Review Article

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SCIENTIFIC RATIONALE FOR HEMAGGLUTININ (HA) BASED DIAGNOSTIC AND VACCINE DEVELOPMENT FOR INFLUENZA VIRUSES

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ABSTRACT Flu outbreaks have been identified as a significant cause of illness and mortality over the previous ten years. Humans, pigs, and birds can all contract influenza viruses with H1 HA, and millions of people died from the Spanish Flu pandemic in 1918. Nearly 18000 people died from an influenza outbreak in 2009, and from 2016 to 2021, India reported 87,786 illnesses and 4,931 deaths. A trimetric surface glycoprotein called influenza hemagglutinin (HA) aids in the entrance of the virus into host cells. The monosaccharide sialic acid and HA have a strong affinity for one another, which facilitates the attachment of the virus to the surface of the host cell. Through endocytosis, HA controls viral entrance into the host cell as well. Under pH 6.0, lysozome-mediated unfolding of HA in the endosome releases hydrophobic antigenic peptide chains. Inside the endosome, these fusion peptides undergo a second folding and release into the cytoplasm of the cell. HA proteins are linked to a number of additional tasks, including infectivity. The antigenic sites of HA are crucial in triggering an immune response during host-pathogen contact. The HA amino acid sequence of influenza viruses evolved to have a higher level of genomic plasticity during molecular evolution. Hemagglutinin is a significant protein targeted for vaccine manufacturing and is always under positive selection pressure to prevent host immunity and vaccination. It is found on the surface of the virus and is used in molecular, immunological, and serological methods to diagnose influenza. Here, we go through the virus's structure and point of entry, its use as a potential recombinant vaccine, its function in diagnosis, its evolution, and its influence on hemagglutinin's antigenic characteristics.

KEYWORDS : Hemagglutinin, Influenza, antigenic variation, Host pathogen interaction, antigenicity, vaccine and diagnostics.

Introduction

The Orthomyxoviridae family of the Monengivirales order contains influenza A viruses. They have eight negative-sense RNA segments that code for 12 proteins, including the surface proteins HA (Hemagglutinin), M (Matrix), and NA (Neuraminidase), as well as the polymerases PB2 (Basic Polymerase-2), PA (Acid Polymerase), NS1 (Nonstructural Protein-1), and NS2 (Nonstructural Protein-2). A chronic infection with influenza, a common respiratory disease that affects the upper respiratory system, may cause acute respiratory distress. Only strains A and B of the four influenza viruses (A, B, C, and D) can infect people and lead to ARDS [2]. Due to point mutations in the genome, particularly in the HA and NA genes (antigenic drift) and reassortment of gene segments from intra- and inter-species influenza viruses (antigenic shift), influenza viruses have the capacity to undergo rapid and consistent genetic and antigenic evolution [3]. The viruses are divided into 18 hemagglutin (HA) (H1-H18) and 11 neuraminidase (NA) (N1-N11) subtypes based on the variation of their surface glycoproteins [4]. In the past, the influenza virus has been linked to pandemics and epidemics (5). Three global (pandemic) influenza outbreaks took place in the 20th century: in 1918, 1957, and 1968. All three have been widely acknowledged as having, respectively, Spanish, Asian, and Hong Kong influenza as their presumed origins. H1N1, H2N2, and H3N2 are now recognised as three distinct influenza A virus antigenic subtypes [6]. According to estimates, there were 50 million fatalities from influenza pandemics in 1918–1919, 2-3 million during the Asian influenza pandemic of 1957, and 700,000 during the 1968 epidemic [7]. Recent studies have found that influenza causes 99, 000–200, 000 fatalities [8]. Recent studies suggest that, as opposed to the seasonal influenza average of 1.3, the reproduction number (the number of new cases connected to a single established case) during the early mild wave of the 1918-1919 pandemic may have ranged between 2 and 5. The 2009 pandemic's anticipated transmissibility of infection (R0) ranges from 1.4 to 1.6, higher than seasonal flu but lower than the previous three pandemics [7].

The most critical influenza surface protein, hemagglutinin, is crucial to the virus' ability to enter its host. Because HA is a significant protein that the human immune system first targets, its great variability is taken advantage of in the creation of vaccines and diagnostic tools [9]. Around the world, eight genogroups have emerged [10]. The amino acids D187 and D222 for human 2,6 receptors, D187 and G222 for pig 2,6 and 2,3 receptors, and E187 and G222 for avian 2,3 receptors establish the receptor-binding specificity of HA1. The 1918 virus's infectivity and binding affinity for 2,6 receptors were both decreased by a single mutation (D222G), but a double mutation (D187E/D222G) made the HA non-binding to 2,6 receptors and the virus non-infectious [11]. Because it enables the fusion of the viral and cell membranes, cleavage of the HA gene is necessary for viral infectivity. N-glycosylation happens when glycans bind to asparagine (N) residues.

The influenza A (H1N1) viruses have the glycosylation sites N71, N104, N142 (variant 144), N177 (variant 172 and 179), and N286 (H1 numbering). Only one N104 glycosylation site was present in the A/SC/1918 and CA/07/2009 isolates, whereas H1N1 viruses acquired the N286 site after 1918 as well as the N142 and N177 sites before 1957. The other site, N71, made its debut in 1987. Seasonal influenza A (H1N1) viruses had four glycosylation sites before the 2009 pandemic virus, having lost the 286 site [11]. The influenza virus has a significant degree of variability, which alters its behaviour. Therefore, it is crucial to research them in order to prevent pandemics and epidemics in the future. So, in this review, we covered the structure, antigenic locations, evolution, viral entry, vaccine development, and HA protein-based diagnostics.

Structure and entry of virion by Hemagglutinin protein

The structure of hemagglutinin (HA) was initially described in 1981

[12]. The hemagglutinin protein is present as a HA0 trimer that is broken down by proteases into HA1 and HA2. It is a protein that has undergone both acylation and glycosylation. There are four structural components that make up the RBS of influenza A HA. These components are known as the 130-loop, 150-loop, 190-helix, and 220-loop based on where they are found in the core amino acid sequence. Between influenza A and B HAs, Trp153, His183, Leu194, and Tyr195 (H3 numbering; Trp158, His191, Leu201, and Tyr202 in influenza B numbering) are substantially conserved. The influenza A virus also has a highly conserved Tyr at residue 98 [9, 12]. Uncleaved HA0 must first be protease-cleaved into a fusion-competent HA1/HA2 complex since it is unable to initiate membrane fusion [13]. Trypsin-like proteases or other soluble proteases break the extracellular human influenza HA0 proteins into HA1 and HA2. A stalk-like structure with N- and C-terminal domains, HA1 is membrane-proximal. A receptor binding membrane distal domain is present in HA2. The influenza virus connects to sialic acid receptors found on the host membrane via HA1 after cleaving HA0. In contrast to avian influenza, which only binds to alpha 2-3 linked sialic acid receptors, human influenza hemagglutinin protein preferentially interacts to alpha 2-6 linked sialic acid receptors. The hemagglutinin of the swine influenza virus (SIV) binds to both the alpha 2-3 and 2-6 linked sialic acid receptors. The straight presentation of alpha 2-3 sialic acid and the bending presentation of alpha 2-6 sialic acid are used to represent them, respectively [14]. For influenza viruses to enter the host cell, the hemagglutinin protein is crucial. The virion is endocytosed by the host cell when HA binds to its receptor. Endocytosis can be mediated by clathrin or by micropinocytosis [15]. The M2 ion channel is opened by the endosome's low pH (4.6–5.0), which also modifies HA's shape and makes the fusion peptide visible. Virion particles become acidic as a result of the M2 ion channel opening, releasing Vrnp from M1 into the host's cytoplasm. The viral endosomal membrane is fused as a result of host proteases cleaving HA0 into HA1 and HA2. Highly pathogenic avian influenza (HPAI) viruses have polybasic HA0 cleavage sites that have an R-X-R/K-R furin recognition sequence that is recognised intracellularly in the trans-Golgi network. The extracellular HA0 proteins of human and low-pathogenic avian influenza (LPAI) viruses are divided by trypsin-like proteases or other soluble proteases [13, 16].

Hemagglutinin Protein as a Vaccine candidate

An important method for preventing influenza infection is vaccination. Although egg-based vaccinations are frequently used to prevent influenza, this strategy has significant drawbacks. It takes a long time to produce egg-based vaccinations, which is insufficient during an outbreak. Additionally, recipients of vaccinations made with eggs develop allergies [17]. Therefore, other approaches are being looked for in order to reduce production time, costs, and vaccine effectiveness. One important surface protein used in the creation of vaccines is hemagglutinin. To express the HA protein for the creation of vaccines, various methods are employed. The prokaryotic expression system is the one that is most frequently used. Prokaryotic expressed proteins have correct protein folding and cause the generation of antibodies that are neutralising. They are produced quickly and efficiently. The oligomerization of HA is crucial for protecting against live viruses. Kuenstling (2018) reported that the influenza A virus subtype H1N1's HA hemagglutinin (HA) segments (rHA11-326 and rHA153-269) were expressed in Escherichia coli. By using immunoblot, gel filtration, hemagglutination, and competitive binding tests, they examined expressed hemagglutinin fragments. They discovered that rHA11-326 only contains the head of HA with the neutralising epitopes, but rHA153-269 contains both the neutralising epitopes and the trimerization domain. When mice with rHA11-326 or rHA153-269 were challenged with H1N1, the results of a serological research showed that rHA11-326 trimerized but rHA153-269 was unable to form oligomers [18].

Table 1.Current status of HA based vaccine against Influenza virus (19)

S.No	Clinical trials Reg No	Characteristics	Status
1.	NCT01498718	VRC-FLUDNA061-00-VP is composed of 3 closed-circular DNA plasmids that encode for the hemagglutinin (HA) from the following 3 strains: A/California/04/2009 (H1N1); A/Perth/16/2009 (H3N2), and B/Brisbane/60/2008. DNA vaccine vials were being supplied at 4 mg/mL and each dose was 1 mL.	Completed
2.	NCT03460743	Biological: Study group - quadrivalent recombinant hemagglutinin influenza vaccine	Completed
3.	NCT00478816	Two 0.5 mL doses of MF59-adjuvanted A/Vietnam/1194/2004 (H5N1 Clade 1) hemagglutinin (HA) subvirion influenza vaccine, containing 7.5 µg of H5N1 antigen	Completed
4.	NCT01658800	VAX161C vaccine Recombinant H5 influenza vaccine, VAX161C	Completed
5.	NCT01676402	Biological: Seasonal Influenza DNA vaccine	Completed
6.	NCT03598439	Influenza vaccine that uses a hemagglutinin protein manufactured in insect cells with a baculovirus vector Other Name: FluBlok Quadrivalent	Active, not recruiting
7.	NCT01412281	Biological: Virosomal influenza vaccine (AdImmune HA Antigen)Biological: Virosomal influenza vaccine (CSL HA Antigen)	Completed
8.	NCT01229371	Biological: Inflexal V influenza vaccine (CSL HA Antigen) 2010Biological: Inflexal V influenza vaccine (AdImmune HA antigen) 2010/2011	Completed
9.	NCT01229371	Biological: Inflexal V influenza vaccine (CSL HA Antigen) 2010 Inflexal V influenza vaccine (surface antigen, inactivated, virosome, using CSL HA Antigen) 2010/2011, with intramuscular administration, containing per 0.5 mL dose: 15 µg HA antigen of A/California/7/2009 (H1N1)-like virus; 15 µg HA antigen of A/Perth/16/2009 (H3N2)-like virus; HA antigen of B/Brisbane/60/2008-like virus	Completed
10.	NCT02555618	Biological: TAK-850Biological: Influenza HA vaccine	Completed
11.	NCT01195038	Recombinant Influenza HA Vaccine (H5N1)	Completed
12.	NCT03965195	Biological: Recombinant Influenza Vaccine Nursing home residents and staff 18 years and older are allocated to receive quadrivalent recombinant influenza vaccine. Other Names: Flublok RIV4	Active, not recruiting
13.	NCT00349037	Biological: Trivalent DNA vaccine with and without pPJV2012 administered by PMED	Completed
14.	NCT00980447	UMN-0501 is a purified recombinant influenza HA vaccine (A H5N1/Vietnam/1203/2004).	Completed
15.	NCT04523324	Flublok™ Quadrivalent by Sanofi, Inc. 2019-20 recombinant hemagglutinin quadrivalent influenza vaccines (RIV4)	Active, Not recruiting
16.	NCT02600585	Flublok Recombinant influenza vaccine (RIV); Intramuscular injection of vaccine of recombinant uncleaved hemagglutinin (rHA0) derived from influenza A/H1N1, A/H3N2, and B viruses, as identified for the season by the FDA Vaccines and Related Biological Products Advisory Committee (VRBPAC) in a total volume of 0.5ml.	Completed
17.	NCT04144179	Biological: Quadrivalent RIV with H3 strain 1Biological: Quadrivalent RIV with H3 strain 2Biological: Quadrivalent RIV with H3 strain 3Biological: Quadrivalent RIV with H3 strain 4Biological: Quadrivalent RIV with 2018-2019 NH H3 strain	Completed
18.	NCT01587131	Biological: FVH1 - a DNA-based influenza vaccine 0.9 mg FVH1 vaccine	Completed

19.	NCT01142362	Biological: VGX-3400X. VGX-3400X, which includes plasmids targeting the proteins of the H5N1 avian influenza virus.	Completed
20.	NCT01320696	Reverse Genetic (RG) reassortant A/H9N2 influenza vaccine	Completed

The stem region of the A/equine/Argentina/1/93 (H3N8) virus created a genetic vaccination (HAe) and a recombinant protein vaccine expressed in prokaryotic cells (HAp), which has higher reactivity to non-homologous strains than protein alone [20]. Hemagglutination inhibition assays are currently used to assess the immunogenicity of influenza virus vaccines, but these methods only evaluate antibodies against the head region of hemagglutinin and HI titers do not accurately reflect disease immunity. In the effort to create a universal vaccine against the most conserved stalk region of hemagglutinin, significant progress has been achieved. In HI tests, there is no activity in the stalk area. According to research by Jacobsen (2017), protection from weight loss in mice is strongly correlated with antibody levels against hemagglutinin in ELISA. They discovered that HA-specific antibody levels can be measured using ELISA, which can serve as a sign of immunity for all influenza vaccines [21].

The FcRIIIa-based reporter assay can be employed with bigger samples, according to Chromikova, (2020). [22]. The head region of the HA protein serves as the basis for licenced influenza vaccinations. The conserved stalk domain of the HA protein serves as the foundation for all influenza vaccines. The antistalk antibodies in vaccinations used in clinical trials for the A/H1N1, A/H5N1, and A/H9N2 vaccines containing chimeric cH6/1, full length H2, and H18 HA antigens were measured using an enzyme linked immunosorbent assay. High levels of anti-stalk antibodies were discovered to have been evoked, supporting the idea of a universal influenza vaccination based on the HA stalk [22, 23]. The globular portion of the receptor binding domain of the influenza A/H1N1/2009 hemagglutinin HA (63-286)-RBD produced in E. coli was folded correctly and was capable of inducing an immunological response, proving that glycosylation is not a requirement for vaccination effectiveness [25]. According to one of the investigations, a global influenza B virus vaccination strategy based on antibody reactions to conserved head and stalk hemagglutinin epitopes of the virus has been developed. As new vaccine candidates, recombinant viruses and mosaic influenza B hemagglutinin proteins have been created. In the mouse model, this vaccination technique offered widespread cross-protection [26].

Evolution of HA protein

The influenza virus's surface protein called hemagglutinin is crucial to the virus's ability to enter its host cell. The attachment of viruses to various animal hosts is caused by changes in the Hemagglutinin protein, according to Sriwilaijaroen and Suzuki (2012)[26]. The 2009 H1N1 outbreak, which resulted in a pandemic and a significant loss of life globally, was brought on by the accumulation of different HA mutations [28-31]. The host's immune system is another factor putting pressure on HA's evolution. Influenza viruses can avoid host immune responses thanks to antigenic drift. Antigenic drift is the buildup of small changes in influenza virus genes [31-34]. Although a single amino acid change does not significantly alter the HA protein's capacity to avoid polyclonal antibodies, it can alter how avidly the protein binds to receptors. Early research found five antigenic sites on H1N1 HA (Sa, Sb, Ca1, Ca2, and Cb), as well as five on H3 (A to E) [36,37]. The 2009 pandemic virus may have originated from American and Eurasian swine viruses, and pigs aid in the virus' transmission (38). The 6b1 clade is present in Iran and is characterised by S84N, S162N, and I216T, where position 162 got glycosylated, according to a phylogenetic research carried out in 2015–2016. The findings also show that the head region of HA experiences more alterations than the stem area (39). The HA sequences underwent phylogenetic analysis, which revealed that mutations cycle periodically around the world with the vast majority of circulating viruses falling under subclade 6B.1A. The D187A change in the A(H1N1)pdm09 HA's receptor binding site (RBS) has a negative impact on its functionality, according to the PROVEAN investigation (40). In a study by Boonnak et al., 2021, it was discovered that A/H1N1 viruses belonged to clade 6B.1A1 and shared antigens with A/Switzerland/3330/2017. Hemagglutinin protein's three-dimensional (3D) protein structure research suggests that a particular mutation (S200P) may cause altered HA protein interaction to a sialic acid receptor [41].

In other investigations as well, the HA head region was found to have a higher frequency of mutations than the stem. It was discovered that there was a rise in substitution in the head of hemagglutinin during seasons with lower influenza activity [42]. Matsuzaki (2014) discovered a brand-new antigenic site termed Pa of Pdm09 between residue 147 and sites Sa, Sb, and Ca2 (43). The HA gene is under negative selection when examined in viruses from America, Europe, and Asia (P value: HA = -2.253). [43]. Additionally, it was noted that the HA evolution patterns and frequencies varied greatly across the three continents, ranging from 2.36 10-3 in Europe to 3.18 10-3 in Asia. Studies have also shown that Sa and Sb sites are more likely to mutate, which leads to noticeably more infectious serotypes of viruses. In currently prevalent strains of pdm09, amino acid changes (D114N, S179N, S202T, S220T, I233T, K300E, and E391K) become fixed, while A203T, N458K, and E508G alterations are discovered to be dynamic. Disease severity rises with D239G/N. Through S200P and S202T, RBD improves HA's binding affinity to the receptor. H1N1 evolved in a manner resembling that of the influenza virus that caused the 1918 pandemic. In order to avoid neutralising antibody responses, the Hemagglutinin Stalk Domain changes over time, but at a very modest rate [45].

Antigenic sites in HA protein

Since the 1950s, researchers have known that antibodies produced in response to primary influenza virus exposures are highly strain-specific, whereas antibodies produced in response to secondary viral exposures to antigenically distinct strains are more likely to exhibit strong cross-reactivity with the initial strain. Originally known as "initial antigenic sin," this observation has since been referred to as "antigenic seniority" or "immune imprinting." Cross-reactive B lymphocytes that have been activated by prior influenza virus exposures are thought to be preferentially recalled upon exposure to an antigenically dissimilar viral strain, despite the fact that the processes underlying original antigenic sin are still not completely understood [46]. The status of the remaining 329 HA1 residues is uncertain, however there are 131 antigenic sites and 181 non-antigenic sites among them. The antigenic sites of influenza H1N1 viruses are further divided into five antigenic epitopes, which are A, B, C, D, and E in influenza H3N2 viruses. In general, the majority of the antigenic alterations in influenza A (H1N1) and A (H3N2) are caused by at least three substitutions at advantageously selected locations (H3N2). One dominant HA mutation, S206T, was recently found in 2009 H1N1pdm strains collected during the pre-epidemic, early, middle, and late stages, with mutation frequencies of 0%, 27%, 44%, and 83%, respectively. This mutation is novel because neither the 1918 nor the 1977 H1N1pdm viruses nor the human, swine, or avian IAVs included it [47].

On the other hand, the S206T mutation briefly emerged in the HA sequences of swine H1N1 viruses collected in 1976 and 1977 as well as human H1N1 viruses collected in 1934. The 2009 H1N1pdm virus may be directly affected by the HA-206 S to T mutation with regard to human infection and transmission. The HA receptor-binding domain may contain S206 (RBD). Antigenic diversity between the A/Bayern/7/95 and A/Beijing/262/95 influenza A (H1N1) subtypes was produced by a deletion of K at position 130. (K134 in H3 numbering). Lys123Asn, Lys157Asn, Gly158Glu, Asn159Asp, or Lys212Met) and compensatory mutations lowering HA charge (Asp225Gly or Gln226Arg) altered viral receptor-binding selectivity and restored the functional equilibrium between HA and NA (48). The pH of the fusion optimum was dramatically reduced by the N133D (H3 numbering) mutation. While the A198E change is linked to a considerable increase in HA thermostability relative to the wild-type virus, a number of amino acid substitutions, including K163 N, Q192 L, D190E, G228E, and K285 M, lowered the stability of HA as measured by heat inactivation. When the amino acid D190 N was changed, we discovered that viral proliferation in mice and eggs was decreased [49].

HA antigen as a diagnostic

A test for H1N1 infection can be performed using HA protein produced in vivo. Numerous studies have shown that the HA antigen can be used to accurately measure sero-prevalence. According to Park et al. (2018), spontaneous infection also results in the production of anti-HA stalk antibodies [50]. He investigated H1N1 patients, assessed their antibodies against the HA stalk, and discovered a correlation between them. They evaluated H1N1 PDM09's pre- and post-challenge

responses. In 64% of the volunteers, they discovered an increase in antibody production against the anti-HA stalk [50]. Alvarez (2010) showed that bacteria can express the Ha1 fragment (globular head), and he also developed an effective ELISA that could recognise antibodies in human serum samples. This ELISA can determine if a person has full or partial immunity to H1N1 pdm09 [51]. A synthetic HA0 peptide-based indirect ELISA was created by Zhao (2011). The outcome indicates the promise of this ELISA as a diagnosis, with the sensitivity and specificity of the test being 96.5% and 74.4%, respectively, when compared to HI subtype IAVs [52].

Additionally, Lu et al. (2018) established the production of the tri stalk protein (HA403-474) of A/Luxembourg/43/2009 (H1N1) in *Escherichia coli* and its successful application in ELISA using virus-infected mice serum to distinguish between group 1 and group 2 influenza. Tri stalk protein was used as a coating agent in an indirect ELISA assay to measure anti-HA stalk antibodies in human sera. They demonstrated an inverse relationship between IgG titers and HA stalk antibodies against the pandemic H1N109 virus [53]. In 2015, Yang et al. showed that fusion of recombinant HA protein synthesised in baculovirus (2 mc/mouse) with stabilising peptide generated from the C-terminal acidic tail of human synuclein (ATS) increased stability and provided protective immunity [54]. The expression of a fake rHa1 (HA 18-344) gene fragment in baculovirus was shown by Shim et al. in 2019. They created a HA1-specific ELISA that can identify blood levels of anti-HA1 antibodies in both human and mouse samples [55]. For the purpose of determining IgG and IgA antibodies against the influenza A H1N1 virus 2009, ELISA can be employed with synthetic peptides of the neuraminidase and hemagglutinin proteins in addition to prokaryotic, eukaryotic, and insect-expressed HA [56].

Challenges and future perspective

There are three types of influenza vaccinations: inactivated influenza vaccines (IIVs) made from eggs and cells, a live attenuated influenza vaccine (LAIV), and a baculovirus recombinant HA vaccine made from insect cells. In order to prevent seasonal influenza antigenic drift and improve pandemic preparedness, next-generation influenza vaccines are essential. These are intended to trigger stronger intra- and intersubtypic immune responses as well as immune responses that stay longer and are protective. Their production addresses one of the main problems with the embryonated chicken egg technique by aiming for quick and large-scale capacity. The effective development of next-generation influenza vaccine candidates presents major hurdles for the research and development community in terms of eliciting favourable humoral and T-cell-mediated immune responses against conserved epitopes. It's a terrific idea to use diagnostic methods for influenza based on the hemagglutinin gene and protein. However, choosing a conserved region of the HA is difficult because of antigenic drift.

CONCLUSION

Acute respiratory distress syndrome is brought on by influenza viruses, which are infectious and have developed into significant respiratory pathogens. There have been several epidemics in the past, notably the Spanish flu of 1918. In 2009, there was a recent influenza epidemic that contributed to more than 18,000 deaths globally. Since it is an RNA virus, influenza has considerable genomic plasticity and is able to change both its virulence proteins, such as HA, and its genome. Influenza infection is greatly aided by attachment to the host cell surface and internalisation by endocytosis. As a result of the quick mutations that happened in HA, various novel serotypes and variations have been described in the past. Recently, HA has been exploited as a crucial molecule in the development of both vaccines and diagnostic tests. Although an influenza vaccine was created, its effectiveness fell short of 100%. There are one or two influenza B viruses, one influenza A (H1N1), one influenza A (H3N2), and one influenza A (H1N1). According to reports, seasonal fluctuations in the influenza variants that are currently spreading around the world are significant obstacles to the development of a particular vaccine with higher efficacy. Genetic polymorphisms in the HA area are a crucial component for creating reliable and effective vaccinations and diagnostics, and HA is being explored as a superior option (vaccine and treatments). To create a multi-epitopic universal vaccine to replace the yearly influenza shot, conserved areas of epitopic regions of the HA gene should be investigated.

Abbreviation

HA (Hemagglutinin), RBD (Receptor Binding Domain), HPAI (Highly Pathogenic Avian Influenza Virus), LPAI (Low Pathogenic Avian Influenza Virus), Acute Respiratory Distress Syndrome (ARDS).

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Authors' contributions:
AS and RC conceptualize the study, AS and AKY and RBK retrieved the data from different biological databases, AS, RKN and JRK analyzed the data and interpreted. AS and RC wrote the manuscript, RC and HSM revised and edited the manuscript.

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