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GENETIC MUTATIONS IN INFLUENZA VIRUSES AND ITS RELATION TO DRUG RESISTANCE

Submitted By

Nagwa Fouad Abass Elkholy

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Master of Environmental Science, Institute of Environmental Studies & Research

Ain Shams University, 2008

A thesis submitted in Partial Fulfillment
Of
The Requirement for the Doctor of Philosophy Degree
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Department of Environmental Basic Sciences
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Abstract

Background: The respiratory tract can be infected by a diverse group of viruses that produce syndromes ranging in severity from mild colds to pneumonias. Influenza is a zoonotic disease caused by a variety of the RNA flu viruses. Influenza causes annual epidemics in winter and causes illness in temperate climates. Influenza seasons differ each year in length and severity. An epidemic can take an economic toll through lost workforce productivity, and strain health services. Antiviral drugs for influenza are an important adjunct to influenza vaccine for the treatment and prevention of influenza. When taken before infection or during early stage of the disease (within two days of onset of illness), antivirals may help prevent infection or may reduce the duration of symptoms by one or two days. World Health Organization (WHO) monitors antiviral susceptibility in the circulating influenza viruses.

Methods: Samples from influenza-like illness (ILI) patients were collected and treated according to the recommended laboratory tests of World Health Organization (WHO) **(WHO-Manual, 2011**). The total number of ILI subjects included in this study is 490. Influenza virus was isolated in embryonated hen eggs and MDCK cells. The main tests are: Haemagglutination (HA) test, Haemagglutination Inhibition (HAI) test and reverse-transcription polymerase chain reaction (RT-PCR). Diagnostic kits supplied by WHO and Centers for Disease Control and Prevention (CDC) to National Influenza Centres (NICs) is used in identification of viruses and antibodies. Data were analyzed using SPSS program version 17.

Results: This study gave a highlight on influenza activity in Egypt during three seasons 2009 – 2012. The peak was usually in December. It is always moderate. Influenza virus strains circulating and predominating in Egypt through season 2010 was A(H1N1)pdm09 which was genetically similar to California/7/2009 vaccine strain. Viruses predominated through season 2011 are A(H1N1)pdm09 and B which were genetically similar to California/7/2009 vaccine strain & B/Brisbane/60/2008 vaccine strain respectively. Viruses predominated through season 2012 were H3N2 which were genetically similar to A/Victoria/361/2011. Infection with influenza A was higher than influenza B. The number of people, children or adult, infected with influenza A was higher than that infected with influenza B. Regarding the age group of virus

distribution; it appeared that influenza viruses showed a marked tendency to affect younger age groups.

The pandemic H1N1 2009 viruses were resistant to M2 inhibitors (amantadine and rimantadine) but sensitive to neuraminidase inhibitors (oseltamivir and zanamivir). It seemed that most viruses being collected during season 2009-2010 have the substitution S203T and quite a number have the D222E substitution. D222G substitution was observed in the HA of some pandemic (H1N1) 2009 viruses. The clinical significance of these substitutions remains uncertain. B viruses do not show resistance to neuraminidase inhibitors.

Conclusions: Despite limited drift observed in all isolates over the 3 seasons 2010, 2011 and 2012 in Egypt, circulating A(H1N1)pdm09, A(H3N2) and B strains remained antigenically similar to the vaccine strains of that seasons. Some virus isolates were oseltamivir resistant. We need to study and provide information regarding circulating influenza strains. Such information was needed to guide decisions regarding influenza treatment and chemoprophylaxis and to formulate vaccine for the coming year. Ongoing studies were attempting to find new antiinfluenza drugs that target the virus replication cycle at many different steps, but none has yet been approved for clinical use. All these approaches have the major disadvantage that they attack a viral function and/or structure. Hence, even though strongly conserved elements will not change easily, the virus will eventually adapt to and escapes from the selective pressure exerted by the drug**.**

Key words: Influenza, H1N1 pdm2009, H3N2, B, vaccine, antiviral agents.

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Contents

List of Abbreviations

- BSA: Bovin Serum Albumin
- CDC: Centers for Disease Control and Prevention
- Conventional RT-PCR: Conventional reverse-transcription polymerase chain reaction
- D-MEM: Dulbeco's Modified Eagle Medium
- FBS: Fetal Bovin Serum
- GISRS: Global Influenza Surveillance and Response System (GISRS)
- GISN**:** Global Influenza Surveillance System
- HA : Haemagglutination
- HAI: Haemagglutination Inhibition
- HCWs: health-care workers
- HPAI: Highly Pathogenic Avian Influenza
- ILI**:** influenza-like illness
- LAIV: live attenuated influenza vaccines
- MDCK: Madin-Darby Canine Kidney
- Min: minute
- NH: Northern Hemisphere
- PIP**:** Pandemic Influenza Preparedness
- PMK: Primary Monkey Kidney
- RBCs: red blood cells
- RDE: receptor destroying enzyme
- RNP: ribonucleoprotein
- RT: room temperature
- RT-PCR: reverse-transcription polymerase chain reaction
- Sec: second
- SH: Southern Hemisphere
- S-OIV: novel swine-origin influenza A (H1N1) virus
- TIV: Trivalent inactivated vaccine
- UK: United Kingdom
- µl: microlitre

- vol: volume
- WHO: World Health Organization
- WR: WHO EURO Region

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