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# Metamorphosis in surface protein of swine flu: A Prime predicament for H1N1 Inhibitor

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## ABSTRACT

The emergence of new mutant strains of influenza virus like H1N1, H3N2, H1N9, H7N9, etc. in every season of flu is on the whole due to the frequent mutation in eight genes of the flu virus. This cause influenza virus spreads international and turns into pandemic in 2009. Every yr 36000 peoples are infected from flu. Around 123397 people have been examined itself in India in 2010. Drug for influenza treatment now has been resistance due to a mutation in the receptor of neuraminidase. Mutation is the essential hassle for designing inhibitor towards the influenza virus. A quantity of traces produced due to genetic reassortment and mutant property of the virus. This makes the different traces of hemagglutinin (H) and neuraminidase (N). Total eight genes of influenza virus, out of these solely few genes involve for mutation consistently in which may additionally be some advantage or might also some insensitive to flu. Gene mutation causes unique changes in the sequence of the amino acids and changes the conformation of other worried residues which causing the unfitting binding web site for influenza inhibitor. A quantity of different mutation buddies with specific mutation like H274Y, N294S, I223R, E119V, Q136K, and S31N etc. are responsible for changing conformation of the binding site. Some mutation of the floor protein of the virus may motive a negative or effective impact on the binding site. Some caused mutation also helps in antiviral activity. This assessment highlights the mutation which motives resistance and altering residues in the binding website online of in neuraminidase, hemagglutinin, and M2 channel protein which would provide the pathway for designing a rational drug. Figure 2 Mechanism of drug-resistance and mutation in the neuraminidase amino acid (H274Y, R292K, N294S, or E119V. A suggests the interplay of oseltamivir with wild-type neuraminidase receptor. B showing the interaction of oseltamivir with mutant type (274Y), and C showing the mechanism of mutation, how E276 rotate and make a pocket for OST, and binds to R224. Histidine changed with the aid of Tyrosine (bulkier residue) which adjustments the other residues conformation like E276, R292 so that E276 no longer rotate and oseltamivir no longer exact bind.

**Keywords:** Influenza virus, Mutation, Neuraminidase, Hemagglutinin, H274Y.

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## INTRODUCTION

The emergence of influenza drug resistance is a foremost public fitness concern. Bird flu, avian flu, and swine flu have the inherent property of mutation. The reassortment of genetic segments in extraordinary host species from unique subtypes of influenza viruses frequently, which might also generate new lines which may reason flu as epidemic or pandemic. Due to the genetic assortment, they developed new subtypes which purpose seasonal flu in human beings [1, 2]. Inhibitors like (NAIs), such as oseltamivir (OST) and zanamivir, M2-channel inhibitors, were the tablets of preference against influenza A or B. Substitutions of amino acids in primarily Neuraminidase (NA), hemagglutinin (HA), and M2- channel protein cause mutation which leads to resistance for all anti-influenza drugs. There are two most important lessons of antivirals on hand for the cure and prevention of influenza, the M2 inhibitors and the neuraminidase inhibitors (NAIs). Due to excessive mutation fee and era of new traces of influenza virus, it is a principal trouble for growing contemporary therapeutics, even vaccine is additionally ineffective due to antigenic waft and shift which causes point mutation. Avian influenza viruses carrying molecular markers for resistant which are additionally responsible for point mutations which arise due to natural fluctuations and triple reassortment [3].

On the groundwork of floor proteins, influenza virus has been categorised into two types, A and B. Both have a bad feel of RNA virus with a low fidelity of RNA polymerase [4]. Influenza gene is divided into the eight gene segments (hemagglutinin [HA], nucleoprotein [NA], matrix [M1], matrix [M2], M-protein channel [MA], polymerase simple 1 and 2 [PB], polymerase acidic [PA], and non-structural protein [NS] genes). They are all wrapped around a central core which consists of single strand RNA. On the foundation of phylogenetic, again neuraminidase divides into two groups, team A includes subtypes (N1, N4, N5, and N8) and team B includes (N2, N3, N6, N7, and N9) [5]. Influenza A is responsible for greater morbidity and mortality than other types. It reasons 10-20% of the world's population and 250,000- 500,000 deaths per year during the world. During the decade of surveillance, a widespread expand in drug resistance was once noted, from 0•4% in 1994– 1995 to 12•3% in 2003–2004 [3]. This enlarge in the percentage of resistant viruses was once 61% in 2004, and above 90% in 2009 and it became 100% until 2010 against Tamiflu (Oseltamivir carboxylate) [3, 6]. In Taiwan, 1187 tremendous instances were tested for the H274Y substitution in the neuraminidase (NA) N2 gene that confers resistance to oseltamivir [7]. Drug oseltamivir resistance has conferred due to single point missense mutation from histidine to tyrosine at role 274 (H274Y).

This trade is a different place in extraordinary traces at 273 in kind B, 274 in type N2 and 275 in type A N1 amino acids numbering. All the viruses have a mutation in the NA gene and confirming OST resistance and cross-resistance against other antivirals [7, 8]. Studies confirmed

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that NA amino acids sequences from America, Asia, Europe, Oceania, and Mexico have comparable molecular distribution patterns and among them only chosen amino acids of NA affected by way of mutations [9]. Molecular distribution patterns of amino acids may also exchange in each pressure of the virus. So that role of the mutation in each stress may additionally one of a kind in every strain of the influenza virus. Some position of the amino acid is also responsible for the change in the NA active web page in another continental which is not associated to resistance but helps to a conformational alternate in some other amino acid. Many amino acids substitution is associated to virus confer OST-resistance might also be due to variation in seasonal influenza virus [10]. The mutation influences the residues to compensate the molecular alternate so that purpose the destructive situation for any inhibitors. Some mutation in traces may also help for new strains, direct or indirect involvement in resistance, decrease the susceptibility to inhibitors and some have interfered the inhibitor as nicely as residues. In wild-type influenza proteins amino acids are surrounded with steady and ideal isomer to inhibitors and any alternate in amino acids of surroundings may additionally disturb the binding site, end result causes the resistance to drugs [11].

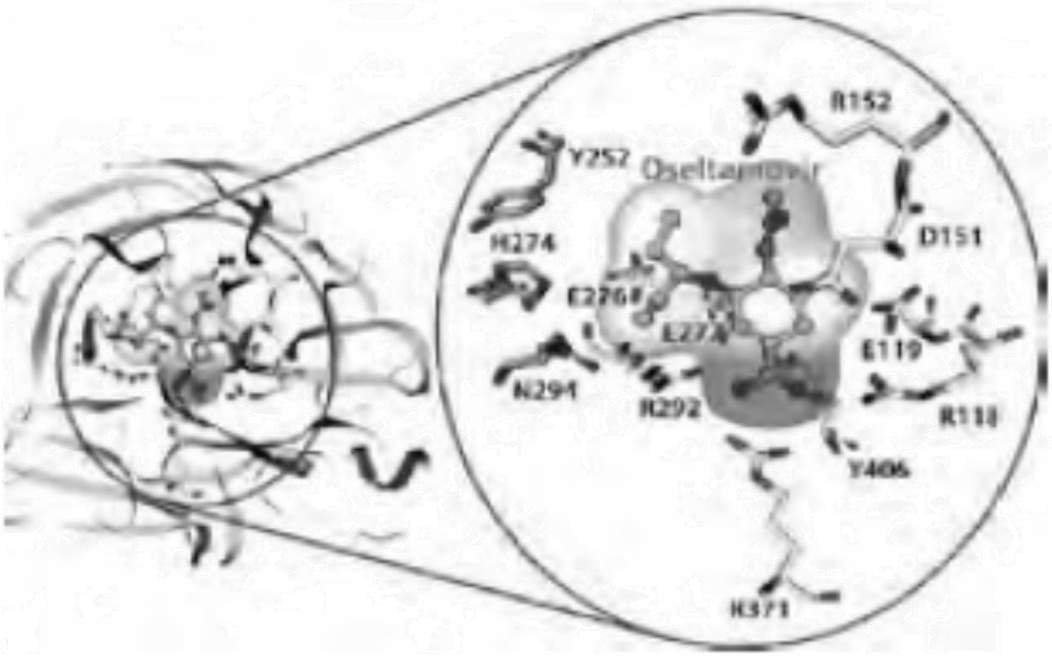


**Fig.1 :Shows the communication of oseltamivir in the lively site of feral type neuraminidase (NA1). AAs R118, D151, R152, E276, R292 and 371, make the catalytic site for OST in fig.1B [12].**

Among these surroundings amino acids of active sites, only some of the amino acids are responsible for mutation, which causes the conformational change to other AAs and caused improper binding [13]. 150-loop cavity which formed by 147- 156 in N2 numbering an open conformation adjacent to the active site, also novel target for other NAI [14]. 150- loop NA active site, T148I substitution have a major role in reduced NA activity towards inhibitor, OST have efficacy that opens the loop [15]. Due to a mutation in 150-loop the residue, R152 loss the interaction with OST but it strongly interact with 156 residue of loop-150 [16, 14]. Interaction shows that closed conformations of NA enzyme have a strong interaction with OST with R156 and these conformations may be used for the design of future inhibitors.

**Mechanism of resistance:**

According to the Influenza Virus Sequence Database, NA sequence E119V, R292K H274Y and N294S were associated with resistance to oseltamivir in H3N2 and H275Y and I223R were potentially associated with oseltamivir and zanamivir confrontation in H1N1 and H5N1 influenza type A. Mutation H273Y in influenza type B, also associated with resistance to oseltamivir and paramivir, but sensitive to zanamivir [17, 18, 19, 8]. In 2007 H5N1-NA resistance is owing to change in bulkier residues at position Y274 which replaced the smaller side chain residue H274. Tyrosine (Y) have different side chain and larger in size then histidine which changes the conformer of active sites. These alterations disturb the orientation of other residues like E276 (fig-2 C) come closer to a binding site which is unable to form a salt bridge with arginine 224 [20, 21, 8]. This causes improper fitness of OST in the active site. Due to mutation and substitution of bulkier residue tyrosine (Y), change the orientation of Glu276 which cause less hydrogen bond forming with OST. The hydrophobic nature of binding pocket of OST is changed, resultant shrinkage of hydrophobic pocket and it makes unfavorable for OST in the active site of neuraminidase and this makes a mutant structure in viral neuraminidase. Phenylalanine (F), Tyrosine (Y) like high hydrophobic residues in place of smaller residues like Histidine (H) reduced the susceptibility of OST due to bulkier

**Substitution**

The study showed that H274Y decreases the amount of neuraminidase that reaches the cell surface and that this defect can be stabilized by V234M and R222Q secondary mutations that also restore viral fitness and confirming the H274Y support the OST-resistance growth. V241I, V234M, R222Q, D344N, D354G, and N369K mutation compensate the defect of NA level and help to the reaches cell surface and produced new strains of NA, some are compensated negative effects with the H275Y [23, 24, 25]. Mutation was unlikely to be caused by other viral mutations based on genetic sequences like L607V, K660R, F103S, W104G, but not such sensitive to OST

[26]. Common mutations in N1 are detected at N294S and H274Y, while the E119V R292K and N294S (fig-2) mutations are mostly found in the N2 and N9 subtypes. R152K, D198N, I222V, and R371K are specific mutation found in the influenza B-type [8, 27]. It has originated that R292K NA mutation confers resistance to zanamivir, peramivir, and oseltamivir. Mutation in R292K, N294S, I223R, and H274Y confer high-level resistance in N1 as well as N2 they all are single nucleotide polymorphism [7]. R292K is the most common NA mutation in subtypes N2 and N9 whereas N294S mutation also associated with N1 (H1N1, H5N1) and N2 (H3N2) [28]. I223R mutation with the H275Y in N1- NA gives the synergetic effect on IC50 which caused contraction of the active site of the enzyme which potentiates the resistance or reduced susceptibility to antiviral drugs [18].

In the novel strains like H7N9, H3N2 and H1N9 virus also reduced the sensitivity of NAIs against influenza flu [24, 25]. H274Y, N294S, and R292K are the inhibiting reorientation of Glu276 which was unable to interact with Arg224 and prevent pocket formation for binding and they associated with OST resistance [19]. E119V occurs with I222V mutation also give greater change in IC50 value as Compared with the susceptible virus, that interferes with OST molecules because water molecule binds with OST side and valine molecule at lively site [26, 27]. Recently it was found that E119G mutation in NA confers that both direct and indirect effect the drug binding and reduce the affinity to the inhibitor, it may cause cross-resistance against zanamivir, paramivir [29, 30]. All these substitutions are associated with catalytic residues in the active site of the neuraminidase protein. From NA inhibition assay it had been confirmed that zanamivir selected residues 119 and 292, and oseltamivir select residue at 274 and 292 are mutant which acquired by virus [30]. In fig-2B, its find that in binding pocket only a few amino acids involve for interactions with inhibitor rather all the amino acids interact with each other for stabilization of binding pocket, favorable residues include R118, E119, D151, R152, W178, S179, I222, R224, E227, S246, E276, E277, R292, N294, R371, and Y406, so that makes format for active site [12]. Mutation in amino acids concerns the casing of pocket and pocket size which caused the indecent for inhibitors. Recently it was found that mutation S247N is highly resistant to oseltamivir when along with H274Y it highly pandemic clinically. S247N also change the active site for zanamivir with I223R. A novel mixture of NA mutations Q313R and I427T caused resistance to both oseltamivir and zanamivir [30, 31]. All the changes in residue take place in presence of antiviral drugs which cause adverse environments for the virus; resulting changes in genes for surviving and save from adverse condition. These survivals for the fittest condition in virus gene cause monomer-monomer interface impede to NA inhibitors mutation in the gene and result changes in proteins and produce new potent twists with modifications of surface protein (H and N). Studies show that even in the absence of oseltamivir used, there is a rapid wide spread of H1N1 resistance strains transmission takes place [32, 33, 34, 35].

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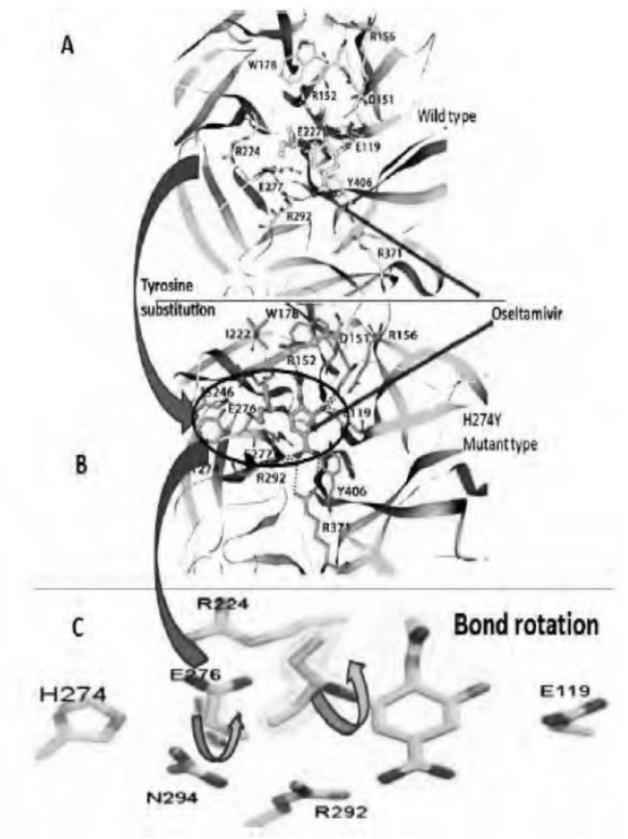


Figure. 2 : Apparatus of drug-resistance and mutation in the neuraminidase amino acid (H274Y, R292K, N294S, or E119V. A illustrates the communication of oseltamivir with feral type neuraminidase receptor. B presentation the communication of oseltamivir with mutant type (274Y), and C showing the mechanism of mutation, how E276 rotate and make pocket for OST, and binds to R224. Histidine restored by Tyrosine (bulkier residue) which transforms the other residues conformation like E276, R292 so that E276 not rotate and oseltamivir not properly bind.

In fig-2, its find that in the binding pocket only a few amino acids involve for interactions with inhibitor rather all the amino acids interact with each other for stabilization of binding pocket, so that makes a format for the active site. Mutation in amino acids perturbs the frame of pocket and pocket size which caused the improper for inhibitors.

Table-1: Showing the all effective mutation with substitute amino-acid at a specific position and effect of the mutation on enzymes and related drugs resistance. Mutation shows extremely frequent in influenza strains.

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VOL 1 ISSUE 1 (2016) PAGES 71 - 85

Received: 12/03/2016. Published: 29/07/2016

| Mutation | Amino acid change | Location | Replaced by | Effect of mutation |
|---|---|---|---|---|
| H275Y<br>H274Y<br>H273Y | Histidine (H) | 274 | Tyrosine (Y) | Cause oseltamivir resistance to H1N1. Same amino acids change in binding site in influenza type A N1, N2 and influenza type B respectively. |
| N294S | Aspargine (N) | 294 | Serine (S) | Antagonistic Effect the OST resistance in H3N2. |
| I222R | Isoleusine(I) | 222 | Arginine (R) | Reduced the susceptibility to OST and zanamvir to NA in H1N1 with H274Y act synergistically. |
| Q313R I427T | Glutamine<br>Isoleucine | 313<br>427 | Arginine<br>Threonine | Increase the resistance to both oseltamivir and zanamivir. |
| V241I V234M<br>D344N<br>D354G<br>N369K | Valine<br>Aspartic acid<br>Aspartic acid<br>Valine<br>Asperginine | 241<br>234<br>344<br>354<br>369 | isoleucine<br>Methienine Asperginine<br>Glycine<br>Lysine | Helps new strains of NA with compensate the defect of OST mutation. Helps for new virions. They cause negative effects with H275Y and reduce the fitness of virus. |
| S247N | Serine (S) | 247 | Asperginine (N) | Recent find mutation in H1N1 pandemic, highly resistance to OST. |
| R371K | Arginine (R) | 371 | Lysine | Associated with resistance of OST/ Zanamivir |
| E119V<br>E119G | Glutamic Acid (E) | 119<br>119 | Valine (V)<br>Glycine | Cause Zanamivir as well as OST resistance in N2-subtypes of NA.<br>Direct and indirect influence drug binding to NA receptor. |
| T148I | Threonine | 148 | Isoleucine | Reduced the NA activity to OST by 50 %. |
| E276<br>R224 | glutamic acid<br>Arginine | 276<br>224 | No change | Rotation takes place when OST bind and Glu276 make bond with Arg224 to form pocket for OST.it is main source key for mutation in H274 |
| **R292K** | Arginine (R) | 292 | Lysine (K) | All NA inhibitors resistance in N9- subtypes of NA, it confer high level resistance even in novel strains of swine |
| L607V, | Leucine (L) | 607 | Valine (V) | Mark on mutation but not sensitive for OST |
| K660R | Lysine (k) | 660 | Arginine (R) | Not effective, but mark on mutation |
| F103S, | Phenylalanine(F) | 103 | Serine (S) | Not effective, but mark on mutation |

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| | | | | |
|---|---|---|---|---|
| Q136K | Glutamate (Q) | 136 | Lysine (K) | Mutation in NA which confer zanamvir resistance and it give negative effect on viral growth |
| Q138R,<br>P139S<br>G140R | Glutamine (Q)<br>Proline (P)<br>Glycine (G) | 138<br>139<br>140 | Arginine (R)<br>Serine (S)<br>Arginine (R) | Monomer – monomer interaction show single mutation, which interfere OST and Zanamivir binding. |
| D187E | Aspartic acid (D) | 187 | Glutamic acid | In HA, Decrease virus affinity to linked with NAI to OST. |
| Y252H<br>Q248G | Tyrosine (Y)<br>Glutamate (Q) | 252<br>248 | Histidine (H)<br>Glycine (G) | Hypothesis that both mutation increased the affinity to NA with OST |
| T220S,<br>Q223R<br>E275V, T333A, D239G | Threonine (T)<br>Glutamate (Q)<br>Glutamic acid(E), Threonine (T) Aspartic acid(D) | 220<br>223<br>275<br>333<br>239 | Serine (s)<br>Arginine<br>Valine (V)<br>Alanine (A)<br>Glycine (G) | Mutation in HA but not interfere the antiviral resistance. Decrease the viral affinity to NAI. |
| S31N | Serine | 31 | Aspareginine | Mutation takes place in M2- channel, cause amantadine resistance. |
| K660R, L607V, V292I | Lycine (l)<br>Leucine (L)<br>Valine (V) | 660<br>607<br>292 | Arginine (R)<br>Valine (V)<br>Isoleucine (I) | Mutation in PB2 protein but not affect resistance. |
| F103S | Phenylalanine (F) | 103 | Serine (S) | Mutation in non-structural protein but no any effect on resistance. |
| W104G | Tryptophan (W) | 104 | Glycine (G) | Mutation in nucleoprotein but no any effect on anti-viral resistance. |

Other study showed that mutation in NA of H1N1 strains like Q136K, Q138R, P139S, G140R shows the monomer-monomer interaction in NA which alter the hydrogen bond interaction with R156 and D151 with zanamivir which confers zanamivir resistance. But research shows that Q136K benefitted for the H274Y mutation NA by reduced the enzymatic activity as well as reduced NA levels in viral particles. It shows the key site that can affect the susceptibility of neuraminidase inhibitors.

When Q136K mutation and H274Y mutation had introduced together give a negative effect on virus growth. This mutation increases the 86 fold IC50 against zanamivir as wild-type virus [35, 36]. Even in H5N1 the Y252H and Q248G mutation with the H274Y substitution in NA has been hypothesized to be responsible for an increased affinity of NA for oseltamivir. Y252H mutation does not affect the binding energy and interaction of oseltamivir with N1 but it theoretically important for oseltamivir resistance.

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Tyr is a larger side chain amino acid residue and it interacts with Arg224 and Glu276 by hydrogen bonding but not interacts with OST. It changes the other orientation of adjacent residues, which cause the shrinkage of the active site [13, 37].

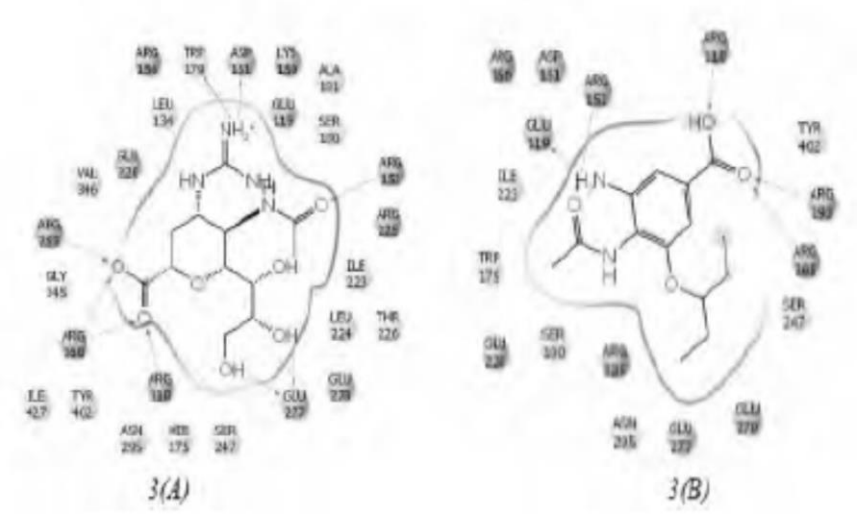


**Fig. 3, Communication of Zanamivir (3A) and oseltamivir (3B) with resistant neuraminidase receptor PDB id- 4B7Q. No any amino acids are the best pose for OST, but Asp151, Arg152 Arg156, Ser179, Ser180, Arg225, Glu228, Glu277, Glu278, Arg293, Arg368 and Tyr402 (according to N1-subtype numbering) binding site for the inhibitors. Other hand Ser180, Ile223, Ser247, His275, Asp295, and Try402 show stabilization effects on docked site.**

The new mutant sequence (PDB- 4B7Q) which are resistant against existing inhibitors but they do not bind the proper as wild-type NA receptor, so these are not effective against new strains. Structure-based drug design (SBDD) and homology modeling help for the new ligands against the new mutant sequence.

**Hemagglutinin**

Mutation in Hemagglutinin (HA) subtypes (H1- H16) which circulates in the human population through antigenic drift and shift. It also forms numbers of strains like H1N1, H1N2, H2N2, H3N2, H4N6, H10H8, H5N1, H5N7, etc. from HA. HA protein plays an important role to attachments, penetration, and neutralization of the host antibody response [39]. More than a few amino acid substitutions in the HA1 domain from 2004 to 2009: takes place like D35N, T82K, Y94H, K141E, N125D, D187E, R189K, R209K, D222G, Q223R, and E274K. Hemagglutinin residue 223 plays a critical role in the binding affinity of the galactose moiety of sialosaccharides

for the receptor binding site of HA [40, 41]. Mutation D187E and Q223R of HA would decrease virus affinity for the 2, 6-linked receptor because the salt-bridge between E187 and R223 would lead to narrowing of the receptor binding pocket. But Q223R mutants showed low rates of human-to-human transmission, whereas D187E mutation shows the human to avian type receptor switch. In 2004- 2008 presence of NA H274Y mutation with the HA proteins mutation at residues 82, 141, or 189 promotes virus replication so that new virions are produced [42]. In 2009 H1N1 pandemic HA receptor showed mutation D222G and Q223R to be critical for receptor binding and to cause a shift from alpha 2, 6 to alpha 2, 3 sialic acid receptor specificity [41, 42, 43].

**M2 channel**

M2 protein in virus helps the transfer of viral RNA to the nucleus to the human cells. It has two type pores, first is ion channel, the second one is lipid face pore. Ion channel has more affinity to the amantadine and rimantadine [44]. More than 7000 Influenza A field isolates were screened for specific amino acid substitutions in the M2 gene known to confer drug resistance towards amantadine rimantadine [2]. Among them, V27A L26F and S31N mutant show 98- 100 % against the M2- channel inhibitors like amantadine and rimantadine. S31N mutant in M-2 channel has a frequent, vulnerable effect on limited size, polarity, and dynamic nature of its amantadine-binding site changes so that it's the more vulnerable effect on vaccine formation, as well as in 27 positions of H1N1. But other mutations like V27A and L26F are less frequent. Until there are no effective vaccines developed against these influenza strains. Several mutations at surface protein, HA (T220S, E275V, T333A, D239G), PB2 (K660R, L607V, V292I), Nonstructural protein (F103S), and Nucleoprotein (W104G) were identified but none of them were likely to result in anti-viral resistance. Non-structural protein plays important role in identified mutation, the spread of species, viral tropism, and infectivity inhuman. Consequently these proteins have not been found very important role in influenza so that is not targeted as inhibitors.

**Mutation problems:**

Mutation in surface protein neuraminidase is a major problem for the design of drugs for influenza virus. This is due to change in one amino acid at the active site it changes all adjacent amino acids and results resistance. The conserved residues that interact with NAIs are under selective pressure, but only a few have been linked to resistance. Literature analyzed that NA active site consists of four conserved binding site and 12 residues, site-1 have positive charge Arg118, 227, and 371, site-2 negatively charged Glu-151, 119 and 227 site-3 Ile222, Trp178 , trp406, and site-4 consist of Glu276, and 277. The amino acid R118, D151, R152, E227,, R224,

E276, R371, and Y406 that directly interact with the NAls but have not reported to confer resistance to NAIs. They form the active site or pocket for inhibitors. Acquainted mutant H274Y, N294S, I223R, E119G are the not directly interact with NAI, they are located distant from active site but they affect the binding mode of inhibitors. They are only associated with resistance. In amalgamation with Y274, I117V, I119V, I223V mutation give synergetic outcome on oseltamivir resistance whereas addition to N294S to Y275 give antagonistic effect on oseltamivir resistance [12,].

Some accessories mutations help virus for infection and the majority of mutations to NAIs are caused by mutations within the NA gene itself. Changes to the HA gene and M2-gene can also lead to decreased susceptibility to NAIs. These changes to turn to decrease the need for NA activity substitutions, to more infectivity and new strains for virulence. But researchers say that induced mutation may help as unfavorable conditions and act as an inhibitor for NA. In Table -1 showing that all possible important mutations which take place in surface enzyme and M2-ion channel of influenza with its amino acids and its position. Overall mutation H274Y, N294S, I223R, E119V, E276V, S247N, Q136K, and S31N are the main frequent mutation which takes place in all types if surface protein as shown in table- 1 as bold letters.

Pharmacophore model of the existing molecule is also given a great contribution to understanding the interaction with the side chain of NA to NAI. In OST structure C1- position interacts the guanidine of arginine 118, 292, 371 of NA C4-position should be in the range of electrostatic interaction and maintain positive groups with Asp151, Glu119, and Glu227, like guanidine group in cyclopentene ring and dihydro-pyran derivatives. But C5-position having acetamido group unchangeable but it could be substituted by bulky hydrophilic segment so that binding pocket is NA can be filled. C6-position formed a hydrophilic region which formed the bond side chain of Glu277, Ser179, Arg156, Glu277, Arg292 [23, 38].

For new lead compounds the best pharmacophore characterized by five features, namely, one positive ionizable group, one negative ionizable group, one hydrophobic the point and two hydrogen-bond donors have a correlation coefficient of 0.902, root means a square deviation of 1.392, and a cost difference of 72.88, suggesting that a highly predictive pharmacophore model for highly conserved domain [19]. From this pharmacophore state expand new lead for the resistant active site

**CONCLUSIONS:**

There are so many mutations takes place in both surface glycoprotein neuraminidase hemagglutinin and M2- protein but only selected alteration mark the susceptibility of antiviral drugs. The result shows that the position 274, 294, 276, 277, 223 and 119 of NA has more effect

active site and also predisposed for mutation. Same as in HA residue 223 and 187 position and at 31 positions in M2 has more susceptible than others and help the high resistance towards antiviral drugs. This study showed that most frequent oseltamivir- resistant NA mutations including E119V, H274Y, R292K, I223R, and N294S which impact more susceptible for substitution in all strains and it gives the defenselessness profile to a narrative neuraminidase inhibitor and significant resistance to oseltamivir. 150-loop may also take as a future target for drug design. Pharmacophore model of OST and active site give proper information about the mutation and inhibitors for the NA. New anti-influenza drugs may inhibit the oseltamivir-resistant strains such as H274Y mutant and urgently need to battle against the pandemic influenza. There should be further research for targeting this important mutation by the help of structure-based or ligand-based method and design the drug against resistant flu strains.

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