

ORIGINAL ARTICLE

Development of lipid nanoparticles mRNA vaccine against viral Influenza infection

Mohammed M Kassab*

Instructor of microbiology and immunology, Faculty of pharmacy, Cairo University, Egypt.

ABSTRACT

Keywords: Influenza; RNA vaccine; virus; infection.

*Corresponding Author:
Mohammed Kassab
Instructor of microbiology and immunology, Faculty of pharmacy, Cairo university, Egypt.
Email:
Mohammed.Kassab676@gmail.com.
Mobile number:
+201032579044.

Background: Influenza is regarded as an over-serious evoke infection present which necessitate the evolution of novel improved vaccines. **The aim of the study:** Production of nanolipid particles of mRNA influenza vaccine by bioinformatics followed by careful categorization of the dose-reaction qualitative analysis. **Type of Study:** Screening experimental study. **Methodology:** The present study was performed in the faculty of pharmacy, Cairo University, Egypt. In our study, we designed an RNA vaccine of haemagglutinin (HA) and neuraminidase (NA) of the *Influenza A(H1N1 and H3N2) and Influenza B(Yamagata and Victoria) viruses* by bioinformatics and genetic engineering. The particle size of the lipid nanoparticles vaccine delivery system was approximately 100 nm. **Results:** The vaccine showed 86% efficacy during preclinical trials while 78% during human clinical trials phases 1/2. It showed superior biological activity and fewer side effects than other vaccines such as the Influxac vaccine. The efficacy lasted for 12-14 months. Molecular mass of Haemagglutinin was approximately 66 KDa as determined by a mass spectrometer. As well molecular mass of neuraminidase was found to be about 70 KDa. The protective neutralizing antibodies were produced against haemagglutinin. **Conclusion:** The vaccine, in our study, was effective as prophylaxis against viral infection with Influenza.

INTRODUCTION

The *influenza A(H1N1 and H3N2) and Influenza B(Yamagata and Victoria) viruses* are starring zoonotic infectious agents that ground extreme signs and symptoms and possess the opportunity to result in an overflowing range of deaths and awesome budget friendly loss.¹ Immunization remains the high-grade choice to prevent the influenza virus pathological procedure due to the fact few tablets are beneficial towards viral infections. Prevention of viral illnesses may be completed via way of means of the management of preformed antibody that gives passive immunity or via way of means of using vaccines that set off energetic immunity.²

Influenza viruses are the simplest participants of the Orthomyxovirus family. The Orthomyxoviruses has a segmented RNA genome (normally 8 pieces). The term "myxo" refers back to the remark that those viruses have interaction with mucins (glycoproteins at the surface of cells).³ In addition, the Orthomyxoviruses are small (110 nm in diameter). Influenza A virus is the principle motive of global epidemics (pandemics).⁴

Characteristics: Enveloped virus with a helical nucleocapsid and segmented, single-stranded RNA of negative polarity. RNA polymerase within the virion. The essential antigens are hemagglutinin (HA) and neuraminidase (NA) on separate surface spikes.⁵ The antigenic shift of *Influenza A and B* types proteins are caused by the reassortment of RNA segments which leads to the epidemics of influenza. *Influenza A* viruses of animals are the source of the brand new RNA segments. Antigenic drift because of mutations additionally contributes. The virus has many serotypes due to those antigenic shifts and drifts. The antigenicity of the inner nucleocapsid protein determines whether or not the virus is an A, B, or C influenza virus.

Transmission occurs via respiratory droplets.⁶

Pathogenesis: Infection is constrained typically to the epithelium of the breathing tract.⁷ **Laboratory Diagnosis:** The virus grows in culture of cells and embryonated eggs and may be detected via way of means of hemadsorption or hemagglutination.⁸ It is recognized via way of means of hemagglutination inhibition or complement fixation.⁹ A fourfold or more antibody titer upward push in convalescent-serum is diagnostic.¹⁰

Treatment includes amantadine or rimantadine may be used. The neuraminidase inhibitors e.g. zanamivir and oseltamivir also are available.¹¹ Two vaccines are available: a killed (subunit) vaccine containing HA and NA and one containing a live, temperature-sensitive mutant of the influenza virus. Live vaccine replicates in cool nasal passages in which it induces secretory IgA.¹² Both vaccines include the traces of *influenza A and B* virus presently inflicting disease. The killed vaccine isn't always an excellent immunogen and ought to be given annually.¹³ Recommended for human beings older than age sixty five years and people with continual illnesses, in particular of the coronary heart and lungs. Amantadine, rimantadine, zanamivir, or oseltamivir may be used for prevention in unimmunized human beings who've been exposed.¹⁴ **Global human clinical trials:**

Phase 1: A section 1 trial includes cautious assessment of the dose-reaction dating and the pharmacokinetics of the brand new vaccine in a small range of regular human volunteers (eg, 20–100). In section 1 study, the intense results of the agent are studied over a broad variety of dosages, beginning with one which produces no detectable impact and progressing to one which produces both a significant physiologic reaction and a minor poisonous impact.¹⁵

Phase 2: attempt regard assessment of a drug in a average number of human volunteers (eg, 100–200) with the mark sickness. A placebo or positive control drug is enclosed in a single-blind or double-blind design. The destination is to find out whether the causal agent has the desired efficacy (i.e., consequences sufficient therapeutic consequence) at doses that are tolerated by ill patients. Elaborated information are collected paying attention to the pharmacodynamics and pharmacokinetics of the medicine in this long-suffering population.¹⁶

Phase 3: attempt commonly regard numerous natural human volunteers (eg, 1000–6000 or more, in numerous centers) and numerous clinicians who are exploiting the vaccine in the mode planned for its crowning broad use (such as, in outpatients). Such surveys remarkably consider positive controls and placebo in a double-blind biological process design. The destination are to investigate boost, under the conditions of the projected clinical utilization, the scope of advantageous activity of the new vaccine, to analyze it with placebo (negative control) and older therapy (positive control), and to detect toxicities that take place scarcely as to be insensible in stage 2 examination.

Phase 4 represents the post-marketing stage of the assessment, in which very rare toxicities are detected and reported early enough to prevent serious therapeutic catastrophes. Enzyme-Linked Immunosorbent Assay (ELISA) can be utilized to quantify either antigens or antibodies in test samples. It is based on covalently linking an enzyme to a known antigen or antibody, reacting the enzyme-linked material with a patient sample, adding a substrate for the enzyme and assaying for enzymatic activity. This performing is virtually as sensitive as the

radioimmunoassay (RIA), but does not need particular instrumentation or radiolabels.¹⁷ Our study aimed to evolve new mRNA influenza vaccine lipid nanoparticles to overcome influenza A and B strains infections. The present study regarded restrained persuasion of the dose-reaction chemical analysis and the pharmacokinetics of the marker new RNA *Influenza* vaccine in a small range of lawful humanlike voluntary.

METHODOLOGY

This study was a patent with a registration number 1356/2021 authorized by the ministry of scientific education, Egypt and was carried out in a research project number 46362/2021 funded by STDF.

Ethical statement:

In the existing study, we followed All applicable institutional, international and/or national guidelines for the attention and utilization of humans and animals. All processes carried out in study including humans and animals were authorized by the local authorities, Ethical committee for human and animal handling at Cairo university(ECAHCU), at the Pharmacy faculty, University of Cairo, Egypt in agreement with the recommendations of the weathrall report with approval number P-1-2-2021. All efforts were performed to ablate the number of humans and animals utilized and their suffering during study.

Source of animal models:

One hundred male transgenic mice weighing 180-190 gm were obtained and sanctioned for legalization from pharmacology and toxicology department of faculty of pharmacy, Cairo university, Egypt.

Inclusion criteria for animal models are :

Adult animal; Can be induced by Influenza virus infection(1×10^3 TCID₅₀ for *Influenza A H1N1*, 1×10^7 TCID₅₀ for *Influenza A H3N2* , 1×10^4 TCID₅₀ for *Influenza B Victoria* and 1×10^6 TCID₅₀ for *Influenza B Yamagata*) through intranasal route of administration such as transgenic mice humanized by lung human cells for increasing expression of viral proteins and evoking strong humoral and cell mediated immunity. Incubation time ranged from 3-5 days for appearance of influenza symptoms to occur.

Exclusion criteria are: Young animal; Pregnant female animal

Place and date of the study: This study was done in faculty of pharmacy, Cairo university, Egypt between January 2021 and November 2022.

Type of study: Screening experimental study.

Methods:

The potential open reading frames for HA and NA were identified via bioinformatics. RNA of HA and NA of Influenza virus were expressed and purified. Genes of interest were cloned using PCR then inserted into PUC18 exploiting Bam HI and SphI restriction endonucleases II for the digestion of the plasmid, followed by ligation by ligase enzyme. Linearization of the pDNA template was done via the restriction enzymes EcoRI, Eam11041 and Lugal (obtained from ThermoFisher scientific company, USA). The linearized pDNA template was in vitro transcribed into mRNA in a mixture containing T7 recombinant RNA polymerase and nucleoside triphosphates, RNase inhibitor and 5X transcription buffer (obtained from ThermoFisher scientific company, USA). mRNA transcripts were capped by transcription through addition of a cap analog like the dinucleotide m7G(5,)-ppp-(5,)-G (called regular cap analog obtained from ThermoFisher scientific company, USA); furthermore mRNA transcripts were modified via mRNA tailing by Poly(A) polymerase (obtained from ThermoFisher scientific company, USA). Then DNase I and protein kinase K (obtained from ThermoFisher scientific company, USA) were added for post in vitro transcription clean up. Purification was performed

via high performance liquid chromatography(HPLC) or mRNA extraction method. mRNA purification was carried out through organic extraction method titled acid guanidinium thiocyanate-phenol-chloroform extraction. It is a liquid extraction technique for isolating RNA. It has a high purity and recovery of RNA. Chloroform solutions consist of 96%chloroform and 4% isoamyl alcohol(Isoamyl alcohol reduces foaming and ensures deactivation of RNases) mixture was blended with an equal volume of phenol to obtain 25:24:1 solution. For mRNA purification, the PH was retained at about 5 via addition of 65 μ L of lactate solution or 140 μ L of 1 M HCl to 100 mL of medium, which keeps mRNA in the aqueous phase preferentially. This performing relies on phase separation by centrifugation of a mixture of aqueous sample and a solution containing water-saturated phenol and chloroform, resulting in an upper aqueous phase and a lower organic phase(mainly phenol). Guanidinium thiocyanate a chaotropic agent, was added to organic phase to assist in the denaturation of RNases that abase mRNA .The RNA partition in aqueous phase,while protein partitions into organic phase. Under acidic conditions(PH 5) DNA partitions into the organic phase while mRNA remains in aqueous phase. RNA was yielded from the aqueous phase by the precipitation with 2-propanol; then propanol was washed with ethanol and the pellet was briefly air-dried and dissolved in RNase free water. The ratio of the absorbance at 260 and 280nm was victimized to evaluate the purity of mRNA transcripts. The quantities and sizes of recombinant mRNA transcripts were boost analyzed via Northern blot technique: mRNA transcripts were separated via agarose gel electrophoresis according to their sizes postdated by denaturation and transfered to nitrocellulose membrane where they were intercrossed to radio-labeled probes. The purified mRNA was enclosed with vaccine delivery system consisting of lipid nano-particles of dimethyl dioctadecyl ammonium bromide(DDAB) forming lipid bubbles surrounding recombinant mRNA transcripts .The particle size of lipid nanoparticles vaccine delivery system was approximately 100 nm. Formulation was prepared which comprised a sterile suspension of Lipid nanoparticles -mRNA Influenza vaccine for intramuscular injection. Each 1 ml dose is formulated to incorporated 10mcg of HA, 5mcg NA, 50 mcg of dimethyl dioctadecyl ammonium bromide lipid (DDAB), 0.5 mg of Aluminium hydroxide. As well each dose restrained 4.4mg of sodium chloride and 0.61 mg of sodium dihydrogen phosphate dihydrate.

Immunogenicity in animal models were assessed via injecting the purified RNA in transgenic mice by intraperitoneal route of administration:

In vitro evaluation of vaccine on transgenic animals(mice).

Transgenic mice are those whose genes were edited by recombinant DNA technology exploiting tissue culturing technique. A transgenic animal is one that possess an integrated gene of DNA sequence (a trans-gene) which was transported by human involvement into a cell genome. The purpose of the transgenic mice was to rise the expression of HA and NA antigenic proteins inside the human host cells. One hundred transgenic mice (weighing180-190gm) were administered with the vaccine. They standardized two doses, 21 days were apart from each other. The first dose was half the second booster dose.¹⁸

Screening and bio-assay of the biological activity and toxicological effects of the vaccine.

Protection tests were used to determine the potency of vaccines .

Succeeding protection with the immunizing agent that was being tried, groups of transgenic mice were administered with increasing numbers of Organisms. The lowest number of microorganisms fatal for fifty in percent of animals (LD50) was ascertained and analyzed to LD 50 in non-immunized animals in order to touchstone the preventive ability of the immunizing agent. Stratified quantities of blood serum from vaccinated individuals were transported to natural mice, which were then vaccinated with the transmissible factor. The overflowing solution of serum efficacious at defending 50% of animals (ED50%) was dictated as a standard of the effectiveness of the immunizing agent.

Human evaluation of the mRNA influenza vaccine via randomized human clinical trials phases 1-2:

3 groups of human volunteers were included in our study. Each group consisted of 100 subjects: (Group 1) (Negative control group) were administered the placebo intramuscularly (Group 2) (Positive control group) were administered the standard Influxac Influenza killed vaccine intramuscularly. (Group 3) (Test group) were administered the test mRNA Influenza vaccine intramuscularly. The 3 groups were challenged with graded amounts of the infectious microorganisms after 2 weeks to allow the appearance of protective neutralizing antibodies. After 21 days the 3 groups administered booster doses. The protection power of the test vaccine was evaluated during 2 years. The detection of the protective antibodies was done via enzyme linked immunosorbent (ELISA) assay.

ELISA procedure for detection of the neutralizing antibodies to mRNA Influenza vaccine:

To the bottom of the well, antigens(HA and NA) were connected. Purified influenza virus preparations were utilized in ELISA. Purification of viruses was finished via eggs injected with 450 plaque establishing units(PFU) of virus. Later an incubation period of 48 hours at 37⁰C, cooling of eggs down to 4⁰C for 10 hours. Fluid of allantoic was harvested and cleared by centrifuge at 4000 rpm(Beckman L7-65 ultracentrifuge with SW-28 rotor) for 3 hours at 4⁰C utilizing a 40% sucrose cushion solution. Furthermore, the aspiration of the supernatant was achieved and in 3 ml of phosphate buffered saline, the refined virus preparation enclosing antigens on its surface was afterwards inactivated exploiting neutral buffered 0.04% formaldehyde for 48 hours at 4⁰C.

Antigens were attached to antibody in patient's liquid body substance. Antibody to human IgG linked to patient's IgG, the antibody to human IgG was enzyme-bonded(enzyme of Horseradish per-oxidase). Addition of substrate for the enzyme was made altering colour when enacted upon via the enzyme. Activity of Enzyme was deliberated via addition of the substrate for the enzyme and computation of the reaction of colour at 450nm wave length utilizing a UV spectrophotometer.

Flow cytometry technology:

(Invitrogen Attune CytPix flow cytometer, USA) was utilized to observe and investigate mRNA HA and NA vaccine-specific CD+4 and CD+8 T lymphocytes. In this test, the patient's cells were tagged with a monoclonal antibody. These antibodies were against proteins specific to the cells of involvement (e.g. CD4 protein when measuring the number of T helper cells). Monoclonal antibodies were tagged with fluorescent dyes such as fluorescein and rhodamine. Individual cells were passed via the beam of laser driving them to fluoresce. The fluorescence was assessed by a machine known as Fluorescence activated cell sorter (FACS).

Statistical analysis

All cultures were conducted in triplets. Their presentation was by means and standard deviation. One way analysis of variance (p value \leq .05) was used as means for performing statistical analysis and also, statistical analysis based on excel-spreadsheet-software.

RESULTS

The immunizing agent stated eighty six in percent of the efficacy in preclinical studies(table 3) and seventy eight in percent during Phases 1/2 clinical trials(table 4). It presented improved biological activity and less side effects than other vaccines such as the Influxac vaccine. The consequence continued for 12-14 months. The neuraminidase had a molecular weight of about 70 kDa. As well the molecular weight of hemagglutinin was approximately 66 kDa as observed aside utilizing mass spectrometry. Moreover Protective neutralizing antibodies were obviously created against hemagglutinin as shown in table 1. CD+4

and CD+8 T lymphocytes counts were assessed in clinical trials phases 1/2 and the results were demonstrated in table 2. The LD50% of influenza virus was found to be greater than 70 mcg/ml. The ED50% for the mRNA influenza vaccine was found to be 10 mcg/ml. mRNA influenza vaccine formulation was 15 mcg/ml.

DISCUSSION

The vaccine resulted in 86% efficacy during preclinical trials on animal models, while showed 78% protection power during human clinical trials phases 1/2. It showed better biological activity and fewer side effects than other standard vaccines such as Influvac vaccine. The ratio of the absorbance of mRNA transcripts at 260 and 280nm using UV spectrophotometer was nearly 2 indicating high purity of recombinant mRNA transcripts which were purified by organic extraction method. Its efficacy lasted for 12-14 months and the vaccine needs to be updated periodically due to the high rate of mutations of this virus. During human clinical trials 94 subjects were infected in negative control group, while 41 infected in positive control group and 22 infected in test group. The LD50% of Influenza virus was found to be greater than 70 mcg/ml. The ED50% of the mRNA influenza vaccine was found to be 10mcg/ml. The formulation of mRNA influenza vaccine was 15 mcg/ml. RNA Influenza vaccine is recommended for people older than 6 months of age. The recommended dosage of this vaccine is 15 mcg/ml dose given intramuscularly annually during September. The test vaccine is contraindicated for persons having allergy against eggs. The adverse effects noticed was mild pain at the site of intramuscular injection and mild fever for few days which were relieved by simple analgesics such as paracetamol and ibuprofen. Our vaccine contains both influenza A and B viruses mRNA of haemagglutinin and neuraminidase which were the main mode of prevention. Preceding 2013, the immunizing agent was trivalent and restrained recent isolates of two A strains (H1N1 and H3N2) and one B strain. During 2013, quadrivalent vaccines incorporating two A strains and two B strains became available. The vaccine is usually reformulated each year to comprise the present antigenic strains. Two principal kinds of vaccines of influenza are accessible, a live attenuated

Vaccine and a killed vaccine. A killed vaccine holding refined protein subunits of the virus (hemagglutinin and neuraminidase) was exploited for numerous years. The virus was triggered off with formaldehyde moreover treated with a lipid solvent that dis-aggregated the viruses.¹⁹ HA was the most important antigen due to its provocation of neutralizing antibodies. The immunizing agent was administered via intramuscular route. A great-dose killed vaccine that restrained 4 times as more hemagglutinin as the accepted vaccine was recommended for those over 65 years of age. The else vaccine was a live, attenuated immunizing agent comprising temperature-sensitive mutants of *influenza* A and B viruses. In the cooler (33°C) nasal mucosa; These temperature-sensitive mutants could replicate where they stimulated IgA. The live virus in the vaccine hence vaccinated but did not ground illness. The immunizing agent was challenged via dispersion into the nose. The inactivated vaccine and our mRNA vaccine were advisable for adults; whereas The live immunizing agent was suggested for children. The live vaccine should not be given to pregnant women or to immunocompromised individuals to avoid the infection. Most of the immunizing agents were ready-made in chicken eggs, and any person who had a evidential hypersensitivity reaction to proteins of egg should avert these immunizing agents. Nevertheless, during 2012, FDA sanctioned a killed influenza immunizing agent (Flucelvax) ready-made in calf kidney cell culture. The immunizing agent possess two merits: It can be conferred to those with allergy of egg, and it shows a abbreviated turnaround time, thus the current drift mutant can be utilized. As well during 2012, the food drug administration American organization authorized a recombinant immunizing agent (Flublok) ready-made by inserting the gene encrypting the viral hemagglutinin into an insect virus (baculovirus) that was

propagated in cell culture of insect. The immunizing agent restrained Sublimate hemagglutinin as the immunogen. It could too be bestowed to those with allergy of egg. Because little IgA was made and the titer of IgG was relatively low, The inactivated immunizing agent was not a good immunogen. Protection continued solely six months. Before the flu season (e.g., in October) Yearly boosters are suggested and should be granted soon. The boosters besides render a possibility to vaccinate against the up-to-the-minute antigenic alterations. The immunizing agents should be given to all persons 6 months and older who do not have a contraindication to get the vaccine. This is peculiarly crucial that people with chronic diseases, in particular cardiovascular and respiratory conditions, obtain the immunizing agent. It should as well be surrendered to health care personnel who are liable to transfer the virus to those at high probability of infection. One adverse consequence of the vaccine of influenza used in the 1970s comprising the swine influenza strain that crusaded influenza in humans was an exaggerated risk of Guillain-Barré syndrome, which is defined by an ascending paralysis. Investigation of the adverse actions of the influenza vaccines in utilization during the last 10 years demonstrated no augmented hazard of Guillain-Barré syndrome. Our RNA influenza vaccine does not drive Guillain-Barre syndrome because RNA vaccines do not enter the nucleus of the human cells and remain extracellular; besides RNA vaccine contains only two portions of the infectious agent which are sufficient to cause this autoimmune disorder. Our mRNA influenza vaccine is a Quadrivalent vaccine containing the mRNA of haemagglutinin and neuramindase of the strains of Influenza A and B viruses. It stipulates protective covering against these strains effectively.

The role of immunity in prevention of the infection:

This vaccine strongly stimulated the humoral immunity and moderately stimulated the cell mediated immunity.

The humoral immunity was the main body defense mechanism that prevented the infection. The main neutralizing antibodies in blood were IgM, IgG1 against HA and NA of the virus. Few IgA antibodies against HA and NA of the virus were produced because it was not relinquished by the natural route of infection. This vaccine moderately excited cell mediated immunity (both helper CD4+ and cytotoxic CD8+ T. lymphocytes) against the pathological process. Advantages: No reversion to virulence is attainable. Disadvantages: Excretion of immunizing agent virus and transmission to nonimmune contacts is not achievable, thus it does not impart to the evolution of the herd immunity against this viral pathological process; Shorter duration of action than live attenuated vaccine was observed. It inevitably requires to be stored at -70°C in a refrigerator to stave off spoilage and impurity.

CONCLUSION

An mRNA immunizing agent of Influenza HA and NA was efficacious as prophylaxis agent against viral pathological process of *Influenza* virus and mutant forms of its mutant forms. It can be given for people older than 6 months. we suggest to explore contentiously brand-new approaches for the advance of new vaccines against the mutant types of this lethal viral infection worldwide whenever possible. It efficaciously aggravated both the humoral and the cell mediated immunity.

REFERENCES

1. Parveen Kumar.Kumar, Clark's clinical medicine ; 2020.p.774-811.
2. Caroline S,Zeind Michael G.Applied therapeutics, the clinical use of drugs; 2018; 16:1002-1031.
3. Trevor Anthony,Katzung Bertram,Kruidering-Hall Marieke.Katzung Trevor pharmacology examination board review ;2022.p.1142-1257.
4. Bardal Stan,Waechter Jason, Martin Douglas.Applied pharmacology; 2021; 9:276-328.
5. Olson James. Clinical pharmacology made ridiculously simple; 2020; 12:93-109.
6. Levinson Warren.Review of medical microbiology and immunology; 2021; 9:211-244.
7. Swanson Larry N, Souney Paul F, Muntnick Alan H, Shargel Leon.Comprehensive Pharmacy Review for NAPLEX; 2019.p.678-707.
8. Fisher Bruce, Champe Pamela, Harvey Richard. Lippincott illustrated reviews microbiology; 2021; 45:1475-1533.
9. Dipro Cecily, Schwinghammer Terry, Dipro Joseph, Well Barbara.pharmacotherapy handbook; 2021; 5:99-114.
10. Golderg Stephen. Clinical physiology is made ridiculously simple; 2020; 11:404-4436.
11. Wilson Golder N.Biochemistry and genetics; 2019; 23:598-649.
12. Metting Patricia J.Physiology; 2019; 14:437-505.
13. Tanja Becker et al(2021). Influenza vaccines: success and continuing challenges. The journal of infectious diseases, volume 224,issue 4,pages S540-S419.
14. Sook-San Wong et al(2013). Traditional and new Influenza vaccines. Journal of clinical microbiology reviews. 26(3): 476-492.
15. Barry C Buckland(2015). The development and manufacture of influenza vaccines. Journal of human vaccine immunotherapy. 2015;11(6):1357-60.
16. Hossam Waleed et al(2022). The effect of of influenza vaccine in reducing the severity of clinical outcomes in patients with COVID-19: a systematic review and meta-analysis. Nature journal. Article number: 14266(2022).
17. Angiezel Merced-Morales et al(2022). Activity and composition of the 2022-23 Influenza vaccine-United states, 2021-22 season. Journal of MMWR Morbidity and Mortality weekly report. 2022 Jul 22; 71(29): 913-919.
18. Emma L. Rawlins et al(2012). The amazing world of lung-specific transgenic mice. Am j Respir Cell Mol Biol. 2012 Mar; 46(3): 269-282.
19. Jidang Chen et al(2021). Advances in development and application of Influenza vaccines. Journal of frontiers in immunology. 12:711997.

LIST OF TABLES AND FIGURES:

Table 1. It represents the absorbance of different serum neutralizing antibodies to Influenza RNA vaccine via ELISA:

Conc.(ng/ml)	Absorbance
1.55	0.187
3.07	0.259
6.19	0.349
12.5	0.567
25	1.029
50	1.809
100	3.034

Table 2. VACCINATION AGAINST INFLUENZA VIRAL INFECTION:

Description	Vaccinated	Pre-vaccinated
CD+4 COUNT	1050	1000
CD+8 COUNT	720	600
Total	1770	1600

Table 3. VACCINATION OF TRANSGENIC MICE WITH RNA INFLUENZA VACCINE DURING ANIMAL TESTING PRECLINICAL TRIALS:

Description	Vaccinated	Non-vaccinated
Alive	86	31
Dead	14	69
Total	100	100

Table 4. It represents protection power of RNA Influenza vaccine in randomized clinical trials:

Description	Vaccinated		Nonvaccinated
	Test	Standard	Control
Infected%	22	41	94
Noninfected%	78	59	6

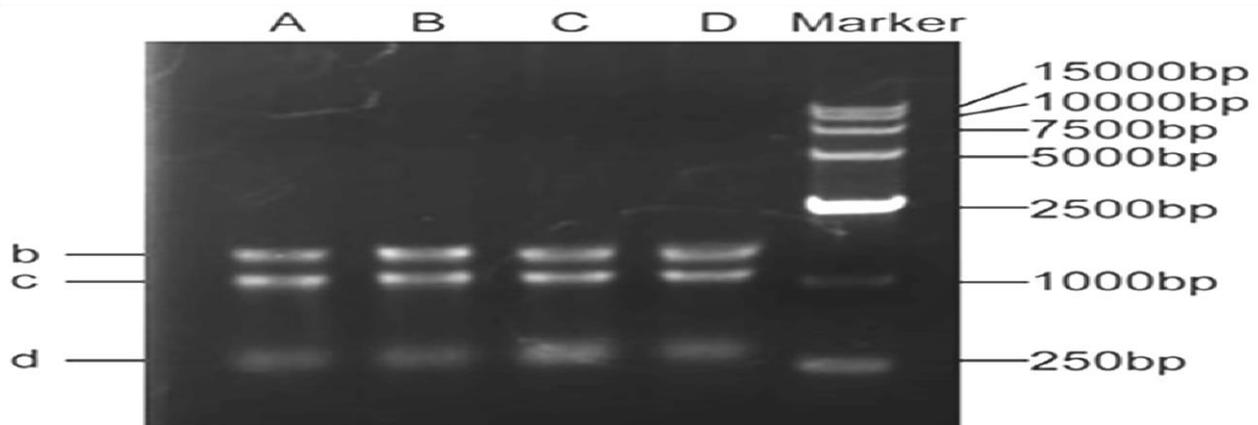


Figure 1. It displays different amounts and sizes of mRNA transcripts of HA and NA measured via Northern blot technique.¹

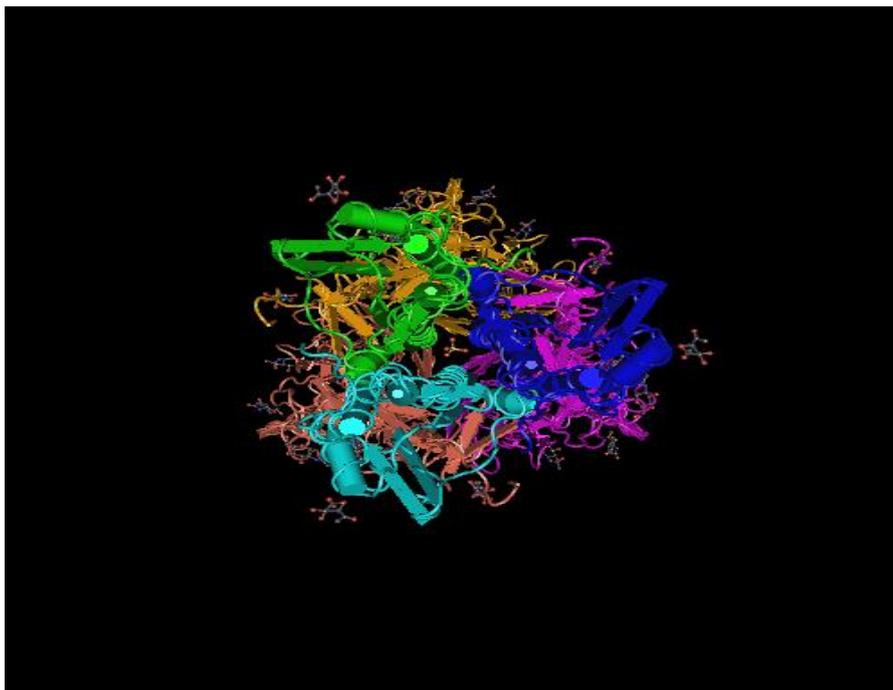


Figure 2. It represents 3D structure of *Influenza* haemagglutinin. It was composed of 342 amino acids.

¹ Molecular mass of neuraminidase was found to be about 70 KDa; while Molecular mass of Haemagglutinin was approximately 66 KDa as ascertained by a mass spectrometer. The neutralizing antibodies were yielded against haemagglutinin. The percentage of the sublimate proteins were nearly 86% via northern blot technique.

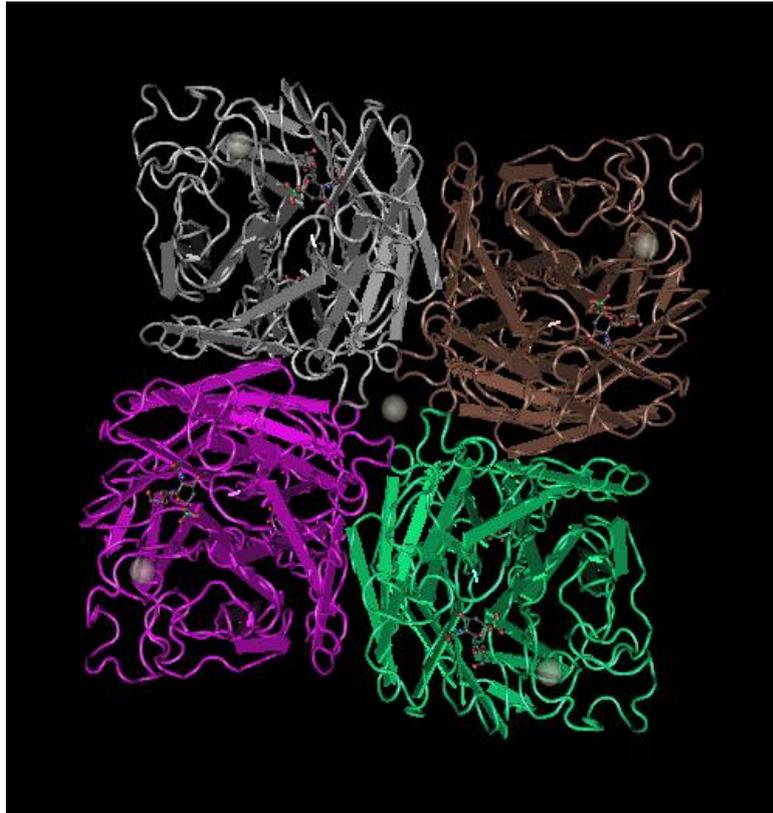


Figure 3. It represents 3D structure of *Influenza* neuraminidase. It was composed of 366 amino acids.

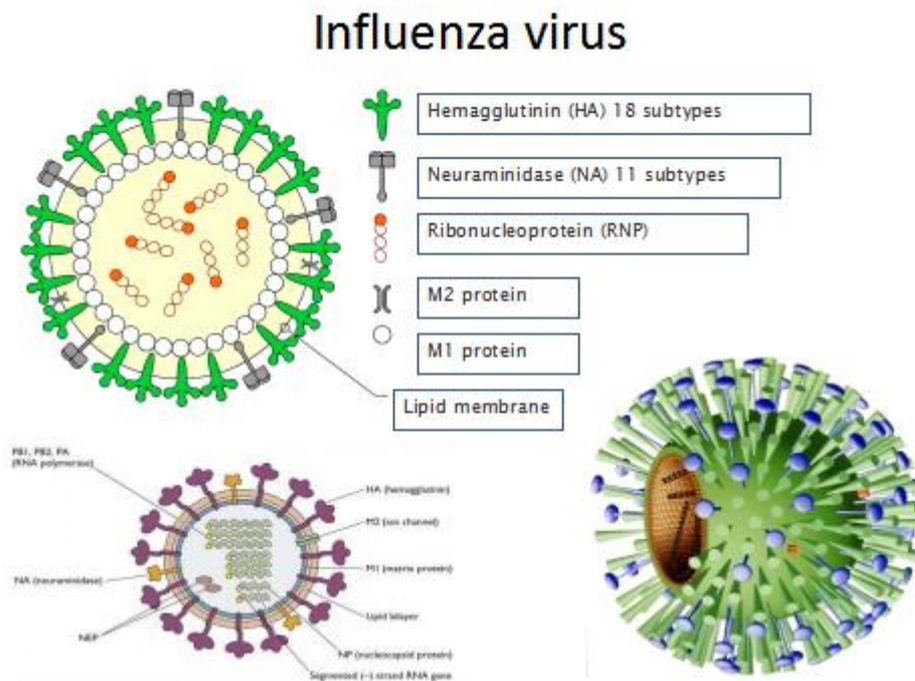
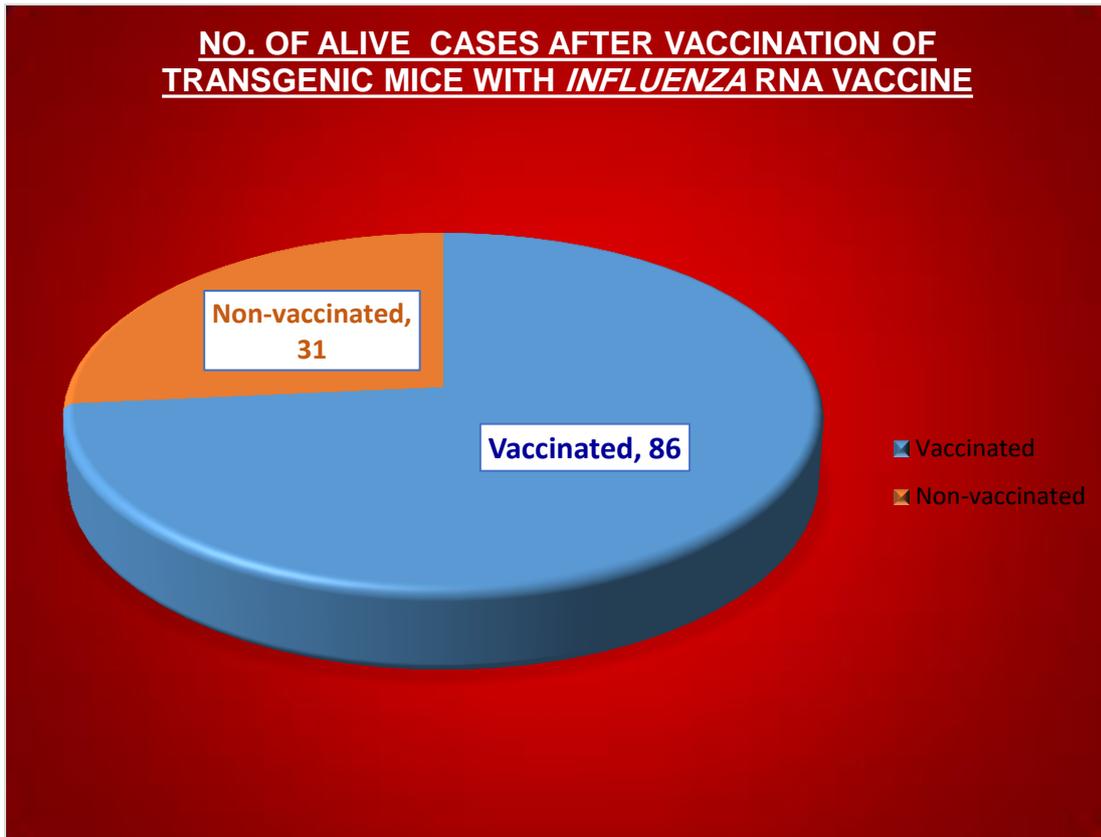
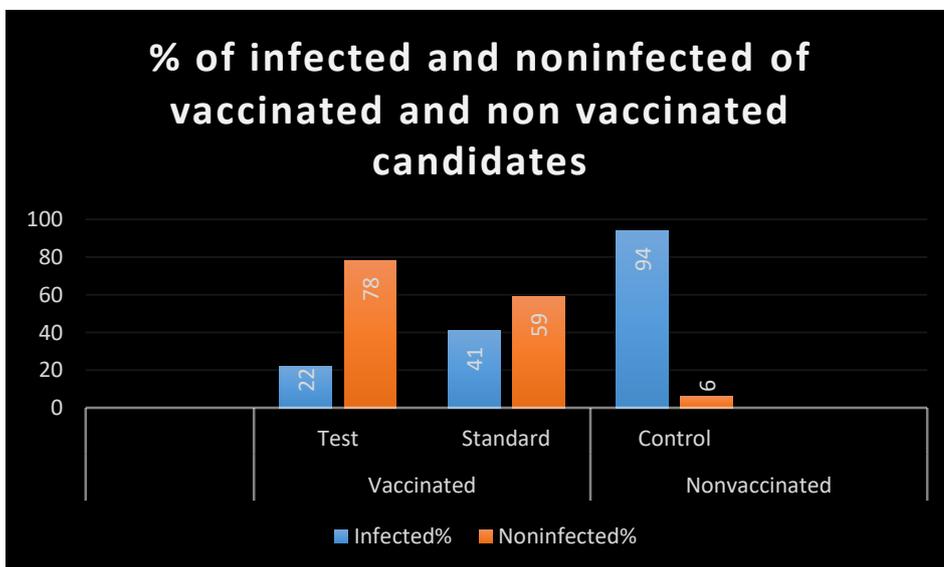


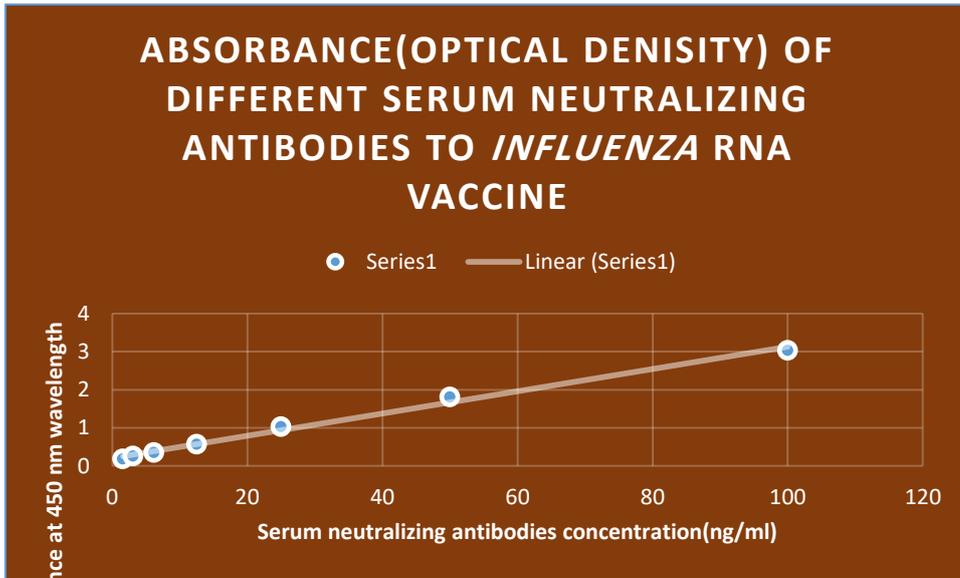
Figure 4. It displays the structure of *influenza A(H1N1 and H3N2)* virus.



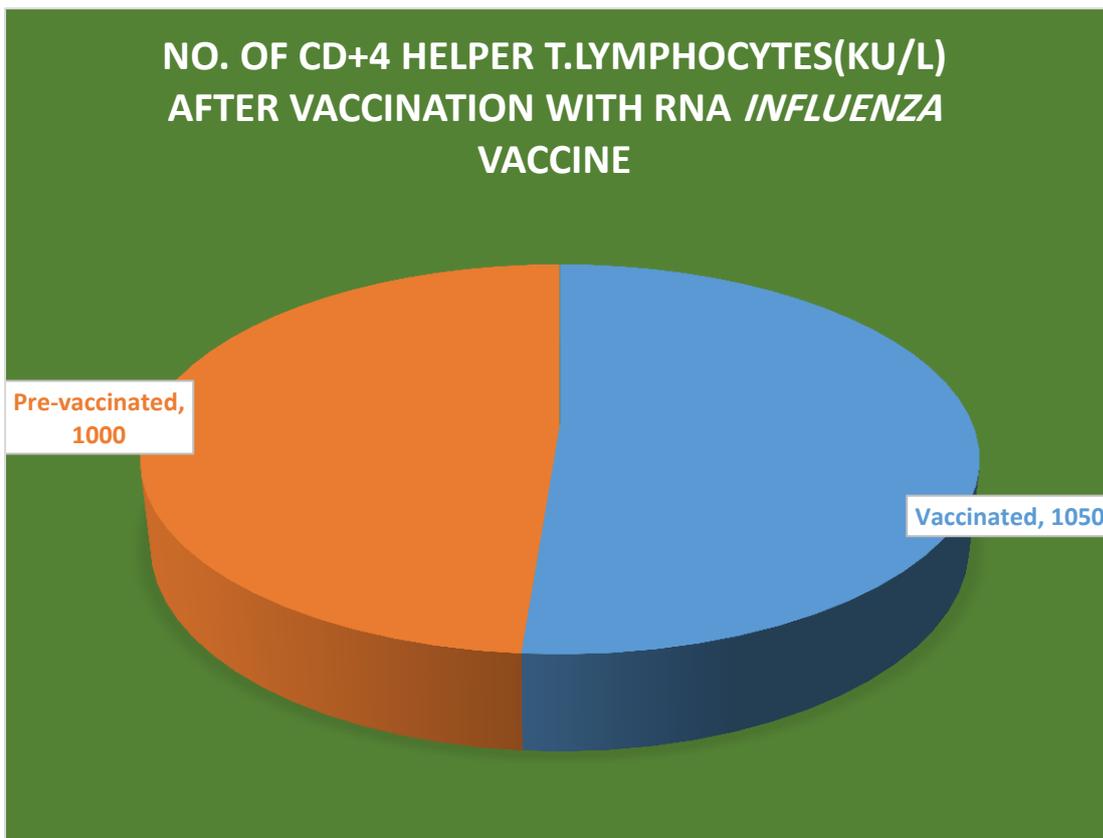
Graph 1. It displays the protection power mRNA *influenza* vaccine during preclinical animal testing was 86%.



Graph 2. It shows that the protection power of mRNA *influenza* vaccine during human clinical trials phases 1/2 was 78%.

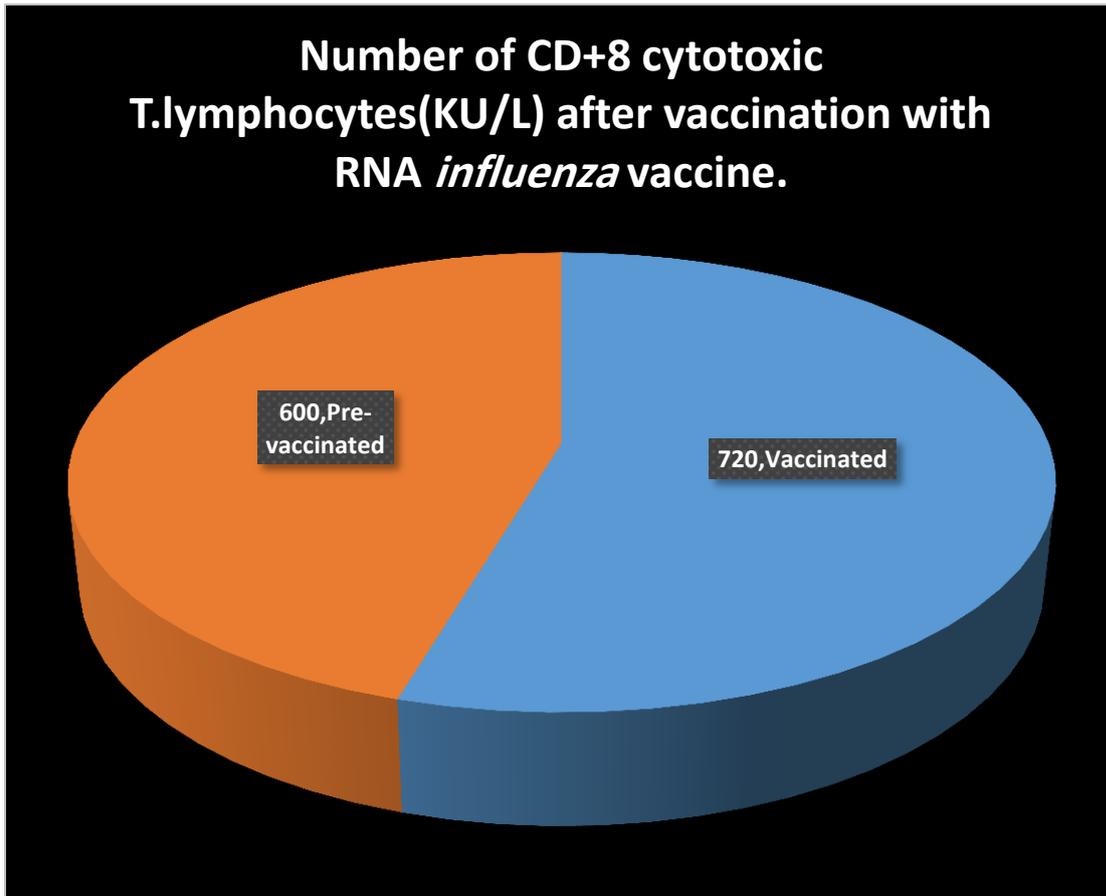


Graph 3. It represents the absorbance of different serum concentrations of neutralizing antibodies to *Influenza* RNA vaccine via ELISA.



Graph 4. It represents the ability of RNA influenza vaccine to evoke the cell mediated immunity during human clinical trials phases 1/2.²

² KU/L: One thousand unit per litre.



Graph 5. It demonstrates the ability of RNA *influenza* vaccine to elicit the cell mediated immunity during human clinical trials stages 1/2.³

³ KU/L: One kilo unit per litre.