The prediction of Influenza A/H3N2 Vaccine Efficacy using samples obtained from Indonesian Hajj pilgrims in 2013

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Abstrak

Latar Belakang: *Vaksinasi merupakan salah satu cara efektif dalam mengontrol dan mengurangi beban penyakit yang disebabkan oleh Influenza. Akan tetapi, efikasi vaksin bisa bervariasi jika strain yang digunakan untuk vaksin berbeda dengan strain yang bersirkulasi di dunia. Hal ini menunjukan pentingnya melakukan analisa prediksi efikasi vaksin. Pada studi ini, prediksi efikasi vaksin Influenza A/H3N2 dilakukan berdasarkan perhitungan* antigenic distance strain *vaksin WHO dengan virus influenza yang berasal dari jemaah Haji Indonesia pada tahun 2013.*

Metode: *Sekuensing gen HA dilakukan terhadap dua sampel tersimpan yang terkonfirmasi positif Influenza A/H3N2 yang berasal dari jemaah Haji Indonesia tahun 2013.* $P_{epitope}$ *Calculator digunakan untuk menghitung* antigenic distance *dari dua strain virus influenza dan dilanjutkan dengan perhitungan* $P_{epitope}$ *value. Vaksin strain yang direkomendasikan oleh WHO; A/Texas/50/2012, A/Switzerland/9715293/2013, A/HongKong/4801/2014 dan dua virus yang diambil dari jemaah Haji Indonesia pada tahun 2013 dianalisa pada studi ini.*

Hasil: *Prediksi efikasi vaksin yang direkomendasikan WHO tahun 2013 (A/Texas/50/2012) dengan sampel yang berasal dari jemaah Haji Indonesia tahun 2013 menunjukkan hasil lebih rendah dibandingkan dengan strain vaksin untuk musim flu pada tahun selanjutnya. Hasil ini sesuai dengan hasil analisis filogenetik dan perbandingan asam amino dimana sampel pada studi ini berkerabat lebih dekat dengan strain vaksin untuk musim flu selanjutnya dengan perbedaan asam amino yang lebih sedikit di bagian epitope protein HA dibandingkan dengan vaksin tahun 2013.*

Kesimpulan: *Perhitungan efikasi vaksin menggunakan* antigenic distance *antara strain vaksin WHO dan virus yang menginfeksi jemaah haji Indonesia pada tahun 2013 menunjukkan hasil yang rendah.* ***(Health Science Journal of Indonesia 2018;9(1):1-7)***

Keywords: *Efikasi vaksin, Influenza A/H3N2, jemaah Haji, Indonesia*

Abstract

Background: Influenza vaccination is an effective approach to control and reduce the disease burden of influenza viruses. However, the efficacy of influenza vaccine varies every year due to the different antigenic distance between vaccine and the circulating influenza strains globally and therefore necessitates the study of vaccine efficacy (VE). This study describes the prediction of Influenza A/H3N2 VE based on antigenic distances WHO vaccine strains and the virus obtained from Indonesian Hajj pilgrims in 2013.

Methods: Coding between Sequence of HA gene of Influenza A/H3N2 virus was obtained from archival samples of Indonesian Hajj Pilgrims in 2013. $P_{epitope}$ value calculation using $P_{epitope}$ Calculator to measure the antigenic distance of HA sequences of two influenza strains was implemented. The HA sequences of WHO vaccine strains: A/Texas/50/2012, A/Switzerland/9715293/2013, A/HongKong/4801/2014 and two influenza viruses from Indonesian Hajj pilgrims in 2013 were analyzed.

Results: This study predicted that influenza vaccine strain recommended by WHO for 2013 (A/Texas/50/2012) have low efficacy to the influenza virus obtained from Indonesian Hajj Pilgrim in 2013 while showing higher efficacy to vaccine strain recommended for the following year. This result was in line with phylogenetic analysis and amino acid differences in which the samples in this study were grouped together with vaccine strain in following years and had less amino acid differences in epitope located in HA protein compared with 2013 vaccine strain.

Conclusion: The prediction of VE using the antigenic distance measurement between WHO vaccine strain and Indonesian Hajj pilgrim collected in 2013, is considered low. ***(Health Science Journal of Indonesia 2018;9(1):1-7)***

Keywords: Vaccine efficacy, influenza A/H3N2 virus, Hajj pilgrim, Indonesia

The Influenza virus has become a global public health concern as million cases with mild to severe respiratory syndromes and approximately half a million death worldwide were reported annually.[1] The natural characteristics of Influenza A virus with its segmented RNA genome emphasizes the rapid viral evolution. The Influenza virus has a potential being a global threat in the form of Influenza epidemic and pandemic due to the sequential accumulation of genetic and antigenic changes overtime.[2, 3]

Vaccination is believed to be an effective approach to control and reduce the disease burden caused by influenza viruses.[4] However, there are variations on the efficacy of influenza vaccine from year to year due to the different antigenic distance between vaccine and the circulating influenza strains.[5, 6] The selection of component strains for annual influenza vaccine is essential as the recommended viral strains should offer optimal immunity from numerous variants of influenza virus in global circulation. The main focus of genetic and antigenic surveillance is on haemagglutinin (HA) since the antibodies to other protein such as Neuraminidase (NA) were reported not to have neutralizing ability toward Influenza virus infection.[7] Hence, the official recommendation for influenza vaccine formulations should be based on the HA gene of global circulating influenza strains.[4, 8]

The roles of mass gathering events such as Hajj pilgrimage in the transmission of infectious diseases have been described in several publications.[9, 10] Since influenza viruses can spread rapidly, the possibility of new emerging influenza virus strains spreading to countries across the globe is alarming. This likelihood event highlights the need to vaccinate the pilgrims before they leave their own country. Ministry of Health Republic of Indonesia, suggests the administration of influenza vaccine to Indonesian hajj pilgrims. The problem arises when the influenza vaccine strain received by Hajj pilgrims in their country is different to the circulating strain in Kingdom of Saudi Arabia (KSA) during the pilgrim season. In fact, such mismatch often happened as the influenza viruses undergo antigenic changes, causing several times reformulation of vaccine composition[11] and has been described comprehensively in a recent study.[12]

To investigate the mismatch possibility, we conduct a study on specimens obtained from Indonesian Hajj pilgrims in 2013 with influenza-like illness (ILI) symptoms and get hospitalized during or after the Hajj pilgrimage. This study has objective to predict the Vaccine Efficacy (VE) of Influenza A/H3N2 vaccine based on antigenic distances between WHO vaccine strains and virus obtained from Hajj pilgrims in 2013 that had ILI symptoms after they have arrived in Indonesia.

METHODS

Ethical Declaration

This study has been approved by the ethics committee of National Institute of Health Research and Development number LB.02.01/5.2/KE.082/2015.

Population study and sampling strategy

The samples used in this study were archival clinical specimens (nasal swabs or tracheal swabs) of Indonesian Hajj Pilgrims with ILI symptoms in 2013. A total of two samples were confirmed as positive for Influenza A/H3N2 virus; 13015 and 13087, using real-time RT-PCR by Virology laboratory, NIHRD, Jakarta, following the WHO protocols.[13] The samples were suspended in Hank Balance Salt Solution (HBSS) transport medium and stored properly in -80°C.

RNA isolation and RT-PCR

Viral RNA was extracted directly from 140 µL of samples using QIAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Archived clinical samples with a volume less than 140 µL were excluded from the study. Reverse transcription and amplification of complete coding DNA sequence (CDS) of HA gene was conducted using SuperScript™ III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA) as described previously.[14]

Direct sequencing

PCR products which purified using QIAquick™ PCR Purification Kit (QIAGEN, Hilden Germany) then sequenced using Big Dye Terminator V.3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA), as described by the manufacturer. After purification using Big Dye X Terminator Purification Kit (Applied Biosystems, Foster City, CA), the reactions were resolved in an automatic sequencer ABI-Prism 3130xl Genetic Analyzer (Applied Biosystems Foster City, CA). The nucleotide sequences were edited and assembled using Sequencer version 5.2.4.

Antigenic Distance

The vaccine strain and the circulating strain's antigenic distances are the input for the vaccine efficacy prediction. The specific measure of antigenic distance between two influenza strains was calculated as $P_{epitope}$ value by the method described in previous studies.[6, 15] $P_{epitope}$ value was defined as the fractional change of mutated amino acid between the epitope of one strain compared to the vaccine strain, as shown in equation below.[6, 16]

$$P\ epitope\ value = \frac{\text{(Number of amino acid differences in the epitope)}}{\text{(Total number of amino acid in the epitope)}}$$

It is assumed that an antigenic epitope which has the greatest percentage of mutations is the dominant epitope, because it is influenced by the greatest selective pressure from the immune system. The HA1 domain of H3 virus contains five non-overlapping epitopes, namely epitopes A (amino acid position 140-146), B (amino acid position 155-160, 188-198), C (amino acid position 276-281), D (amino acid position 204-220) and E (amino acid position 170-174, 259-265).[17] The dominant epitope is defined as the epitope with the greatest $P_{epitope}$ value.

$P_{epitope}$ Calculator, a Microsoft Excel-based file, was applied for H3 sequence and used to calculate $P_{epitope}$ value between WHO vaccine and Indonesian viruses.[6, 16] This study used vaccine strains obtained from GISAID: A/Texas/50/2012 (accession number EPI377499), A/Switzerland/9715293/2013 (accession number EPI-530687), A/HongKong/ 4801/2014 (accession number EPI539576), the recommended vaccine in 2013 to 2016, respectively.

Based on the $P_{epitope}$ Calculator output, the dominant epitope with the greatest $P_{epitope}$ value and the amino acid discrepancies between two influenza viruses were obtained to analyze the antigenic distance.

Vaccine Efficacy (VE)

The antigenic distance between vaccine and the circulating strains correlates well with the influenza vaccine efficacy. Thus, the vaccine efficacy can be predicted by calculating the antigenic distance (as $P_{epitope}$ value).[5, 18] The quantitative definition of the correlation of $P_{epitope}$ and VE is given by $E = -2.47 \times P_{epitope} + 0.47$. This equation predicts the vaccine effectiveness of 47% when $P_{epitope} = 0$ for the H3N2 virus.[6, 19]

Phylogenetic analysis

Phylogenetic tree of HA gene was generated using Neighbor Joining in MEGA 6.0 with 1000 replicates. WHO vaccine strains A/Texas/50/2012, A/Switzerland/9715293/2013, A/HongKong/4801/2014, and H3N2 sequences originated from Northern and Southern Hemispheres were included in the analysis. These sequences were obtained from GenBank. Influenza virus activity had different pattern in each part of the world. In Northern Hemisphere, patients with influenza were reported during winter season in December into February, while in Southern Hemisphere the peak of influenza activity is during winter season in June to August. Therefore it is necessary to include influenza virus collected from countries located in Northern and Southern Hemispheres in phylogenetic analysis.

RESULTS

The antigenic distance followed by the prediction of Influenza A/H3N2 vaccine efficacy by $P_{epitope}$ value analysis between concurrent WHO vaccine strains and Indonesian viruses obtained from Hajj pilgrims in 2013, is reported in this study.

As described in Table 1, sample 13015 had the highest VE against A/Switzerland/9715293/2013 while the 13087 had the highest VE against A/HongKong/4801/2014. Although both of the Indonesian H3N2 viruses were collected in 2013, the analysis using $P_{epitope}$ value indicates that both samples had the higher VE against WHO recommended vaccine strains for 2015-2016 (A/Switzerland/9715293/2013) and 2016 (A/HongKong/4801/2014) seasons, respectively.

Table 1. Vaccine Efficacy Prediction of Influenza Vaccine against Indonesian Influenza A/H3N2 virus obtained from Hajj Pilgrims

Vaccine strain	Against 13015	Against 13087
2013-2015 influenza season: A/Texas/50/2012		
- Dominant epitope	B	B
- $P_{epitope}$ value	0,142857143	0,142857143
- VE (%)	0.117 (11.7)	0,117 (11.7)
2015-2016 influenza season: A/Switzerland/9715293/2013		
- Dominant epitope	A	A
- $P_{epitope}$ value	0,105263158	0,157894737
- VE (%)	0.211 (21.1)	0.08 (8.0)
2016 influenza season: A/HongKong/4801/2014		
- Dominant epitope	B	B
- $P_{epitope}$ value	0,142857143	0,095238095
- VE (%)	0.117(11.7)	0.235 (23.5)

Analysis by comparing the amino acids of the five epitopes (epitopes A to E) between WHO vaccine strains and virus from Indonesian Hajj pilgrims was performed to investigate the mutations occurred in these five epitopes (Table 2). In line with the findings described in Table 1, the dominant epitope in the analysis was epitope A and B, suggesting the amino acids discrepancies between WHO vaccine and influenza virus from Indonesian Hajj pilgrims were located in epitope A and B of HA gene.

To support these finding, we carried out phylogenetic analysis of Indonesian strains together with WHO vaccine strains and other influenza A/H3N2 viruses from Northern and Southern Hemispheres. The phylogenetic tree of HA gene illustrated that the Indonesian Hajj sequences obtained in 2013 were not clustered with A/Texas/50/2012 but with the vaccine strains for the next seasons (Figure 1). Sample 13015 was grouped together with A/Switzerland/9715293/2013 and sample 13087 was clustered with A/Hong Kong/4801/2014. The sample 13015 had the highest VE against A/Switzerland/9715293/2013 (21.1%) while the 13087 had the highest VE against A/HongKong/4801/2014 (23.5%).

Table 2. Amino Acid Discrepancies in Epitope A to E between WHO Vaccine and Indonesian Influenza A/H3N2 viruses

Vaccine strain	Sample 13015					Sample 13087				
	Epitope A	**Epitope B**	**Epitope C**	**Epitope D**	**Epitope E**	**Epitope A**	**Epitope B**	**Epitope C**	**Epitope D**	**Epitope E**
A/Texas/50/2012	R142G, N145S	N128A, V186G, P198S	-	-	-	N145S	N128T, V186G, P198S	-	-	-
A/Switzerland/9715293/2013	S138A, R140I	S159F, V186G	-	-	-	S138A, R140I, G142R	A128T, S159F, V186G	-	-	-
A/HongKong/4801/2014	R142G, S144N	T128A, Y159F, P194L	-	S96N	-	S144N	Y159F, P194L	-	S96N	-

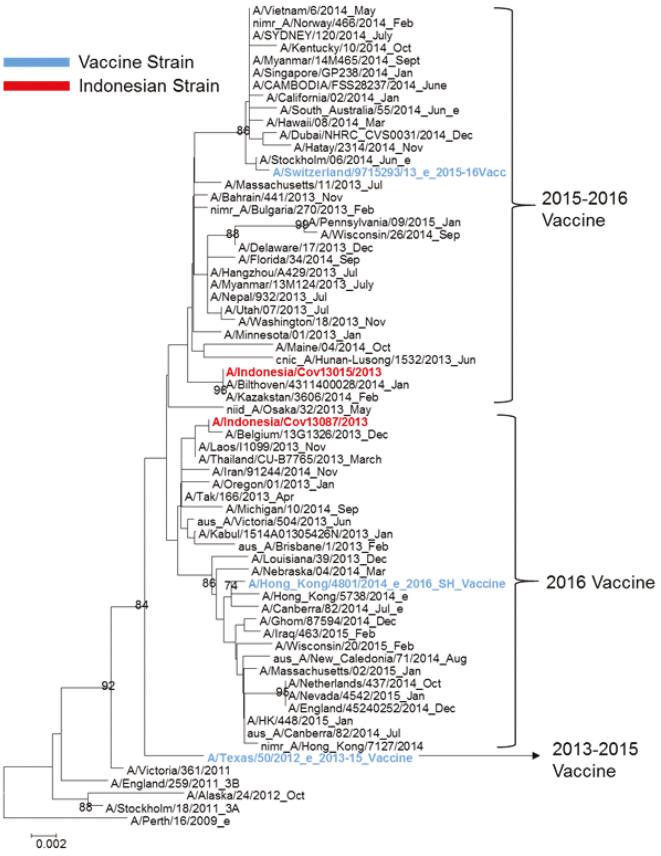

Figure 1. Phylogenetic tree of HA gene of H3N2 virus. Indonesian sequences were in red while the WHO vaccine strains were in blue.

DISCUSSIONS

The instability of influenza virus genome that causes sequential accumulation of genetic and antigenic changes in the surface glycoproteins allows influenza virus to evade the neutralizing antibodies.[20, 21] WHO regularly monitors influenza circulating strains globally to determine the update genetic and antigenic changes within these viruses that may lead to Influenza vaccine reformulation.

Hemagglutinin Inhibition (HI) assay was commonly used as epidemiological surveys and widely used for estimating the efficacy of influenza vaccine as this method only requires patient's serum for the assay.[22] However, this assay is laborious and needs more time to perform. Moreover, the antibody measured by HI assay could not be concluded as a result of previous exposure to disease or vaccination, but only correlates with the level of protection.[23] Study using different vaccine types also gives different results of HI titers.[24]

The estimation of the antigenic distance between two influenza strains followed by the calculation of $P_{epitope}$ value is a novel approach to predict the VE. The VE estimation by $P_{epitope}$ model could be utilized to predict the antigenic distance based on the HA sequence of the virus and vaccine efficacy[6]. This method can also be an effective tool to define the closest vaccine strain against potential circulating viruses. $P_{epitope}$ value calculation on the antigenic distance between two Influenza strains may be used to predict the VE of influenza vaccine strains in Indonesia.

Our data shows that based on the prediction using $P_{epitope}$ value, WHO recommended vaccine strain for 2013 season, A/Texas/2012, has low VE against our samples (Indonesian Hajj pilgrims virus strains collected in 2013). The result suggests that the viruses in this study had closest amino acid similarity to WHO vaccine strain for the next year. This finding is parallel with the previous report from Indonesian ILI surveillance that detected antigenically drifted viruses similar to the WHO vaccine strains earlier than the date of their designation by WHO.[25]

As the natural hallmark of influenza virus, continuous and accumulation of mutations within its genome lead to antigenic drift and generate different influenza strains from the previous circulating strains. If the mutations located within the epitope of the surface glycoprotein of the virus, it will cause vaccine mismatching due to low VE and therefore necessitates yearly vaccine strain update.[4, 11, 26]

The vaccine VE results in this study is limited only for the estimation. Experimental studies with real VE data using patient sera are still needed to confirm these results. General conclusion could not be drawn with only limited samples since as this study only used two samples. Furthermore, in depth genetic and antigenic study of Influenza A/H3N2 virus obtained from a larger number of patients could give more information regarding the evolution of this virus and the vaccine efficacy.

Influenza A/H3N2 virus has five antigenic sites in the HA1 domain, namely epitope A to E. Of the five epitopes in HA, the epitope with highest $P_{epitope}$ value has the biggest antigenic distance and, therefore, determined as a dominant epitope.[6] The epidemiology of the important drift-variants usually display four or more amino acid substitutions which located at two or more antigenic sites of the HA1 domain of HA. [27] In this study, the dominant epitopes were epitope A and B (Table 2). The similar mutations within these epitopes were also reported in the comparison of Influenza A/H3N2 virus from Thailand and A/Switzerland/9715293/2013.[28]

The important antigenic sites that had major significance located within antigenic site A and B. Mutations in this location may act as a key substitution that causing the antigenic drift. The antigenic drift of the circulating strains in the 2014–2015 influenza season in the northern hemisphere has been reported to decrease the vaccine effectiveness.[29] The amino acid substitution at the position 128 may relate to the loss of an N-linked glycosylation site.[30] The amino acid differences within the antigenic site A position 140 to 146 in HA are the characteristics of antigenically distinct viruses of epidemic significance.[30] The substitutions in more than four of the antibody binding sites have been predicted to give an antigenically different virus.[27]

Phylogenetic tree analysis supported VE analysis. The Indonesian Influenza A/H3N2 virus was different with the current vaccine strains but similar with the next season recommended vaccine strains. The two vaccine strains, A/Switzerland/9715293/2013 and A/HongKong/4801/2014 belonged into different clades and Indonesian samples grouped into different clades although collected in the same years. This phenomenon may suggest the diversity of the Influenza A/H3N2 virus as well as the global circulation of this virus.

The Hajj pilgrimage is where the Muslim all around the world gather in KSA and this event enhances

the possibility of the transmission of the respiratory infection including influenza among pilgrims.[9, 10] The vaccination of Hajj pilgrims is still highly recommended. Further study of influenza evolution and transmission among Hajj Pilgrims, including Indonesian Hajj Pilgrims over period of time is needed for directing public health measures.

In conclusion, based on the prediction using the antigenic distance measurement against WHO vaccine strains and Indonesian Hajj pilgrim collected in 2013, the VE in this study is considered low. This recent study have limitations as the analysis was performed in the small number of samples and the estimation based on the modeling calculation. Further invitro studies to determine the biological function of the recent analysis are needed.

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