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من قبل

منال عبد المنعم احمد

بكالوريوس علوم حياة – الجامعة المستنصرية (1998)

بإشراف

أ.م.د. نادرة سلمان محمد

أ.د. عبد الرزاق شفيق حسن

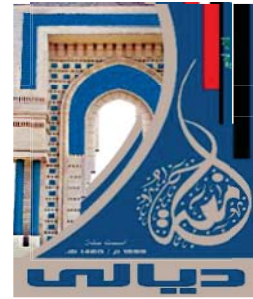
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Comparative Molecular Study on Influenza Type A and B with derived Vaccine in Iraq

A Thesis

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By

Manal Abid almoneem Ahmed

B.Sc. Biology Al-Mustansiriyah University (1998)

Supervised by

Professor Dr.

Abdulrazak Shafiq Hasan

Ph.D. In Medical Microbiology/ Virology

Asisstant Professor Dr.

Nadira Salman Mohamed .

Ph.D In Microbiology/ Molcular Virology

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Chapter One

Introduction

1.1 Introduction.

Influenza is a viral disease 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. Viruses can be transmitted by virus soiled and affected the nose or the eye, in early stage when the patient is a symptomatic and up to 5 to 7 days after infection (Alguacil *et al.*, 2019).

Four types of influenza viruses are included A, B, C and D, types A and B of flu cause human infections every year during the epidemic season, according to the combination of Hemagglutinin (H), and Neuraminidase (N) proteins, influenza A has several subtypes which are expressed on the surface of viruses (Havlickova *et al.*, 2019)

Under the International Health Regulations (IHR, 2005), all human infections caused by a new influenza subtype must be reported, including any influenza A virus that has shown the ability to infect a human and its Hemagglutinin gene (or protein) is not a mutated form, i.e. A(H1) or A(H3), which are common in the human population, antigenic virus investigations have shown some cross-reactivity with seasonal influenza A(H1N1) pdm09 viruses, suggesting that there may be immunity to infection with similar swine viruses in the human population due to the circulation of A(H1N1)pdm09 viruses.(W.H.O. 2020)

In many regions of the world, swine influenza viruses circulate in swine populations. The genetic characteristics of these viruses differ depending on geographical location, most human cases are exposed by contact with infected pigs or contaminated environments to swine influenza viruses, in most instances, human infection tends to result in mild clinical illness, since these viruses continue to be detected in swine populations; it is possible to expect additional human cases.

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 Up to 1 million (Jordà *et al.* , 2020)

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.(Glover *et al.* , 2020).

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).

Influenza spreads fast and efficiently in seasonal epidemics. In temperate regions, every fall and winter, 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 and mortality are common in certain. These high- risk groups include young kids, senior citizens, women and pregnant women with immune-affection (Kennant *et al.*, 2019).

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 (Harper *et al.*, 2005).

1.2 Aims of study.

The Study aims at :

1. Detection genotypes of influenza virus among Iraqi people.
2. Comparative Molecular study on influenza genotypes with international vaccine strains .

Summery

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) subtype existed, by using advanced methods for diagnosis. A cross sectional study was conducted in Iraq from January 2019 to February 2020.

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 RNA extraction, then submitted to revers transcription Real-time polymerase chain reaction (RT-qPCR) 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, for subtyping after sequencing and sequence data alignment. 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 B11.96%. 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. 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. In addition to that, the study detects a non-significant percentage 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 rate 65.92%, and 52.16% respectively of the total tested cases, followed by Dohuk Governorate by rate 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%, TheQar 1.83% respectively, while the subtype H3N2 prevailed in the governorates Anbar 30.4%, Baghdad 17.2%, Erbil 14.70%, Dohuk 13.72%, Basra 9.8/5, 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.

Amplification of hemagglutinin gene flanking regions produced bands were sized (833bp 1266and 599 bp) respectively. Two sequences were determined as Influenza A subtype H1N1 when compared with reference isolates from NCBI. It turns out, that it is not due to Influenza type A subtype H1N1pdm2009 epidemiological isolation, but rather closer identical 99% to USA and Europe seasonal Influenza type A isolates 2019-2020, and the Seq2 located along with Asian lineage such as India, Agwanghi in china. Indicated Influenza B subtype Victoria. The sequenced Hemagglutinin gene showed 4 single nucleotides polymorphism substitution (snp) (C>T, G>A, C>T, C>T) in H1N1, and 4snp in position (C>A, C>T,G>A,G>T). The season's vaccine 2019-2020 includes strains for influenza A H1N1, influenza A H3N2, and 2 influenza B strains (Victoria and Yamagata) matching the circulating flu strains.