Pandemic (H1N1) Influenza 2009

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Abstract

Influenza A viruses cause recurrent epidemics and global pandemics which claim millions of lives. The recent emergence of swine-origin H1N1 virus has transmitted to and spread among humans, resulting in outbreaks internationally. The introduction of improved methods for genetically manipulating influenza virus promises to revolutionize our understanding of viral replication and its interaction with host innate and acquired immune systems, and will also enable the improvement of vaccines. The large scale use of drugs for chemoprophylaxis and treatment will impose strong selection for the evolution of drug-resistant strains. This article reviews the origin of current H1N1 virus, epidemiology of the pandemic, treatment of the disease, research focus and possible developments in the future related to the disease.

Keywords: H1N1, 2009 influenza A pandemic, Swine, Oseltamivir, PB1-F2, Antigenic drift and shift

1. Introduction

An outbreak of influenza A virus subtype H1N1, also known as swine flu, occurred in Mexico in March 2009. According to World Health Organization (WHO) there have been over 489,229 confirmed cases and at least 18239 deaths due to swine-origin flu worldwide from 19th April 2009 to 19th June 2010.[1] In India, cases started emerging early June 2009, and thereafter 33,453 confirmed cases and 1601 deaths due to swine-origin flu have been reported by Ministry of Health and family Welfare (MoHFW) as of July 4, 2010 report. Maharashtra has been the worst hit with 6748 confirmed cases and 487 fatalities till date.[2] WHO raised the level of influenza pandemic alert from phase 5 to phase 6, thereby declaring it the Pandemic (H1N1) 2009. During a normal flu season, the seasonal flu typically causes about 36,000 deaths and 200,000 hospitalizations, mostly among people 65 and older. The H1N1 flu continues to strike children and young adults the hardest, probably because it's been more than a half century since the H1N1 virus was at a pandemic level, leaving younger people with little or no immunity to the current strain.[3]

The name 'swine flu' is potentially misleading. Calling it 'swine flu' was confusing people into thinking they could get infected by eating pig products and this affected the pork industry. WHO stopped using the term 'swine flu' to avoid creating the misconception among the public that the new influenza is an animal disease. WHO now refers to the illness as H1N1 influenza A.[1]

Influenza's endemic reservoir is in aquatic wildfowl, many of which are migratory. Containment of disease outside of mammalian is therefore virtually impossible. Once a novel strain of influenza has crossed the species barrier from birds into a mammalian host, it may persist in that new host species for many decades.[4] The novel influenza A (H1N1) virus has the genetic structure resulting from reassortment of genes from four influenza viruses viz., North American swine influenza, Asia/Europe swine influenza, human influenza and avian influenza. The virus has a unique genetic composition that has never been seen earlier.[5]

2. History so far

Influenza A caused three well-documented pandemics in the twentieth century, in 1918 – 19 (subtype A/ H1N1), 1957 – 58 (A/H2N2) and 1968 – 69 (A/ H3N2). The 1918-1919 'Spanish' flu pandemic killed 50 million people worldwide, and remains unprecedented in its severity. Reverse genetics allowed re-creation of the Spanish influenza virus and its characterization which revealed an avian-like H1N1 virus that contains human-like signature amino acids in several proteins.[6,7] The 1957 'Asian' influenza (H2N2) pandemic killed an estimated 2 million people, and the 1968-1969 'Hong Kong' influenza (H3N2) pandemic killed an estimated 1 million people. Of the three influenza pandemics, the 1957 (H2N2) and 1968 (H3N2) pandemic viruses were avian-human reassortments in which three and two of the eight avian gene segments, respectively, were reassorted into an already circulating, human-adapted virus. The origin of genes of 1918 (H1N1) influenza virus is unknown. In May 1977, 'Russian' influenza (H1N1) virus outbreak was reported in China that affected young adults in the northern hemisphere in the winter of 1977-1978. The re-emerging H1N1 virus did not replace the H3N2 viruses circulating at the time, and both subtypes are co-circulating in humans to this day. The infection of 18 individuals in Hong Kong in 1997 with highly pathogenic avian influenza viruses of the H5N1 subtype, which resulted in six fatalities, marked the first reported fatal infections of humans with avian influenza viruses. After a period of local and sporadic outbreaks, a new H5N1 influenza outbreak started in 2003.[6,8] The H5N1 influenza, commonly called 'bird flu' emerged from poultry and was highly contagious in humans, but human to human transmission has not been reported except in one situation. Till July 29, 2009 this H5N1 virus had caused 433 laboratory confirmed cases and 262 deaths in 15 countries. While the world was grappling with H5N1 avian flu and preparing for a pandemic that could emerge, another influenza virus made a dramatic appearance in Mexico in March 2009 in the form of a novel H1N1 subtype.[5]

3. The Influenza Virus

Influenza A viruses belong to the family of orthomyxoviridae. Diameter of the influenza A virus is around 80-120 nm. Two glycoprotein antigens called hemagglutinin (HA) and neuraminidase (NA) on the surface of the influenza virion are important for pathogenesis. On the basis of antigenicity of their HA and NA molecules, they are classified into 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9). The viruses are named by their structure and geography. For instance, an isolate might be characterized influenza A/Leningrad/360/86 (H3N2). The name means that the strain was the 360th isolate of influenza A in leningrad in 1986 and it contains the third antigenic type of hemagglutinin and second type of neuraminidase.[9,10,11]

The virus consist of a nucleocapsid containing segments of negative-sense, single-stranded RNA, which is enveloped in a glycolipid membrane derived from the host cell plasma membrane. Nucleocapsid assembly takes place in the cell nucleus, but final virus assembly takes place at the plasma membrane. The ribonucleoproteins are enveloped by the plasma membrane which by then contains HA and NA. Virus 'buds' are formed, and intact virions are released from the cell surface. The dominant antigen is the hemagglutinin, which is responsible for attachment to respiratory epithelial cells and therefore an important target for influenza vaccines. The surface neuraminidase enzyme participates in the entry of virus into the cell. Neuraminidase NA is an antigenic enzyme that acts on the mucoprotein HA receptors by splitting off the terminal neuraminic acid. The result is destruction of receptor activity. NA probably serves several functions. It may inactivate a free mucoprotein receptor substance in respiratory secretions that could otherwise bind to viral HA and prevent access of the virus to the cell surface. It may be important in fusion of the viral envelope with the host cell membrane as a prerequisite to viral entry. It also aids in the release of newly formed virus particles from infected cells. Type-specific antibodies to NA appear to inhibit the spread of virus in the infected host and to limit the amount of virus released from host cells.[9] Figure 1 shows the novel H1N1 influenza virus. HA and NA antigens can be seen clearly on the surface of the virus.

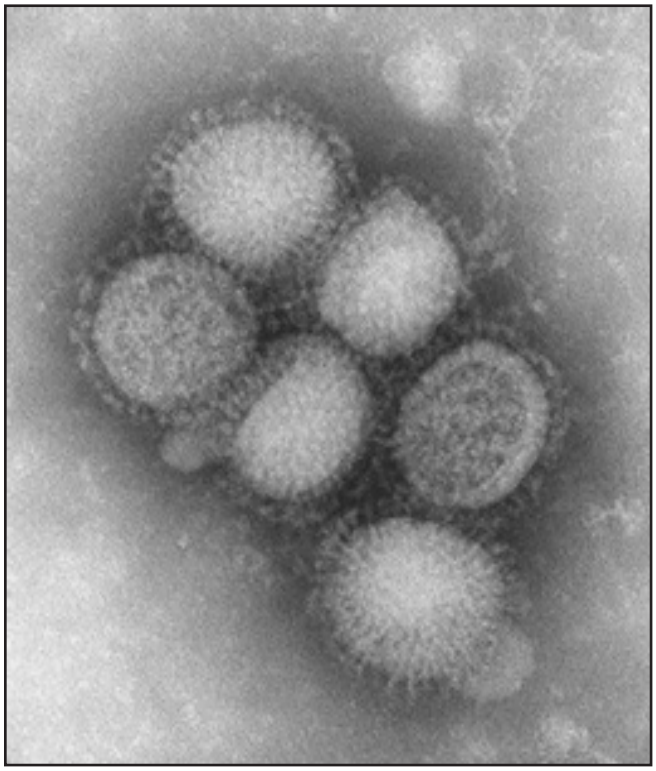


Figure 1 : H1N1 influenza virus, imaged in the Influenza Laboratory of the U.S. Centers for Disease Control and Prevention. Public domain images courtesy of the CDC

Ref. 3: Centers for Disease Control & Prevention CDC http://www.cdc.gov/flu/about/season/index.htm

3.1 Antigenic Drift and Shift

The surface antigens HA and NA are constantly changing, so that they are a moving target for therapeutic and diagnostic antibodies. It is the constant, incremental change (antigenic drift) and periodic, quantum change (antigenic shift) that allow recurring epidemics of influenza.[11] This antigenic drift is due to accumulation of amino acid substitutions in HA epitopes recognized by antibodies that neutralize viral infectivity by blocking interaction of HA with sialic acid residues on host-cell membranes. Hemagglutinin in particular has a high amino acid substitution rate in its epitope regions. [13]

Simultaneously infecting a cell with two different influenza A subtypes yields progeny that contain antigen derived from either of the original viruses may cause an antigenic shift. For example, a cell infected simultaneously with influenza A (H3N2) and A (H1N1) may produce a mixture of influenza viruses of the following subtypes: H3N2, H1N1, H1N2 and H3N1. The concept of antigenic shift and drift may be summarized as follows: Periodic shifts in major antigenic components appear, normally resulting in major epidemics in population with little or no immunologic experience with the subtype. As the population of susceptible individuals is exhausted (i.e., subtype specific immunity is acquired by increasing number of people) the subtype continues to circulate for a time, undergoing mutation with subtle antigenic drift from season to season. This allows some degree of infection to continue to occur. Infectivity persists because subtypes-specific immunity is not entirely protective against drifting changes.[9]

In the rare event of a double infection with two different strains of influenza into a single host, re-assortment of the genome segments can occur, producing a series of completely novel combination of genome segments in the progeny viruses.[14]

3.2 Inter-Species Transmission

All subtyes of influenza A can be found in birds. Avian viruses do not usually infect humans, although several cases of human infection with bird flu viruses have occurred since 1997. Avian influenza viruses may be transmitted to humans in two main ways:

(1) Directly from birds or from avian virus – contaminated environments to people or
(2) Through an intermediate host, such as a pig. Pigs are an important carrier because they can be infected with both human and avian flu, and also come in contact with both species.[10]

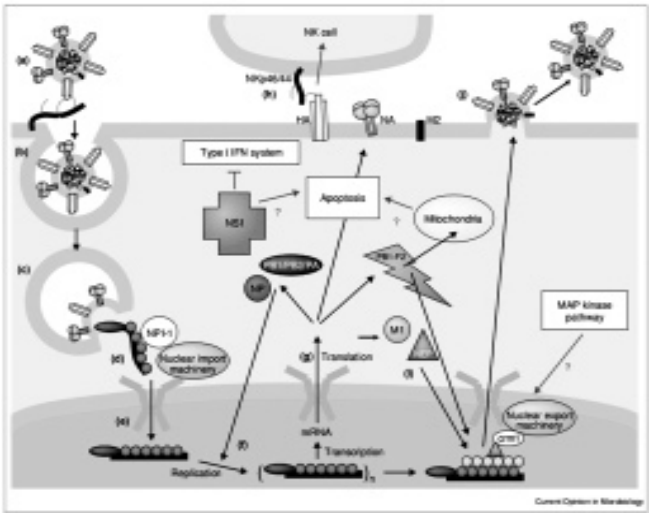
3.3 Replication

Influenza A viruses contain a genome composed of eight segments of single-stranded, negative sense RNA that each encode one

or two proteins. Replication and transcription of viral RNAs ae carried out by the three polymerase subunits PB2, PB1 and PA, and the nucleoprotein NP (Figure 2). Newly synthesized viral ribonucleoprotein (vRNP) complexes are exported from the nucleus to the cytoplasm by the nuclear export protein (NEP) and the matrix protein M1, and are assembled into virions at the plasma membrane. The NA protein facilitates virus release from infected cells by removing sialic acids from cellular and viral HA and NA proteins.[6]

Once the virus has entered a cell of the new host, the virus must successfully co-opt host cell processes to replicate there. If the virus does succeed in replicating, it needs to be released from the host cell to infect more cells or be shed from the host. Even if progeny virus exits one host cell, host innate immune responses may hinder infection of other cells. Interferons may induce uninfected cells to enter an antiviral state that inhibits viral replication.[15] To counter host responses, influenza virus has developed strategies for evading innate immunity: The viral NS1 polypeptide acts as an interferon antagonist that blocks the activation of transcription factors and IFN-β-stimulated gene products, and binds to double-stranded RNA (dsRNA) to prevent the dsRNA-dependent activation of 2'-5' oligo (A) synthetase, and the subsequent activation of RNase L, an important player in the innate immune response.[6,15] A typical replication cycle of the influenza A virus is shown in figure 2.[12]

Figure 2 : Schematic Representation of the Infectious Cycle of Influenza A virus (IV) and the Interactions of Viral Proteins with the Host.



(a) IV binds through its hemagglutinin (HA) protein to sialic-acid-containing receptors on host-cell plasma membranes. (b) Virions are internalized into endosomes. (c) When the endosomal pH becomes sufficiently acid (around pH 5–6), a conformational alteration in the HA induces fusion between the viral envelope and the endosomal membrane. (d) Viral nucleic acid is delivered into the cytosol in the form of ribonucleoproteins (RNPs). Efficient uncoating requires the ion channel activity of the M2 protein. (e) A nuclear localization signal in the nucleoprotein (NP) facilitates the import of the incoming viral RNPs into the nucleus of the host cell through interactions with the cellular NPI-1 protein or karyopherin-α. (f) Once in the nucleus, RNA replication and transcription is initiated by the viral-RNAdependent RNA polymerase, which is a complex of three viral proteins (basic polymerase subunit 1 [PB1], basic polymerase subunit 2 [PB2] and acidic polymerase subunit [PA]). (g) Viral mRNAs are transported to the cytosol and translated into 11 defined proteins. Newly synthesized NP and polymerase proteins move back to the nucleus to further amplify RNA replication. The HA, neuraminidase (NA) and M2 proteins enter the endoplasmic reticulum (ER) and are transported to the plasma membrane. (h) HA binds to NKp46 and NKp44 proteins, activating natural killer (NK) cells, which mobilize the innate immune system against IV. Meanwhile, non-structural protein 1 (NS1) has been disarming the antiviral host defense mechanism mediated by the type I interferon system, and is also playing havoc with cellular apoptotic pathways. The recently discovered PB1-F2 localizes in mitochondria and nuclei, apparently inducing apoptosis in a cell-type-specific manner. As the infection cycle progresses, M1 and nuclear export protein (NEP) migrate to the nucleus (i) and trigger RNP export to the cytosol. This process seems to be mediated by the interaction of NEP with the cellular CRM1 protein, and is dependent on the activation of cellular MAP kinases. Viral budding occurs at the plasma membrane (j) through the interaction of M1-coated RNP cores with viral plasma membrane proteins, excluding host proteins in the process. Viral aggregation through interaction of HA with sialic acids is prevented by NA-mediated desialylation of the viral glycoproteins, which also enables the release of virus from the cell surface. The red arrows indicate poorly defined relationships.

Ref. 12: Yewdell J., García-Sastre A., Influenza virus still surprises, Current Opinion in Microbiology 5 (2002) pg 416

3.3.1 Role of PB1-F2 in Pathogenicity

PB1-F2 is a small viral protein that is one molecular marker in pathogenicity. PB1-F2 appears to accelerate apoptosis in monocytes, but not in fibroblast or epithelial cell lines. A simple notion is that this protein enhances viral fitness by disabling monocytes and perhaps other immune cells.[12]

It makes patients sicker and prone to catch bacterial pneumonia.[16] The swine-origin influenza virus S-OIVs encode a truncated PB1-F2 protein of 11 amino acids (Table 1) whereas Eurasian avian-like swine viruses possess full length PB1-F2 proteins (87-89 amino acids).[6] The absence of a full-length PB1-F2 protein has been suggested as one possible determinant for the low pathogenicity of the 2009 influenza H1N1 pandemic strain, with a case fatality rate comparable to seasonal influenza.[16]

4. Emergence of Swine-Origin H1N1 Influenza Virus

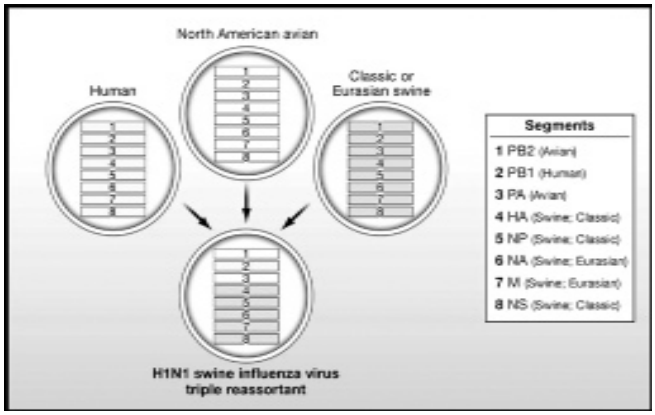
The S-OIV probably resulted from the reassortment of recent North American H3N2 and H1N2 swine viruses (i.e., avian/human/swine 'triple' reassortment viruses) with Eurasian avian – like swine viruses (see figure 3). As a result these viruses possess PB2 and PA genes of North American avian virus origin, a PB1 gene of human H3N2 origin, HA (H1), NP, and NS genes of classical swine virus origin, and NA (N1) and M genes of Eurasian avian-like swine-virus origin (hence their original description as 'quadruple' reassortants). However, the human-like PB1 gene and the avian-like PB2 and PA genes have been circulating in pigs since 1997-1998 (when triple reassortant swine viruses were first isolated), and have probably undergone adaptation to pigs.[6]

5. Receptor Secificity and Mutation

Hemagglutinin mediates cell-surface sialic acid receptor binding to initiate virus infection. Sialic acids (Sias) are regarded as receptors for influenza viruses and are usually bound to galactose (Gal) in an α2–3 or α2–6 configuration. After virus replication, neuraminidase removes sialic acid from virus and cellular glycoproteins to facilitate virus release and the spread of infection to new cells.[14]

The HA of human influenza virus isolates typically binds preferentially to sialic acid SA-α-2,6-Gal-terminated saccharides, whereas that of avian influenza virus isolates has a higher affinity for SA-α-2,3-Gal-terminated saccharides. Interestingly, sialic acid receptors are distributed differently in the respiratory tracts of humans and other host species. This difference, together with the predominance of

Figure 3 : Lineage of the 2009 Swine origin influenza A virus.



Influenza A viruses have a segmented negative-sense RNA genome that encodes up to 11 proteins including the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) and the virulence factors NS1 (host interferon antagonist) and PB1-F2 (proapoptotic factor). The 2009 H1N1 swine virus is a triple reassortant; it includes segments from swine, avian, and human influenza viruses.

Ref. 33: Wang T.T., Palese P., Unraveling the Mystery of Swine Influenza Virus, Cell 137, June 2009, pg 984

SA-α-2,6-Gal-terminated saccharides in the human trachea, may explain why replication of avian influenza viruses in humans generally tends to be restricted. The rare occurrences of fatal pneumonia in humans infected with H5N1 virus isolated from China in 2005 and H7N7 virus from the Netherlands in 2003 are likely due to the ability of these viruses to attach to and replicate in lower respiratory tract cells, which do have SA-α-2,3-Gal-terminated saccharides.[8,15,34]

Interestingly, influenza virus may change its sialic acid receptor binding preference and amino acid change in viral protein to cause increased virulence in the host.[6] These changes have been summarized in Table 1.

6. Population Dynamical Considerations

Epidemiologists frequently use the concept of basic reproductive number, R_o as a measure of the transmission fitness of an infected pathogen. R_o is defined as the average number of persons infected by one case in a totally susceptible population in absence of interventions aimed at controlling the infection. Consequently, a pathogen with Ro < 1 cannot sustain transmission, and thus an epidemic caused by such a pathogen would inevitably die out.[17] Estimates of R_o for pandemic flu strains range from 1.8 to 2.4 for the 1981 Spanish flu, and around 1.5-1.8 for H3N2 Hong Kong flu pandemic. Early estimates of the recent H1N1 pandemic before April 2009 come out above unity (1.0) with their stated range of R_o being 1.4 to 1.6 while a genetic approach based on evolutionary biology giving a result of 1.2 [18]

7. Symptoms of a Possible H1N1 Infection

The influenza syndrome starts with the abrupt onset of headache, fever, chills and dry cough; later, high fever, myalgias, malaise, rhinitis, sore throat and anorexia appear. In young children, gastrointestinal symptoms may be prominent.[11]

Influenza can be envisaged in 3 stages. First stage represents infection and virus replication on mucosa of the respiratory tract and alveoli, resulting in respiratory symptoms in the majority. This lasts about 7 days. Second, the cytokine excess phase, which starts about 2-3 days after infection and continues for a few to several days even after virus multiplication stops. The headache and bodyache that accompany onset of fever are mainly on account of the pro-inflammatory cytokines in excess. The viral pneumonia or acute respiratory distress syndrome (ARDS) of influenza has a major causative contribution from this cytokine storm. The third stage is bacterial super-infection of the alveoli – in other words, secondary bacterial pneumonia that complicates influenza in a small proportion of subjects.[19]

8. Controlling Spread of H1N1 Virus

Influenza viruses are chiefly spread from person to person by airborne droplets expelled by an infected person coughing, sneezing or speaking. Facemasks are of doubtful efficacy and complete interruption of airborne transmission is impossible in most settings, so adherence to hand hygiene, droplet and contact precautions, as well as behavioral conditioning, i.e. to avoid touching one's nose, eyes or mouth, must be given priority in the prevention and control of influenza.[20,21]

Table 1 : Determinants of Viral Pathogenicity

Protein	Position	Pathogenicity		Swine-origin H1N1 viruses	Function
		High	Low		
PB2	627	Glu	Lys	Glu	Replicative ability in some mammals, including humans
	701	Asp	Asn	Asp	Nuclear import kinetics affecting replicative ability in mice
PB1-F2	66	Asn	Ser	Truncated (11 aa)	Induction of apoptosis
HA	Cleavage site	Single basic aa	Multiple basic aa	Single basic aa	HA cleavage
NS1	92	Asp	Glu	Asp	Unknown
	C-terminus	Arg-Ser-Glu-Val, deletion	Glu-Ser-Glu-Val	11 C-terminal aa truncated	Unknown

aa, amino acid(s)

Ref. 6: Neumann G., Nado T., Kawaoka Y., Emergence and pandemic potential of swine-origin H1N1 influenza virus, Nature, 459, June 2009, pg 935

9. Detection of Swine-origin H1N1 Influenza

Accurate and rapid diagnosis of novel influenza A (H1N1) infection is critical for minimizing further spread through timely implementation of antiviral treatment and other public health based measures.

Upper respiratory specimens such as nasopharyngeal aspirates or nasopharyngeal swabs, throat or nose swabs are suitable for the detection of S-OIV (Swine – origin influenza virus). Word Heath Organization recommends that suspected clinical cases of swine-like H1N1 influenza A infection are confirmed by (1) specific RT-PCR assays that differentiate S-OIV from seasonal influenza viruses, (2) the isolation and identification of swine-like H1N1 influenza, or (3) the detection of a fourfold rise of neutralization or HAI antibodies to S-OIV.

Real-time polymerase chain reaction (RT - PCR) is more rapid and sensitive than traditional techniques including virus isolation by cell culture.[22]

10. Treatment (Antiviral Drugs)

Two classes of antiviral drugs are currently available for chemoprophylaxis and treatment of influenza. The neuraminidase inhibitors oseltamivir (brand name Tamiflu) and zanamivir (brand name Relenza) impair the efficient release of virus from infected cells. The M2 protein inhibitors adamantanes such as amantadine (known under several brand names) and rimantadine (brand name Flumadine) target the viral M2 protein, which is required for efficient uncoating of the virus inside the cell.[6] Table 2 mentions the pharmacological characteristics of antiviral influenza drugs.

Oseltamivir phosphate is an ethyl ester prodrug which is hydrolyzed hepatically to the active metabolite oseltamivir carboxylate. The oral bioavailibility of zanamivir is low (<5%), and the commercial form is delivered by oral inhalation of dry powder in a lactose carrier.[23] Unless oseltamivir is given very early in the virus amplification stage, it cannot prevent or cure the viral pneumonia or ARDS directly due to the virus and inflammatory and innate immune responses.[19]

Peramivir, an NA inhibitor that was developed through structure-based design, is active in in vitro tests against viruses of all nine NA subtypes, including highly pathogenic H5N1 viruses. Phase II clinical trials are now under way to assess the efficacy of intramuscularly administered Peramivir against seasonal influenza.[6]

11. Drug Resistance

High-level drug resistance to both types of inhibitors is typically conferred by single amino acid substitutions in the M2 protein and NA. Mutations conferring resistance to M2 inhibitors generally provide full cross-resistance to both amantadine and rimantadine, whereas several mutations conferring resistance to NA inhibitors are drug-specific. H1N1, H1N2 and H3N2 viruses are resistant to adamantanes.[17]

Two key factors affect the epidemiology of drug resistance in influenza. The first factor is the rate at which treatment generates resistance de novo. The second factor is fitness costs associated with drug resistance mutations. Over the recent years, resistance to M2 inhibitors has grown rapidly. This rapid rise of M2 inhibitor resistance is presumably due to higher rates of de novo resistance and low transmission fitness costs. Therefore, the recent findings regarding NA inhibitors that the de novo resistance may be higher and fitness cost may be lower than previously thought raise justifiable concerns that the extensive use of NA inhibitors may induce a greater resistance problem than anticipated so far.[17]

Oseltamivir – resistant human H1N1 viruses may have emerged in immunocompromised patients in which prolonged replication may have resulted in the selection of mutations that increase the fitness of oseltamivir – resistant viruses.[6]

As of 30th June 2010, a total of 298 cases of oseltamivir resistant H1N1 virus have been detected. They are assumed to remain sensitive to zanamivir. All except one of the confirmed cases have a mutation in the NA gene resulting in an amino acid change from histidine to tyrosine at amino acid 275 (referred to as H275Y). [24]

12. Vaccines

Vaccines need to be revised every 1-3 years to account for mutations in the HA and NA proteins of circulating viruses (antigenic drift). Inactivated vaccines have been used for many decades. Typically reassortment is used to generate a seed virus that possesses the HA

Table 2 : Pharmacological Characteristics of Antivirals for Influenza A

	Amantadine	Rimantadine	Zanamivir	Oseltamivir
Formulations	Oral (tablets, syrup)	Oral (tablets, syrup)	Inhaled (powder) Intravenous	Oral (capsules, syrup)
Oral bioavailability	>90%	>90%	<5%[a]	~80%[b]
Effect of food on AUC	Negligible	Negligible	Not Applicable	Negligible
Plasma $t_{1/2}$, h	12-18	24-36	2.5-5	6-10[b]
Protein binding, %	67%	40%	< 10%	3%[b]
Metabolism, %	< 10%	~75%	Negligible	Negligible
Renal Excretion, % (parent drug)	>90%	~25%	100%	99%[b]

[a]Systemic absorption 4% to 17% after inhalation
[b]For antivirally active oseltamivir carboxylate

Ref. 23: Brunton L.L., Lazo J.S., Parker K.L., Goodman & Gilman The pharmacological basis of therapeutics, Tata-McGraw Hill, 11th Ed. pg1257

and NA segments of the circulating virus, and a variable number of segments from a strain of H1N1 virus that confer efficient growth in embroynated chicken eggs. The allantoic fluid of embryonated, virus-infected chicken eggs is purified and concentrated by zonal centrifugation or column chromatography, and inactivated with formalin or β-propiolactone. Treatment with detergents or ether and the removal of vRNP complexes leads to split or subunit vaccines that are administered intramuscularly or subcutaneously.[6,25]

But influenza viruses that thrive in humans are hard to grow in chicken eggs, and the eggs are expensive and in short supply. So different labs worldwide are finding new ways to boost the yield of virus from eggs and are developing innovative methods to avoid the use of eggs altogether by culturing the virus in cell lines. About 10% of currently available swine flu vaccines have been made using new methods.[25,26] A small risk of Guillain–Barré syndrome was associated with the influenza vaccines in both 1976-1977 and 1992–1994.[30] Three manufactures namely Serum Institute of India Ltd., Pune, Panacea Biotech and Bharat Biotech were given licence by DCGI (Drug Controller General of India) to import the WHO approved seed strains and they are in clinical trials. These vaccines will be available for commercial use by May/June 2010. Meanwhile, MoHFW, Government of India has imported 1.5 million doses of Panenza vaccine to vaccinate selected population among the high risk group. Panenza, the pandemic vaccine procured from M/s Sanofi Pasteur, France, is a split virus inactivated, non-adjuvanted monovalent vaccine against pandemic Influenza. [28]

13. Scenario in India

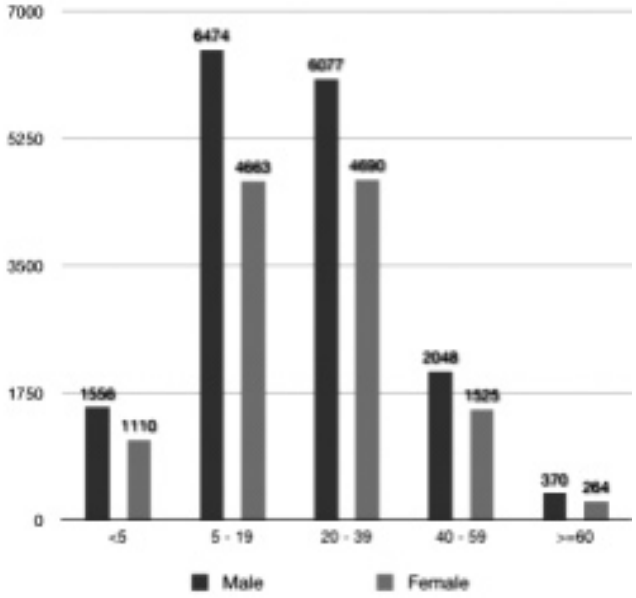
Deaths have sparked a fair amount of concern and panic. Poor communication of risks by the government and the public-health system is largely to blame. Even if this pandemic remains moderate, impact in India is likely to be severe, owning to its high population density, low awareness of the pandemic and the propensity of the virus to infect the young (50% of Indians are under 25 years of age). Moreover, there is a high load of other infectious diseases as well as chronic conditions, groups that are at higher risks of severe forms of pandemic H1N1 disease. The health infrastructure is poor. Despite this bleak outlook, India has strengths for tackling the virus, including that government has pandemic plans in hand, and that we have a vibrant generic – pharmaceutical industry as well as a decent capacity for manufacturing vaccines.[29,30] The chart shows the age - sex pattern of recorded cases in India.[35]

14. In the Future

1) Universal Vaccines

What scientists dream of is a vaccine that can protect against any flu strain for years or even a lifetime. This so-called universal flu vaccine is still a long way off, if it's even possible. One of the most hotly pursued strategies for universal vaccine for influenza A is based on M2 protein. It's an appealing target because the 23 amino acids that make up the ectodomain of M2 (known as M2e) scarcely vary from one human flu strain to the next, even back to the 1918 Spanish flu. Another approach to a universal flu vaccine uses conserved internal proteins such as nucleoprotein (NP) to elicit a different kind of immunity, one based on cytotoxic T lymphocyte

Chart 1: The Age-Sex Distribution of Recorded Cases in India according to MoHFW



(CTL) rather than on antibodies. CTLs recognize and kill infected cells expressing viral antigens, fragments of proteins such as NP. With two main approaches still a question mark, some investigators are working on a seemingly more approachable goal: making HA-specific vaccines that protect against drift strains within the same HA family. It will be several years yet, however, before universal vaccines will be ready for commercial rollout.[31,25]

2) Key questions about influenza virus still remain unanswered: factors that determine interspecies transmission, reassortment and human-to-human transmission, also which genetic changes would allow the circulating H5N1 virus to acquire the characteristic to spread efficiently among humans? [6,15]

3) Experimental and epidemiological studies need to be intensified to allow more accurate quantification of the transmission fitness of the resistant virus.

4) Computer models, based on a realistic human population structure, are required to simulate the emergence of resistance at the outset of a pandemic for various scenarios of drug use.[17,32]

5) Large-scale sequencing efforts, bioinformatics analyses, and the ability to experimentally test predictions with recombinant viruses will eventually provide insight into key features for the emergence of pandemic viruses.[6]

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