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Molecular characterization and phylogenetic analysis of Middle East 2009 H1N1 pdm isolates

Ghaleb Adwan*

Department of Biology and Biotechnology, An-Najah National University, P.O.Box (7)-Nablus, Palestine

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ABSTRACT

Objective: To study hemagglutinin genetic evolution of some Middle East (ME) 2009 H1N1 pdm isolates and compared them with prototype vaccine strain [A/California/07/2009 (H1N1)], which is used as a vaccine strain in the Northern Hemisphere 2010-2011. **Methods:** Nucleotide and/or amino acid sequences of HA gene of fifty-four ME 2009 H1N1 pdm isolates were retrieved from GenBank Database by using Basic BLAST engine. Phylogenetic trees were established for both nucleotide and amino acid sequences using the muscle algorithm of the computer program CLC free workbench 5.6.1 JRE software. Amino acids alignment was also done to compare sequences HA1 domains of HA genes of ME 2009 H1N1 pdm isolates (n=39) with amino acid sequence of prototype vaccine strain A/California/07/2009 (H1N1). **Results:** Phylogenetic analysis of amino acids and nucleotides of the HA gene of the ME 2009 H1N1 pdm isolates confirmed their evolutionary position in cluster with prototype vaccine strain (A/California/07/2009 (H1N1)) which is used as vaccine strain in the Northern Hemisphere 2010-2011. Antigenically, the ME 2009 H1N1 pdm isolates were homogeneous and closely related to prototype vaccine. Only a few amino acid substitutions in the HA among the ME 2009 H1N1 pdm isolates were analyzed. **Conclusions:** The current influenza vaccine is expected to provide a good protection against ME 2009 H1N1 pdm because it contains strains with H1 HA [A/California/07/2009 (H1N1)]-like strain.

1. Introduction

The influenza severity ranges from asymptomatic infection to serious illness with systemic features especially in patients with pre-existing respiratory or cardiovascular disease and in frail elderly. Influenza viruses have a segmented genome of negative sense RNA encapsidated by a virally specified nucleoprotein. There are three types of influenza viruses A, B and C that cause illness in human population. Influenza B and C viruses are essentially restricted to humans whereas influenza A viruses infect a wide variety of avian and mammalian species[1,2]. Influenza A viruses can be divided into 16 subtypes of hemagglutinin (HA) and 9 different subtypes of neuraminidase (NA) based on both antigenic and genetic differences[3-5]. Human influenza is a zoonotic disease, and aquatic birds form the major natural reservoir of influenza A viruses of all HA and NA subtypes[6]. Despite that, pigs play an important role in the ecology of human influenza. One of the most important biological properties of influenza A viruses is their antigenic variability which occurs in two forms: antigenic drift and antigenic shift. Pigs, considered as an intermediate host, possess both receptors NeuAc α 2,6Gal linkages and NeuAc α 2,3Gal linkages for human and avian influenza viruses respectively on their respiratory epithelial cells, and this explains the susceptibility of pigs to both avian and human influenza viruses[7]. For this, pigs have been postulated to be as a mixing vessel for the emergence of new isolates with human pandemic potential[8]. Influenza virus caused seasonal epidemics and occasional pandemics in humans. Three major global pandemics caused by novel antigen variants of influenza viruses have affected the human population, the "Spanish flu" in 1918 (H1N1 subtype), the "Asian flu" in 1957 (H2N2 subtype), and the "Hong Kong flu" in 1968 (H3N2 subtype) resulting in significant mortality during the 20th century[9,10]. All these pandemics originated in whole or in part from nonhuman reservoirs, and the HA genes of all of the pandemic viruses ultimately originated from avian influenza viruses[11].

In Spring 2009, a novel reassortant strain of H1N1 influenza A emerged as a lineage distinct from seasonal

*Corresponding author: Ghaleb Adwan, Department of Biology and Biotechnology, An-Najah National University, P. O. Box (7)-Nablus, Palestine.
E-mail: adwang@najah.edu

H1N1. This new H1N1 virus has now migrated to most parts of the Americas, Europe, Australia, Asia and Africa. Its hemagglutinin (HA), nucleoprotein (NP), and nonstructural (NS) protein genes belong to the classical swine lineage, while its neuraminidase (NA) and matrix (M) protein genes derive from a Eurasian swine influenza lineage which entered pigs from avian hosts around 1979, and its polymerase gene segments, PA, PB1 and PB2, descended from the North American triple reassortant swine lineage[11-13]. This genetic combination may contribute to the improved fitness of the H1N1 pdm in humans and its human-to-human transmissibility. There is a serious concern that the virus may further mutate into a more dangerous form, so it is very important to monitor the evolutionary trends of the 2009 H1N1 pdm virus.

On April 17, 2009, CDC determined that two cases of febrile respiratory illness occurring in children who resided in adjacent counties in southern California were caused by infection with a swine influenza A (H1N1) virus[14]. On June 11, 2009, the World Health Organization declared the outbreak of novel H1N1 virus had become the first global pandemic of the 21st century[15]. The virus was found to be an H1N1 virus that was antigenically and genetically unrelated to human seasonal influenza viruses and genetically related to viruses known to circulate in swine. The name of this pandemic virus is influenza A/H1N1 pdm [16].

Each year, WHO publishes recommendations on the composition of influenza vaccine for the Northern and Southern Hemispheres. There are currently two main branches of H1N1 circulating world-wide including the Middle East (ME) region, a seasonal branch and a pandemic branch. In this paper, we focused on hemagglutinin genetic evolution of some ME 2009 H1N1 pdm isolates and compared them with prototype vaccine strain [A/California/07/2009 (H1N1)], which is used as vaccine strain in the Northern Hemisphere 2010-2011[17].

2. Materials and methods

Nucleotide and/or amino acid sequences of HA gene of ME 2009 H1N1 pdm isolates were retrieved from GenBank Database by using Basic BLAST engine (www.ch.embnet.org/software/bBLAST.html). These isolates were recovered from different ME countries: Israel (n=4), Turkey (n=20), Iraq (n=1), Kuwait (n=13), Iran (n=3), Bahrain (n=4), Egypt (n=5), and Afghanistan (n=4). Other references strains were included in this study such as A/California/07/2009 (H1N1), A/South Carolina/1/18 (H1N1), A/Solomon Island/320/2006 (H1N1), A/Brisban/59/2007 (H1N1), A/Korea/01/2009 (H1N1), A/Tokushima/1/2009 (H1N1), A/Beijing/3/2009 (H1N1) and A/Turkey/1558/2007 (H1N1). Phylogenetic trees were constructed based on the continuous nucleotide sequences and deduced amino acid sequences of the HA gene region including nucleotides 51-1184 (1133 nucleotides) and amino acids 17-394 (377 amino acids). Numbering of nucleotides and amino acids corresponding to positions of A/California/07/2009 (H1N1) strain sequence. The phylogenetic analysis was established by comparison with the representative seasonal strain from the ME region, prototype vaccine strain [A/California/07/2009 (H1N1)], H1N1 influenza A seasonal vaccines, and other 2009 H1N1 pdm isolates. Multiple alignments were done using the muscle algorithm of the computer program CLC free workbench 5.6.1 JRE software (developed by CLC bio A/S, www.clcbio.com). Phylogenetic trees were constructed using the program Neighbor Joining in the same software. The robustness of the groupings in the Neighbor Joining analysis was assessed with 1 000 bootstrap resamplings. Amino acids alignment was done to compare sequences HA1 domains of HA genes of ME 2009 H1N1 pdm isolates (n=39) with amino acid sequence of prototype vaccine strain A/California/07/2009 (H1N1). ME 2009 H1N1 pdm isolates included in amino acid alignment are A/Israel/644/2009 (H1N1), A/Israel/276/2009 (H1N1), A/Israel/277/2009 (H1N1), A/Israel/70/2009(H1N1), A/Sulaimani/05/2009 (H1N1), A/Ghom/1550/2009 (H1N1), A/Khorasan/1583/2009 (H1N1), A/Lorestan/1599/2009 (H1N1), A/Ankara/29/2009 (H1N1), A/Ankara/28/2009 (H1N1), A/Ankara/27/2009 (H1N1), A/Ankara/22/2009 (H1N1), A/Ankara/21/2009 (H1N1), A/Ankara/20/2009 (H1N1), A/Ankara/19/2009(H1N1), A/Ankara/18/2009(H1N1), A/Ankara/17/2009 (H1N1), A/Ankara/16/2009 (H1N1), A/Ankara/15/2009 (H1N1), A/Ankara/13/2009 (H1N1), Ankara/12/2009 (H1N1), A/Ankara/11/2009 (H1N1), A/Ankara/10/2009 (H1N1), A/Ankara/08/2009(H1N1), A/Ankara/06/2009(H1N1), A/Ankara/05/2009(H1N1), A/Ankara/04/2009 (H1N1), A/Ankara/03/2009 (H1N1), A/Kuwait/6379/2009 (H1N1), A/Kuwait/N13111/2009 (H1N1), A/Kuwait/N13039/2009 (H1N1), A/Kuwait/N13055/2009 (H1N1), A/Egypt/N11640/2009 (H1N1), A/Egypt/N11640/2009 (H1N1), A/Egypt/N14644/2009 (H1N1), A/Bahrain/N11892/2009 (H1N1), A/Bahrain/N11660/2009 (H1N1), A/Afghanistan/N11216/2009 (H1N1), A/Afghanistan/N10725/2009 (H1N1). Seasonal A/Turkey/1558/2007(H1N1) isolate was included as a representative strain.

3. Results

1 133 nucleotides of HA nucleotide sequence and 377 deduced amino acids of HA region sequences of ME 2009 H1N1 pdm isolates were compared with prototype vaccine strain [A/California/07/2009 (H1N1)], seasonal influenza A H1N1strains and 2009 H1N1 pdm isolates. Influenza A virus strain A/California/07/2009 (H1N1) is used as a vaccine strain in the Northern Hemisphere 2010-2011. Phylogenetic analysis showed that the sequences of deduced amino acids and DNA nucleotide sequences of HA genes of ME 2009 H1N1 pdm isolates were closely related to the recent prototype vaccine strain A/California/07/2009 (H1N1)-like strain (Figure 1 and 2).

The putative antigenic sites, receptor-binding sites and potential N-linked glycosylation sites were analyzed (Data not shown). Four out five conserved amino acid residues in H1 influenza A virus have Tyr(Y)-94, Trp(W)-153, His(H)-183 and Tyr(Y)-195 at the HA receptor-binding site. While the conserved amino acid number 5, which is amino acid Ser(S)-136 in ME 2009 H1N1 pdm isolates, mutated to amino

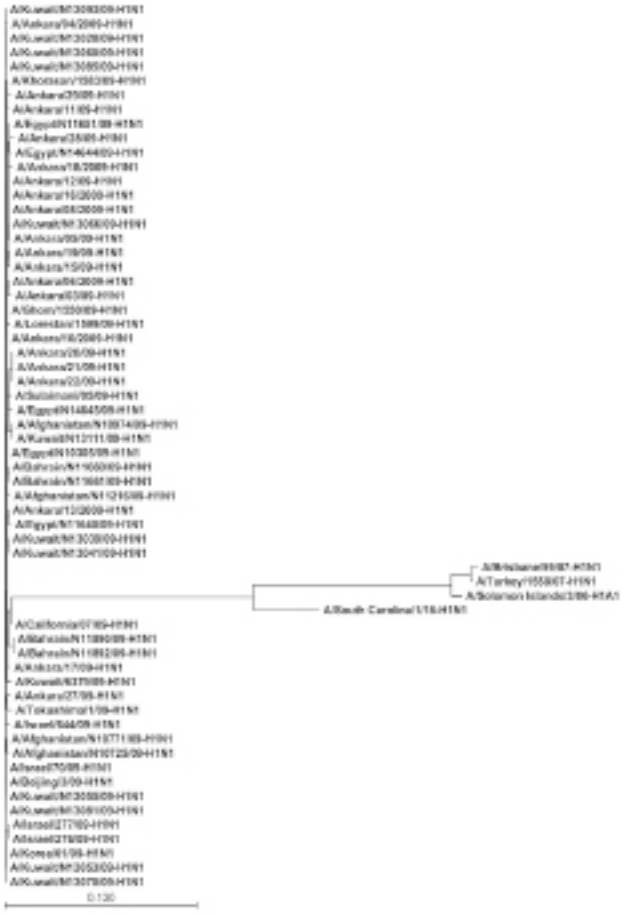

Figure 1. Neighbour-joining phylogenetic tree based on the partial nucleotide sequence of HA genes of selected 54 ME 2009 H1N1 pdm isolates.
Prototype vaccine strain (A/California/07/2009 (H1N1)) and other H1N1 influenza viruses were included. The tree was bootstrapped with 1 000 replicates, and the genetic distance corresponding is shown by the bar. Sequences in this study were obtained from GenBank.

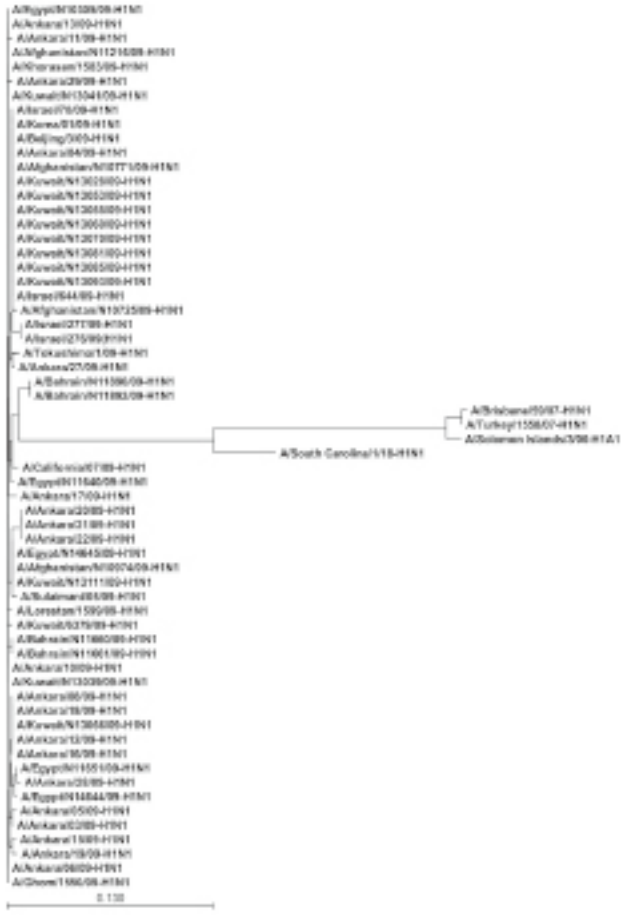

Figure 2. Phylogenetic tree based on the amino acid sequence of HA genes of selected 54 ME 2009 H1N1 pdm isolates.
Prototype vaccine strain [A/California/07/2009 (H1N1)] and other H1N1 influenza viruses were included. The tree was bootstrapped with 1 000 replicates, and the genetic distance corresponding is shown by the bar. Sequences in this study were obtained from GenBank.

acid Thr(T). In addition to that, HA of the ME 2009 H1N1 pdm isolates possessed Asp(D)-190 and Asp(D)-225 in the receptor binding site except in two isolates, A/Egypt/N14645/2009 (H1N1) and A/Egypt/N11651/2009 (H1N1) which had Gly(G)-225 and Glu(E)-225, respectively. Other critical residues known to confer human specificity in H5 viruses, including E227, P221 and E216 were also noted in H1N1 pdm viruses as well as in the ME 2009 H1N1 pdm isolates. It was also found that 32/39 of the ME 2009 H1N1 pdm isolates have a unique mutation in HA S206T.

The patterns of the antigenic site in the HA gene can be observed by amino acid alignment (Data not shown). As for H1N1, four antigenic sites have already been defined, two strain-specific (Sa and Sb) and common antigenic sites (Ca and Cb) of the virus hemagglutinin. It was found that only 3 strains had amino acid substitutions in antigenic site Ca at HA1 domain. These isolates are A/Egypt/N14645/2009 (H1N1), A/Egypt/N11651/2009 (H1N1) and A/Bahrain/N11892/2009 (H1N1) which had D225G , D225E and R209K, respectively. As expected, the ME 2009 H1N1 pdm isolates contain amino acid substitutions at putative antigenic sites when compared with seasonal H1 HA1 region. These isolates had five potential glycosylation sites (Asn-X-Ser/Thr conserved at positions Asn(N)-14, Asn(N)-26, Asn(N)-90, Asn(N)-279 and Asn(N)-290 in HA1 domain.

4. Discussion

Influenza virus genomes are well known to undergo antigenic drift or antigenic shift that enable escape from preexisting immunity and cause new outbreaks of influenza in animals and even humans[18,19], so influenza A viruses have ability to exhibit the greatest genetic diversity and change every year. Each year WHO recommends the most suitable composition of influenza vaccine strains for the Northern and Southern Hemispheres. Phylogenetic analysis of amino acids and nucleotides of the HA gene of the ME 2009 H1N1 pdm isolates confirmed their evolutionary position in cluster with prototype vaccine strain [(A/California/07/2009 (H1N1)] which is used as a vaccine strain in the Northern Hemisphere 2010-2011. Comparison of the nucleotide and amino acid sequences of the HA region between ME 2009 H1N1 pdm isolates and prototype vaccine strain [A/California/07/2009 (H1N1)] showed they are closely related to the vaccine strain recommended for the Northern

Hemisphere 2010-2011. In general, ME 2009 H1N1 pdm isolates have the same amino acids in antigenic site and are closely related to the prototype vaccine strain, which indicates that these viruses may have the same antigenicity of prototype vaccine strain 2010/2011. Vaccines containing A/California/7/2009 antigens stimulated anti-HA antibodies of similar titers against the vaccine virus and recent pandemic A (H1N1) viruses[17]. Variations in H1N1 isolates were predominantly located at the antigenic site, which is of interest for developing suitable vaccine strains[20]. This may suggest that using A/California/07/2009 (H1N1) as a vaccine will be effective against ME 2009 H1N1 pdm isolates[17].

HA of the ME 2009 H1N1 pdm isolates, as in other H1N1pdm viruses, possessed Asp(D)-190 and Asp(D)-225 in the receptor binding sites, as the terminal sialic acid binding specifically to the NeuAc α2,6Gal amino acid linkage[20-23]. A correlation between the amino acid in position 225 of HA and virus receptor specificity has been described by many investigators[24-27]. Probably, viruses with Asp(D)-190 and/or Asp(D)-225 may support the efficient transmissibility of these viruses in the human respiratory tract[21,28]. The D-225G and D-225E mutations were observed in two ME 2009 H1N1 pdm isolates. Changes occurred at a site HA 225 involved both antigen and receptor binding. In this paper we reported three variants D, G and E at this position. Two variants D and G were reported and also observed during the 1918 pandemic[22,29]. Glycan microarray studies have shown that HA possessing D190 along with G225 had specificity for both α-2,3 linked glycan and α-2,6 linked glycan[22]. The significance of these mutations in terms of pathogenicity needs to be verified[21]. There are five key conserved amino acid residues within the receptor-binding site in HA1 domain H1 influenza A virus, Tyr(Y)-94, Ser(S)-136, Trp(W)-153, His(H)-183 and Tyr(Y)-195[20,22,30,31]. These residues are relatively conserved and predicted to have a role in binding to human receptors. Since both Thr and Ser are polar hydrorhilic amino acids, substitution between them most likely maintains interactions and proper conformation of the binding pocket. Other amino acids in ME 2009 H1N1 pdm isolates possessed at positions (G)-134, (V)-135, (A)-137,138-(A), 155-(V), (L)-194, (R)-224, (Q)-226, 227-(Q), (G)-228 and (R)-229, are components of receptor-binding sites of the HA of H1N1 influenza viruses[32]. Among other critical residues known to confer human specificity in H5 viruses[22], E227, P221 and E216 were also noted in other 2009 H1N1pdm viruses including the ME 2009 H1N1 pdm isolates[21].

Antigenically, the ME 2009 H1N1 pdm isolates are homogeneous. There have been only a few amino acid substitutions in the HA among the Middle East 2009 H1N1 pdm isolates analyzed. Location of these mutations are in Ca antigenic site D-225G, D-225E and R-209-K. Two of these D-225E and R-209-K may have little effect on antigenicity, due to D and E are hydrophilic negatively charged amino acids, while both R and K are hydrophilic positively charged amino acids. A unique mutation in 32/39 HA S206T of the ME 2009 H1N1 pdm isolates was neither found in 1918 and 1977 H1N1 pdm viruses, nor was it found in the human, swine and avian Influenza A viruses[33]. However, It was found that the S206T mutation transiently appeared in the HA sequences of human H1N1 viruses collected in 1934 and in swine H1N1 viruses collected in 1976 and 1977. S206T is located in the receptor-binding domain of HA which is a major determinant of Influenza A virus host specificity; therefore, HA-206 S→T mutation may directly affect the infectivity and transmissibility of 2009 H1N1 pdm in humans[33]. The N-linked glycosylation is conserved among various HA subtypes of influenza A viruses[20]. The ME 2009 H1N1 pdm isolates have five N-linked glycosylation sequences while H1N1 isolates 2007 have seven. It was suggested that the presence or absence of N-linked glycosylation may cause increase or loss of function of the glycoprotein because N-linked glycosylation can initiate and maintain folding, stability, solubility, transportation, antigenicity, and immunogenicity of the protein[34]. The apparent lack of glycosylation at some positions between the ME 2009 H1N1 pdm isolates and seasonal H1N1 2007 may lead to differences in three dimensional structure of these viruses[35], taken into consideration that the oligosaccharides are capable of masking some antigenic determinants on HA surface and therefore modifying the HA reactivity[22,36].

In conclusion, the phylogenetic analysis showed ME 2009 H1N1 pdm isolates are closely related to the recent prototype vaccine strain 2010/2011 A/California/07/2009 (H1N1)-like strain. Taken together, the current influenza vaccine is expected to provide a good protection against ME 2009 H1N1 pdm because it contains strains with H1 HA (A/California/07/2009 (H1N1)-like strain.

Conflict of interest statement

The authors declare no competing interest with the present work.

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