3.42%.
The epidemic spread of the virus showed a high morale relationship, an increase in infection rates in the winter months, which appeared from November until the end of February, and a decline in the summer months. The subtype H2N3 predominated during the year 2019 in January and February of the rest of the species in proportion 51.69% and 15.69% respectively, and a lower percentage of the subtype H1N1 while no recorded for Influenza type B in this two months. On the other hand the virus infection. Viral infections decreased in months from March to October 2019 to most of the influenza type. On the other hand the incidence of the influenza virus increased again, starting from the end of October 2019 and continuing until began of March 2020. There was an increase in the incidence of both the type Influenza type A subtype H1N1 with the highest peak in a December and Influenza type B and a decrease in the subtype H2N3.
Figure (4) distribution of influenza infection according to the month of year

Figure (5). Gel electrophoresis profile of Hemagglutinin gene flunking region 599 bp using 2% Agarose stained with red safe DNA dye and electrophoresed by 5vol/cm in TBA buffer: lane M indicated DNA marker 100-1000, and100-1500bp, lane 2, 5, and 7 indicted Hemagglutinin band of flue B victoria, lanes 1,3,4, 6 non Flue B
Figure (6). Gel electrophoresis profile of Hemagglutinin gene flanking region 833bp using 2% Agarose stained with red safe DNA dye and electrophoresed by 5vol/cm in TBA buffer: lane M indicated DNA marker 100-1500bp, lane 1, and 3 indicated Hemagglutinin band of seasonal flu A, lanes 2, 4, 5, 6, 7 no amplification products.

Figure (7). Gel electrophoresis profile of Hemagglutinin gene flanking region 1264bp using 2% Agarose stained with red safe DNA dye and electrophoresed by 5vol/cm in TBA buffer: lane M indicated DNA marker 100-1500bp, lane 1, 2, and 3 indicated Hemagglutinin band of seasonal flu A (USA 2019), lanes 4, 5, no amplification products.
Discussion
The recent study indicated seasonal flue was caused by the infection of two Influenza types A sub types (H1N1 and H3N2), and B. According to the result of real time reverse transcription PCR, in figure (1) indicated that the subtype H1N1 is the dominant strain in Iraq. It was found through the study that the subtype H3N2 was the dominant one in the winter of 2019 began from January and then gradually receded, so that the sub type H1N1 prevailed over it during the period, and the influenza B Victoria strains tend to appear at the end of the season, the recent results H1N1 17.41%, H3N2 4.05%, and Type B 11.96% were higher than that recorded 2018 by the incidence rate of flu A subtype H1N1 14.9 (227/1359) and 4.7 of flu B, 1.5 for H3N2, Our finding Influenza types A (64.2%), and B (35.8%) converging to European Centre for Disease Prevention and Control finding for the 2019–2020 season that indicated co-circulation of Influenza types A (71%), and B (29%) viruses in the European Region. Influenza A(H3) subtype and Influenza B/Victoria lineage have been mentioned as a dominant in north-western Europe, and Central Asia .Previous studies26,27 have shown that type B influenza affects humans only compared to influenza A, which infects both humans and animals, so the ability of influenza type B to mutate and mid-season antigenic-shift is lower than Influenza type A, means it is evolve slowly. This increases the possibility that immunity to Influenza type B can be developed in the current season. The influenza B Victoria strain did not circulate widely during the 2018-19.

According to the WHO reports (February 2020) the distribution of Influenza virus was not homogeneous in the world. Virus predominance was varied between countries. H1N1pdm09 reported dominance in Japan and Republic of Korea, Mongolia and Russia, while in Central Asia A(H1N1) pdm09 and A(H3N2) viruses were circulated equally 2019-2020(WHO February, 2020). For detailed understanding of influenza infection movement in different age groups and influenza strains in communities and its reflection on the performance of the medical service and the comprehensiveness of the vaccination program. The Iraqi median age is 21.0 years (world meter, 2020) .Depending on the results of the current study showed a highly significant distribution of infection rate with the virus Influenza type B, and Influenza type A sub type H1N1 in the age group below 15 years old, then the age group between 30-45 years old, these finding consistence with 24,25 concluded during the largest five epidemics“2009 A/H1N1, 2012–2013 A/H3N2, 2013–2014 A/H1N1, 2010–2011 A/H3N2 and the 2010–2011 A/H1N1” associated with Influenza A subtypes, the children age range 5–17 years old had the highest estimates of relative risk (RR) among all age groups . Adults aged 50–64 and 65+ had the lowest estimates of RR 15. Influenza Type B had highest estimate RR during outbreaks during the 2012–2013 epidemic, while adults 18–49 had the highest RR during the 2010–2011 epidemic (RR=1.43 (1.09,1.85)).Except for the small, 2011–2012 A/H3N2 epidemic. Although the highest rates of infection are in children and young adults, influenza infections are more severe in the elderly≥65 and lead to a proportion of deaths Approximately 90% of influenza-related deaths and 50-70% of influenza-related hospitalizations occur among people in this age group. Increased hospitalization and the need for intensive care are inherent in age groups 65≥ and deaths due to influenza complications. The infection with influenza virus may cause severe infection and death, particularly among the elderly, pregnant women, very young children and among patients with certain medical conditions weakened immune systems. The current population of Iraq is 40,513,679 as of Friday, October 30, 2020 (Worldometer, 2020); Baghdad is the most densely populated
among provinces. The current study showed that Baghdad has the highly significances incidence of influenza Type A with the percentage 65.92% of the total tested cases, because of the crowded city among the governorates may be facilitated transmission of respiratory viruses these result compatible with result, but the distribution of influenza virus among different provinces take different pattern “Baghdad province 21 (4.6%), followed by Babylon 15 (8.3), Waist 12 (5.6%), and Salahaddin 6 (12.3)”. As Dohuk governorate came after Baghdad in casualty rates the Diyala in our finding. While Baghdad ranked second in Anbar province, in infection rates with H3N2 subtype. The influenza type B infections prevailed in the Baghdad, Duhok, Dayala, and Wasit provinces respectively. Non-significant incidences related to the no cases of the Influenza A were recorded in the governorates Kirkuk, Nineveh, and Muthanna may related with lack of health serves or lack of data records. The relationship between the sex of the infected person and infection with the influenza virus statistically is not significant, which was not consistent with previous findings from \(^2\) WHO, 2010 covered Canada and Denmark, as well as the WHO report. On the other hand, the death rate 3.26% in total is closely related to the sex of the infected person with a significant relationship as the mortality rate was higher in males1.84% than females 1.32% in the infection with H1N1, and type B respectively, and equal rate noted in subtype H2N3. It seems that the season 2019-2020 was higher than season 2018 in the mortality rate 1.6% of total infected cases recorded in study. The GBD 2017 Influenza collaborators, (2019) reports refers to the influenza LRTI mortality rate was highest rate among all ages in Eastern Europe (5.2 per 100 000 population adults older than 70 years was more effected. The WHO, (2010) refers to the incidence of severity and case fatality rates variable between males and females. The outcome of the first wave of the 2009 H1N1 pandemic reveals worse for females, with the affected geographic areas, pregnancy factor, severity of asthma, obesity, diabetes, chronic respiratory condition. Overall, men showed higher excess hospitalization rates than women.

The current study was covered the period of 14 months, from January 2019 until first of March, 2020, influenza seasonality in Iraq was similar to the Northern Hemisphere, with a single large peak. The epidemic spread of the virus showed earlier primary peak began from October until the end of February, an increase in infection rates raising in the winter months in both the type Influenza type A with the highest peak in a December, and a decline in the summer months of type B and a decrease in the subtype H2N3. Consistence with the recorded from Bahrain and Qatar that different from Arabian Peninsula and Jordan, which showed clear secondary peaks. The subtype H2N3 predominated during the year 2019 in January and February. of the rest of the species in proportion51.69%, and 15.69% respectively, and a lower percentage of the subtype H1N1 while no recorded for Influenza type B in this two month. Viral infections decreased in months from March to October 2019 to most of the all influenza type. On the other hand the incidence of the influenza virus increased again, starting from the end of October 2019 and continuing until began of March2020.

Previous studies included 70 532 influenza cases from seventeen countries indicated Influenza Aand B accounted for a median 76.5% and 23.5% of cases in a season and were the dominant type in 86.8% and 13.2% of seasons. The proportion of influenza A cases that were subtyped was 85.9%, while only 4.4% of influenza B cases were characterized.
The study of the genetic variants in the strains of the influenza virus that occur continuously allows us to prepare for vaccines. The Influenza vaccines are based on predicting which "mutants" of H1N1, H3N2, and influenza B will appear and proliferate in the next season. Related to the sequence data of strains in country northern and southern hemispheres separate vaccines are developed for their annual epidemics. In the tropics there is exception because influenza shows no clear seasonality. Our finding showed the dominant strain was H1N1, H3N2, and B Victoria. Data collecting in the past ten years, H3N2 has tended to dominate in prevalence over H1N1, H1N2, and influenza B. in compared with the resent vaccine of southern hemisphere 2020-SH(Quadrivalent-“A/Brisbane/02/2018 (H1N1) pdm09-like virus , A/South Australia/34/2019 (H3N2)-like virus B/Washington /02/2019-like virus [B/Victoria lineage], B/Phuket/3073/2013-like virus [B/Yamagata lineage]”’. This season’s vaccine includes strains for influenza A H1N1, influenza A H3N2, and 2 influenza B strains (Victoria and Yamagata). One of the principal questions each season is the degree to which the viral strains included in the vaccine match the circulating flu strains. At this time, there is limited antigenic data on the most commonly circulating strains, so this analysis is still in the very early stages. Based on the currently available antigenic data, the H1N1 and Yamagata vaccine strains appear to have a high degree of match with the circulating strains, while the H3N2 and Victoria vaccine strains do not match as well. Regardless of the degree of match, vaccination remains the best available intervention for reducing risk of infection as well as severe outcomes from seasonal influenza infection. A study published in Vaccine in 2018 found that influenza patients that had been vaccinated were less likely to be admitted to intensive care units and had shorter hospital stays than unvaccinated seasonal influenza patients. The US CDC continues to recommend that all people over the age of 6 months get vaccinated.

Conclusion: The findings of the study lead to the following conclusions: Molecular detection indicated 2 genotypes including influenza A, and B consist of three subtypes (H1N1, H3N2, and B Victoria). The subtype H1N1 is the dominant strain in Iraq through the study period. The winter months, subtype H3N2 predominated during January and February 2019.While H1N1 was dominant in the October 2019 till March 2020.

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