In April 2009, the first case of the 09 influenza virus is identified in the United States. Since then, the virus was spread globally and marked the outbreak of the 2009 pandemic flu in history. The 2009 pandemic flu virus belongs to the influenza A family Orthomyxoviridae (1). It combines genome from North American avian virus origin, human H3N2 virus origin and Eurasian swine virus origin (1). The common signs from infection include flu-like symptoms such as fever, cough, and runny nose; however, many severe cases cause death due to viral pneumonia (2). The influenza virus spread to the human population employing its viral protein hemagglutinin (HA). H1N1 virus gains entry into human body through binding the surface viral protein HA with the sialic acid receptors on the human epithelial cell surface.

HA is a trimmer which makes up of three monomers with glycans attached to it. Receptor binding domain is believed to be located on top of the globular head where human sialic acid binds with hemagglutinin (17). The term "glycans" refers to the sugar chains that are attached to protein during translation and are modified in the Golgi (4). N-linked glycans are glycans that attached to the nitrogen on the asparagine in a consensus sequence (Asn-Xaa-Ser/Thr) where Xaa is any amino acid except Proline (4- 6). Glycans can be found on human cells and on viral proteins, especially on the 2009 H1N1 pandemic viral protein surface (PDB ID# 3M6S, 3LZG, 3AL4). Glycans have various functions. Some studies demonstrate that glycans can stabilize protein structure while folding (6, 7). Some also show that glycans has the ability to shield epitope from antibodies neutralization (7, 8). Since the outbreak of the 2009 pandemic flu, glycans that are attached to hemagglutinin have been an interest of research. In this study, various U.S.A strains were compared between different periods of time before and after the outbreak. Moreover, the

glycans on the globular head close to the receptor binding domain (17) were carefully inspected because it might have the ability to interfere binding between human glycans receptors and hemagglutinin. All the sequences were evaluated must satisfied the criteria of being in the H1N1 influenza A family and were from human host in the United States. The only dependent variable for all the sequences was that they were organized into different timeframes. All of the identical sequences were collapsed to make the comparison more precise.

Influenza Virus Resources database was employed for sequences search and sequences alignment. The amino acid residues of the Sa, Sb, Ca, and Cb antigenic sites were determined based on a journal published in Science (9).

Four different time periods were chosen to classify the different in terms of the numbers of glycosylation site before and after the outbreak in April 2009. The first period was from August 2008 to March 2009 which was before the outbreak of 2009 H1N1 in the United States. The second period was summer 2009 after the outbreak from April to Jun. The third period was autumn 2009 from July to 21st September. The date 21st September was picked because on this day, the U.S. government ordered additional 2009 H1N1 vaccine (10). The last period was from 22nd September 2009 to 10th August 2010. On the tenth of August 2010, the World Health Organization announced the end of 2009 pandemic flu (11).

All the crystal structures used in this paper were from Protein Data Bank (www.pdb.org). The A/California/ 04/2009 (H1N1) strains was specially chosen as a template to compare with different strains. Glycosylation sites (Asn-Xaa-Ser/Thr) around the four main

antigenic regions: Sa, Sb, Ca, and Cb were inspected and compared for every strains from the influenza virus resources database before and after the outbreak in April 2009.

One of the software developed by the University of California, San Francisco Chimera was applied to check the location of glycosylation sites (12). Another visualization software Discovery Studios viewer was also utilized to check the glycosylation site location of the sequences obtained using the superimpose function (13).

The purpose of this study is to understand the change in the number of glycosylation site and its correlation with the influenza virus' pathogenicity. Through the use of the influenza database resource, the sequences between different time periods could be compared and analyzed. Between August 2008 and March 2009, a total of 195 strains were available for sequence alignment after collapsing all the identical strains. After the outbreak, three timelines were divided. From April 2009 to June 2009, 285 strains were obtained and from July 2009 to the date when the U.S. government ordered the pandemic vaccine on 21st September 2009, a total of 102 strains were acquired. Later, between 22nd September 2009 and the day when WHO announced the end of 2009 pandemic flu on 10th August 2010, 268 strains were collected from the database. Following careful inspection, the data shows that before the pandemic flu outbreak, there was actually more glycans attached to the hemagglutinin on the globular head compared to after the outbreak.

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