

# 4<sup>th</sup> World Congress on Virology

October 06-08, 2014 Hilton San Antonio Airport, TX, USA

## Mutation analysis of haemagglutinin gene of the Influenza A (H1N1) pdm09 virus and its correlation with disease severity

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**Background:** Influenza A (H1N1)pdm09 was the first influenza pandemic of the 21st century. Though the overall global case fatality rate of the 2009 pandemic H1N1 appears to be low (less than 0.5%), the fatality rate in India was relatively higher (0.86%). Elucidating viral determinants of disease severity is important for developing better prevention and treatment strategies.

**Materials and Methods:** Nasal/throat swabs collected from patients presenting with Influenza like Illness (ILI) were tested for the presence of (H1N1)pdm09 virus by real time PCR (CDC Protocol). Positive cases were categorized into mild, moderate or severe group based on clinical presentation of the patients (n=15 per group, total 45 samples). Virus isolation was done using MDCK cell line. The haemagglutinin (HA) gene of the isolates was amplified by one step RT PCR using WHO-CDC primers. Purified PCR products were sequenced. The obtained sequences were analysed using BioEdit v7.0.9.

**Results and Conclusion:** We identified 11 mutations that were present exclusively in the severe category. These are A151T, S200P, D239G/N/E, A273T, D52Y, N55D, A158E, G187E, Q310H, N458K and A532T. It was observed that majority of the mutations cluster around the globular head of the HA protein which could probably alter the receptor binding affinity of the virus, conferring them with an advantage compared to the mild/moderate group viruses. The D239G mutation which is a well-studied marker for severity was observed in one of the severe group samples. In this study, we have also identified three additional mutations i.e. A151T, S200P and A273T as novel markers of severity which were present in 13% of the severe group samples. Supporting this observation, there are previous studies which have suggested a role for these mutations in increased receptor binding affinity or increased growth rate. However, more number of samples would be required to understand the significance of the association of these mutations with severity.

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