Characterization of Hemagglutinin Protein Structure of H5N1 Virus Circulating in Indonesia based on Proteomic Analysis

M. Irfan Hadi[1], Hanik Faizah[1], Misbakhul Munir[1], M Yusuf Alamudi[2], Risa Purnamasari[1] and Nova Lusiana[1]

[1]*UIN Sunan Ampel Surabaya, Indonesia*

[2] *AI Lab-Yayasan Prof Nidom, Surabaya, Indonesia*

Keywords: characterization, hemagglutinin, H5N1, Indonesia, proteomics

Abstract: Bird flu virus or H5N1 is one of the infectious agents that still become a global problem. Based on data from the World Health Organization (WHO) until June 15, 2017, more than 800 people were infected with the H5N1 virus, and nearly 500 people died. Indonesia is the 2nd country in the world with the highest number of infected bird flu virus that is 199 people infected and 167 people died, with more than 70% case fatality rate. Influenza A especially H5N1 has eight genes, one of them is Hemagglutinin. Hemagglutinin (HA) protein present on the surface of the virus. The presence of a "cleavage site" in HA protein will improve the pathogenic nature of the AI virus. HA protein also plays a role in the process of viral infection into cells by interacting directly with receptors on the surface of the host cell. Besides, HA protein also functions in the movement of the virus from one cell to another cell. Through the accumulation of mutations in HA, the AI virus can increase its infectious potency. The purpose of this study was to characterize the Hemagglutinin of bird flu virus in Indonesia by using bioinformatics method. The study discovered the differences in the characterization of bird flu virus circulating in Indonesia based on the proteomic analysis.

1 INTRODUCTION

Influenza is a strand-negative RNA virus belonging to the Orthomyxoviridae family and has four types of influenza viruses, i.e., Influenza A, influenza B, influenza C, and thogoto virus. Influenza A can infect hosts in a wide range including birds, poultry, and mammals, with geographic coverage around the world (Luke and Subbarao, 2006). According to WHO (2017), the cumulative data of avian influenza subtype H5N1 in human cases was 859 cases with 453 deaths overall occurred in 16 countries, while in Indonesia there were 199 cases with 167 deaths.

Hemagglutinin (HA) is a glycoprotein having a molecular weight of 76,000 kDa and a rod-shaped molecule located in the layer of influenza virus. HA protein plays an essential role in determining the pathogenicity of influenza viruses, and this segment often has spontaneous mutations that can lead to a new pandemic and endemic influenza (Li et al., 2004). Hemagglutinin consists of 5 antigenic sites ranging from sites A, B, C, D, and E. The primary function of these sites is as a receptor binding to sialic acid from the target cell of influenza virus infection, in an attempt to initiate the fusion process of virus particle through the cell membrane (Bruce et al., 2010; Lutz et al., 2005). The hemagglutinin consists of two subunits: HA1 and HA2 bound by a disulfide bridge. HA from the avian, horse and pig influenza viruses have specificity to α (2,3) -linkage sialic acid receptors, whereas HA from human influenza virus is specific to α (2,6) -linkage sialic acid receptors. The receptor of α (2, 3) -linkage sialic acid is found in the mucosal tracts of avian, horse and certain marine mammals, whereas α (2, 6) -linkage sialic acid receptor is found in the human respiratory tract mucosa. Specifically, in mucosal cells of the pig trachea can be found both types of receptors, so pigs are the only animals that can be attacked either by human influenza virus or non-human influenza virus (Brooks et al., 2010).

HA protein tends to change as a result of mutations in gene encoding protein synthesis, whereas HA protein is a major determinant of the human immune system to recognize influenza

Hadi, M., Faizah, H., Alamudi, M., Munir, M., Purnamasari, R. and Lusiana, N.
Characterization of Hemagglutinin Protein Structure of H5N1 Virus Circulating in Indonesia based on Proteomic Analysis.
DOI: 10.5220/0008903700002481
In *Proceedings of the Built Environment, Science and Technology International Conference (BEST ICON 2018)*, pages 95-98
ISBN: 978-989-758-414-5

antigens and produce specific antibodies against influenza virus infection. As a result of changes in HA, immune cells will not be able to recognize influenza viruses that infect humans (Brooks et al., 2010; Bruce et al., 2010; Lutz et al., 2005).

Proteolytic activation of hemagglutinin proteins is an important factor for the infectivity and spread of the virus throughout the body. Differences in HA VAI protein sensitivity to host proteases will be associated with the virulence levels (Puthavathana et al., 2005; Shangguan et al., 1998). In addition to its role in antigenic properties and viral pathogenicity level, hemagglutinin proteins also play a role in the specificity of VAI hosts. One of the factors that play a role in this VAI infection is the compatibility between the virus with the receptor on the surface of the host cell. (Harvey et al., 2004). The study aimed to characterize bird flu virus circulating in Indonesia in 2008-2012.

2 METHOD

Hemagglutinin protein of H5N1 virus in 2008-2012 was collected from NCBI GenBank with acecesion number of AKC43930, BAL61222, AJP13841, AGC96167. The data of Hemagglutinin amino acids from GenBank were analyzed using SwissProt software and visualized using Pymol Software.

3 RESULTS AND DISCUSSION

This study was conducted to characterize the Hemagglutinin protein of bird flu virus circulating in Indonesia during 2008 to 2012. It was due to the bird flu virus experienced a change from subclade 2.1.3 to 2.3.2 in those years. Based on the study conducted on subclasses 2.1.3 and 2.3.2 from 2008 to 2012, the Hemagglutinin of avian influenza virus had the form of Homo-Trimer. Similar result was reported by Stevens et al. (2006) and Zuo et al. (2015). Hemagglutinin of influenza virus belongs to transmembrane glycoprotein type 1 and is located on the surface of the virus as a homotrimer. Trimerisation was possible due to proteolytic cleavage unfolding in HA0, as a precursor when the folding process is in the monomer form. Hemagglutinin has two chains: HA1 and HA2, each monomer consists of a globular head (part of HA1) and stem region (part of HA2) (Staneková and Varečková, 2010; Velkov et al., 2013). HA belongs to transmembrane glycoprotein type 1, the sequence of signals that can be removed after translation. HA also belongs to the membrane anchor domain near the C terminal, and has a short cytoplasmic terminal (Steinhauer, 1999), has a size of 13.5 nm and a molecular weight of 76 kDa (Cheng et al., 2012a). Hemagglutinin is a target molecule to neutralize antibodies and is therefore considered as a primary surface antigen (Ducatez et al., 2010). The primary function of HA is initiation in infecting the host, involving in the introduction of host cells and the binding of the virus to host cell receptors, composed of sialic acid (Cheng et al., 2012b; Edinger et al., 2014).

The study was also obtained the number and type of ligand in hemagglutinin on bird flu virus circulating in Indonesia in 2008-2012 (table 1). There were differences in the number and nature of ligands in both subclasses 2.1.3 and 2.3.2. The study had a similar result with the study conducted by Xu and Wilson (2011). The ligand of GAL, NAG, and SIA was associated with RBS (receptor binding site) (Lazniewski et al., 2017). The study was also obtained homologous protein from H5N1 (table 2). Of H5N1 protein suspected H5N1 virus had the possibility of part of Influenza virus more than one subtype. It can be found about the possibility of the origin of influenza virus especially H5N1 in Indonesia although it requires a further study, especially in the meta bioinformatics field.

Table 1: Number and Type of Ligand in Hemagglutinin

Subclade and year of isolat	# of Ligand	Type of Ligand	Nature of Ligand
2.3.2 (2012)	2	GAL (SUGAR (2-MER) NAG (SUGAR (N ACETYL-D-GLUCOSAMINE)	Binding site not conserved Not biologically relevant
2.1.3 (2012)	1	NAG (SUGAR (N-ACETYL-D GLUCOSAMINE)	Binding site not conserved
2.1.3 (2010)	3	NAG (SUGAR (N-ACETYL-D GLUCOSAMINE) NAG (SUGAR (2-MER) SIA (SUGAR (2-MER)	Clashing with protein Binding site not conserved Not biologically relevant Binding site not conserved
2.1.3 (2008)	2	NAG (SUGAR (N-ACETYL-D GLUCOSAMINE) NAG (SUGAR (2-MER)	Clashing with protein Binding site not conserved Not biologically relevant Binding site not conserved

Table 2 : Homolog Protein

Subclade dan Year of isolation	Homolog Protein
2.3.2 (2012)	• H3 HAEMAGGLUTININ HA1 CHAIN • Influenza B hemagglutinin (HA) • PROTEIN (INFLUENZA RECOMBINANT HA2 CHAIN) • HEMAGGLUTININ FUSION PEPTIDE G8A MUTANT • uncharacterized protein • Influenza H5 HA head domain VietNam rdt mutations • HEMAGGLUTININ FUSION PEPTIDE G8A MUTANT
2.1.3 (2012)	• Influenza B hemagglutinin (HA) • Influenza H5 HA head domain VietNam rdt mutations • HEMAGGLUTININ FUSION PEPTIDE G8A MUTANT
2.1.3 (2010)	• H3 HAEMAGGLUTININ HA1 CHAIN • Influenza B hemagglutinin (HA) • Influenza H5 HA head domain VietNam rdt mutations • PROTEIN (INFLUENZA RECOMBINANT HA2 CHAIN) • Gp7-MYH7(1173-1238)-EB1 chimera protein • uncharacterized protein
2.1.3 (2008)	• Influenza B hemagglutinin (HA) • Influenza H5 HA head domain VietNam rdt mutations • H3 HAEMAGGLUTININ HA2 CHAIN • PROTEIN (INFLUENZA RECOMBINANT HA2 CHAIN) • uncharacterized protein

4. CONCLUSIONS

The study demonstrated that H5N1 virus circulating in Indonesia had a form of homotrimer based on the bioinformatic analysis. Besides, H5N1 virus circulating in Indonesia from 2008 to 2012 had the number of ligands and homology based on protein structure.

REFERENCES

Brooks, W.A., Goswami, D., Rahman, M., Nahar, K., Fry, A.M., Balish, A., Iftekharuddin, N., Azim, T., Xu, X., Klimov, A., 2010. Influenza is a major contributor to childhood pneumonia in a tropical developing country. Pediatr. Infect. Dis. J. 29, 216–221.

Bruce, E.A., Digard, P., Stuart, A.D., 2010. The Rab11 pathway is required for influenza A virus budding and filament formation. J. Virol. 84, 5848–5859.

Cheng, X., Zengel, J.R., Xu, Q., Jin, H., 2012a. Surface glycoproteins of influenza A H3N2 virus modulate virus replication in the respiratory tract of ferrets. Virology 432, 91–98.

Cheng, X., Zengel, J.R., Xu, Q., Jin, H., 2012b. Surface glycoproteins of influenza A H3N2 virus modulate virus replication in the respiratory tract of ferrets. Virology 432, 91–98.

Ducatez, M.F., Bahl, J., Griffin, Y., Stigger-Rosser, E., Franks, J., Barman, S., Vijaykrishna, D., Webb, A., Guan, Y., Webster, R.G., 2010. Feasibility of reconstructed ancestral H5N1 influenza viruses for cross-clade protective vaccine development. Proc. Natl. Acad. Sci. 201012457.

Edinger, T.O., Pohl, M.O., Stertz, S., 2014. Entry of influenza A virus: host factors and antiviral targets. J. Gen. Virol. 95, 263–277.

Harvey, R., Martin, A.C., Zambon, M., Barclay, W.S., 2004. Restrictions to the adaptation of influenza a virus h5 hemagglutinin to the human host. J. Virol. 78, 502–507.

Lazniewski, M., Dawson, W.K., Szczepińska, T., Plewczynski, D., 2017. The structural variability of the influenza A hemagglutinin receptor-binding site. Brief. Funct. Genomics.

Li, K., Guan, Y., Wang, J., Smith, G., Xu, K., Duan, L., Rahardjo, A., Puthavathana, P., Buranathai, C., Nguyen, T., 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 430, 209.

Luke, C.J., Subbarao, K., 2006. Vaccines for pandemic influenza. Emerg. Infect. Dis. 12, 66.

Lutz, A., Dyall, J., Olivo, P.D., Pekosz, A., 2005. Virus-inducible reporter genes as a tool for detecting and quantifying influenza A virus replication. J. Virol. Methods 126, 13–20.

Puthavathana, P., Auewarakul, P., Charoenying, P.C., Sangsiriwut, K., Pooruk, P., Boonnak, K., Khanyok, R., Thawachsupa, P., Kijphati, R., Sawanpanyalert, P., 2005. Molecular characterization of the complete genome of human influenza H5N1 virus isolates from Thailand. J. Gen. Virol. 86, 423–433.

Shangguan, T., Siegel, D.P., Lear, J.D., Axelsen, P.H., Alford, D., Bentz, J., 1998. Morphological changes and fusogenic activity of influenza virus hemagglutinin. Biophys. J. 74, 54–62.

Staneková, Z., Varečková, E., 2010. Conserved epitopes of influenza A virus inducing protective immunity and their prospects for universal vaccine development. Virol. J. 7, 351.

Steinhauer, D.A., 1999. Role of hemagglutinin cleavage for the pathogenicity of influenza virus. Virology 258, 1–20.

Stevens, J., James, O.B., Terrence, M.T., Jeffery, K.T., James, C.P., Lan, A.W., 2006. Structure and Receptor Specificity of the Hemagglutinin from an H5N1 Influenza Virus. Science 312, 404–410.

Velkov, T., Ong, C., Baker, M.A., Kim, H., Li, J., Nation, R.L., Huang, J.X., Cooper, M.A., Rockman, S., 2013. The antigenic architecture of the hemagglutinin of influenza H5N1 viruses. Mol. Immunol. 56, 705–719.

WHO, 2017. Cumulative number of confirmed human cases for avian influenza A (H5N1) reported to WHO, 2003-2017. WHO.

Zuo, T., Sun, J., Wang, G., Jiang, L., Zuo, Y., Li, D., Shi, X., Liu, X., Fan, S., Ren, H., Hu, H., 2015. Comprehensive analysis of antibody recognition in convalescent humans from highly pathogenic avian influenza H5N1 infection. , 6, p.8855. Nat. Commun. 6, 8855.