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Short communication

Sequence analysis of haemagglutinin and neuraminidase of H1N1 strain from a patient coinfected with *Mycobacterium tuberculosis*

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ABSTRACT

The 2009 H1N1 pandemic (*H1N1pdm09*) was associated with a considerable influenza-related morbidity and mortality. Among the complications, *Mycobacterial tuberculosis* was recorded as a coinfection with influenza in rare cases. The full-length sequences of the viral haemagglutinin and neuraminidase of *H1N1pdm09* influenza A virus were analyzed from a recently infected patient. The patient was chronically infected with *Mycobacterium tuberculosis*. Molecular modelling and in-silico docking of the virus, and other selected strains with the drug oseltamivir were conducted and compared. Sequence analysis of the viral haemagglutinin revealed it to be closely related to the 6B.1 clade, with high identity to the circulating *H1N1pdm09* strains, and confirmed that the virus still harbouring high affinity to the α-2,6-sialic acid human receptor. The viral neuraminidase showed high identity to the neuraminidase of the recently circulating strains of the virus with no evidence of the development of oseltamivir-resistant mutants. Regular monitoring of the circulating strains is recommended to screen for a possible emergence of drug-resistant strains.

1. Introduction

Since the first detection of the H1N1 in Mexico in March of 2009 [1], the virus began to circulate worldwide in a pandemic manner, hence the name *H1N1pdm09*. There have been more than 18,500 laboratory-confirmed deaths from infection with the *H1N1pdm09* strain. Most of the lethal cases of *H1N1pdm09* have been recorded in young and middle-aged adults [2] rather than in young children and old people as with the seasonal influenza epidemics.

The influenza, haemagglutinin (HA), is the principal surface antigen and the main target of vaccine-induced neutralizing antibodies. The virus frequently mutates vulnerable HA epitopes, with subsequent changes in its antigenic structure, to escape recognition and virus elimination by the immune system [3]. Determining the changes in the antigenic sites provide structural insights into the rationale for optimizing vaccines to match circulating variants.

Viral neuraminidase (NA) is a receptor destroying enzyme that helps to liberate the viral particles from the infected cells, which renders it an attractive target for anti-influenza drugs. Although many neuraminidase inhibitors exists, including oseltamivir, zanamivir, laninamivir and peramivir [4], oseltamivir is considered to be the most commonly used. The early use of oseltamivir can reduce the mortality rate in hospitalized persons infected with *H1N1pdm09* by half [5]. Oseltamivir resistance typically emerges in immune-compromised patients, but is much less frequently found in immunocompetent people [6] and it constitutes a major challenge to the effectiveness of the drug for the treatment of influenza and highlights the necessity for clinical assessment of antiviral resistance.

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*. Active tuberculosis occurs in up to 10% of the infected individuals, while the remaining cases develop a latent infection lasting for years [7]. Reactivation of latent infection may occur, with the subsequent development of active disease in most cases pulmonary TB disease [8].

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The current study describes the full-length sequence analysis of both haemagglutinin and neuraminidase of *H1N1pdm09* strain from TB chronically infected patient. In addition, the virtual docking of the neuraminidase against oseltamivir of the detected strain in comparison with selected pdm09 classical and H275Y mutant strains was assessed.

2. Materials and methods

2.1. Ethical approval

The study was ethically approved by the King Faisal Hospital Ethical and Research Committee. Informed written consent was obtained from the patient.

2.2. Subject

An 86-year-old Pakistani female resident in Al-Taif, Saudi Arabia, infected with *Mycobacterium tuberculosis* was admitted to King Faisal hospital at the end of November 2015. The patient was confirmed to have a recent infection of *H1N1pdm09* by Makkah Regional Laboratory, Saudi Ministry of Health Laboratory. Oseltamivir was used for the treatment of the patient after laboratory confirmation of *H1N1pdm09*.

2.3. RNA extraction

Nasopharyngeal, throat swabs and sputum samples were collected from the patient at day 3 of admission in a virus transport medium. Total viral RNA was extracted from 300 μl of the sample using viral RNA extraction kit (Promega). Extracted RNAs were eluted into a final volume of 60 μl in an elution buffer.

2.4. One-step RT-PCR

One-step RT-PCR was conducted using Script™ RT (Promega) and in-house specific primer sets that flank the HA and NA genes of *H1N1pdm09*. Three overlapping primer sets were used for the HA gene: pH1-F1:CAAAAGCAGGGGAAAACAAA, pH1-R1:TTGATGTCCCCACAAAAACA,pH1-F2:GCTCCGCCAATCCTACATTA, pH1-R2:GATAACCGTACCATCCATCTACC, pH1-F3: CCAGCCTCCCATTTCAGAATA, pH1-R3: GTAGAGACCCATTAGAGCACATC and two for the NA gene: pN1-F1 ACCATTGGTTCGGTCTGTATG, pN1-R1 CACTTGGTCCATCGGTCATTAT, pN1-F2 GGCTGTGTTAAAGTACAATGGC and pN1-R2 AAGACCAACCCACAGTGTC, were used. Reverse transcription was conducted at 45 °C for 45 min followed by 5 min denaturation at 95 °C and 35 cycles of 95 °C for 30 s, 52 °C for 30 s (54 °C for pH1-F1/R1 and 50 °C pN1-F1/R1) and 72 °C for 1.5 min. This was followed by a final elongation step for 10 min at 72 °C. PCR amplicons were gel extracted and sequenced directly in

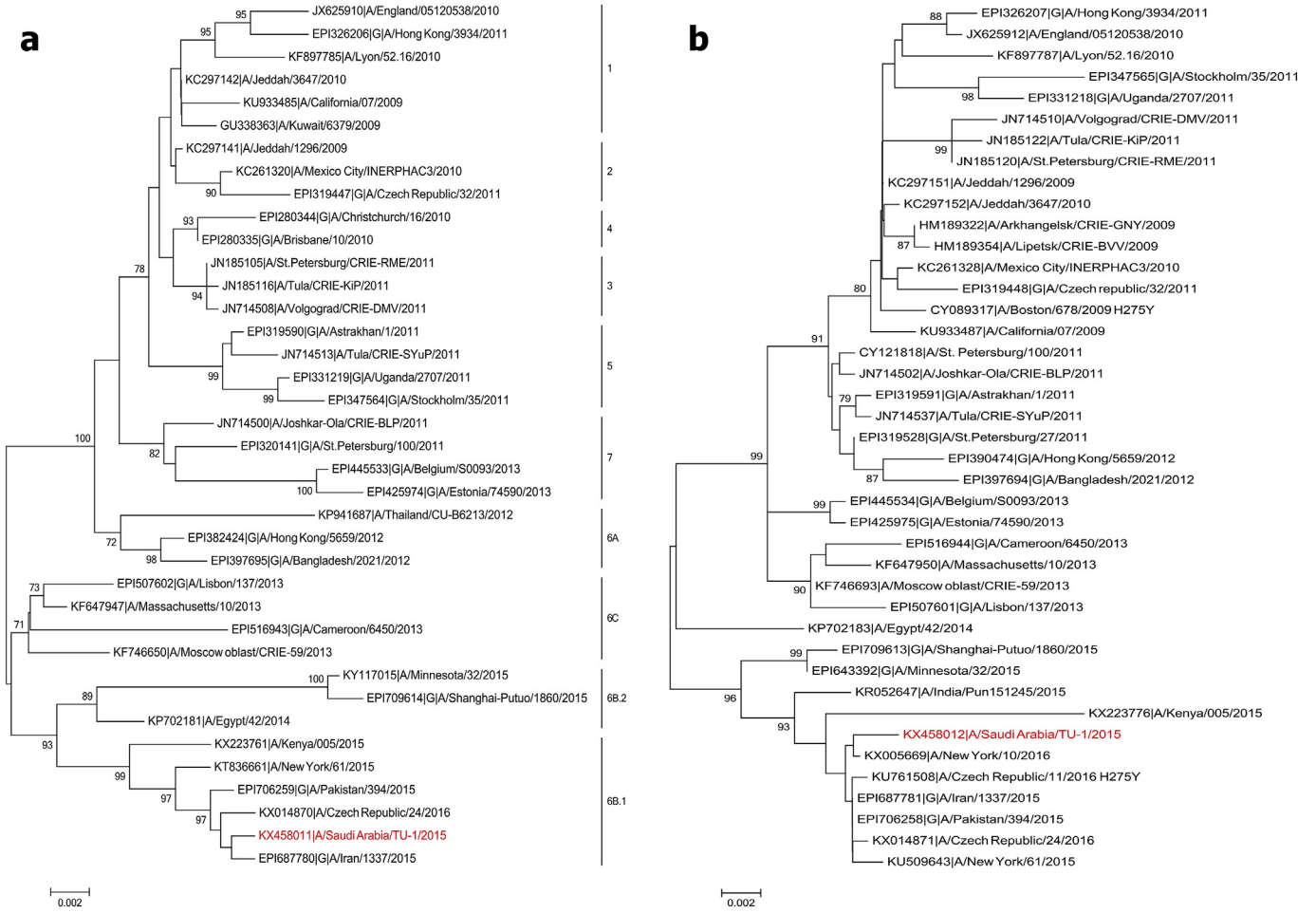

Fig. 1. Phylogenetic trees of the haemagglutinin and neuramindase genes of the *H1N1pdm09*. Maximum likelihood phylogenetic tree with 1000 bootstrap replicates of the different *H1N1pdm09* strains in comparison to the A/Saudi Arabia/TU-1/2015 (red in colour). G symbol denotes strains obtained from GISAID. a) Haemagglutinin, b) Neuraminidase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)