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Evolutionary Analysis of Influenza A (H1N1) Pdm 09 in Post Pandemic Period in Pakistan

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Abstract : In early 2009, Pandemic type A (H1N1) Influenza virus emerged globally. Since then, it has continued circulation causing considerable morbidity and mortality. The purpose of this study was to evaluate the evolutionary changes in Influenza A (H1N1) pdm09 viruses from 2009-15 and their relevance with the current vaccine viruses. Methods: Respiratory specimens were collected with influenza-like illness and Severe Acute Respiratory Illness. Samples were processed according to CDC protocol. Sequencing and phylogenetic analysis of Haemagglutinin (HA) and neuraminidase (NA) genes was carried out comparing representative isolates from Pakistan viruses. Results: Between Jan2009 - Feb 2016, 1870 (13.2%) samples were positive for influenza A out of 14086. During the pandemic period (2009–10), Influenza A/ H1N1pdm 09 was the dominant strain with 366 (45%) of total influenza positives. In the post-pandemic period (2011–2016), a total of 1066 (59.6%) cases were positive Influenza A/ H1N1pdm 09 with co-circulation of different Influenza A subtypes. Overall, the Pakistan A(H1N1) pdm09 viruses grouped in two genetic clades. Influenza A(H1N1)pdm09 viruses only ascribed to Clade 7 during the pandemic period whereas viruses belong to clade 7 (2011) and clade 6B (2015) during the post-pandemic years. Amino acid analysis of the HA gene revealed mutations at positions S220T, I338V and P100S specially associated with outbreaks in all the analyzed strains. Sequence analyses of post-pandemic A(H1N1)pdm09 viruses showed additional substitutions at antigenic sites; S179N,K180Q (SA), D185N, D239G (CA), S202A (SB) and at receptor binding sites; A13T, S200P when compared with pandemic period. Substitution at Genetic markers; A273T (69%), S200P/T (15%) and D239G (7.6%) associated with severity and E391K (69%) associated with virulence was identified in viruses isolated during 2015. Analysis of NA gene revealed outbreak markers; V106I (23%) among pandemic and N248D (100%) during post-pandemic Pakistan viruses. Additional N-Glycosylation site; HA S179N (23%), NA I23T(7.6%) and N44S (77%) in place of N386K(77%) were only found in post-pandemic viruses. All isolates showed histidine (H) at position 275 in NA indicating sensitivity to neuraminidase inhibitors. Conclusion: This study shows that the Influenza A(H1N1)pdm09 viruses from Pakistan clustered into two genetic clades, with co-circulation of some variants. Certain key substitutions in the receptor binding site and few changes indicative of virulence were also detected in post-pandemic strains. Therefore, it is imperative to continue monitoring of the viruses for early identification of potential variants of high virulence or emergence of drug-resistant variants.

Keywords : Influenza A (H1N1) pdm09, evolutionary analysis, post pandemic period, Pakistan
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