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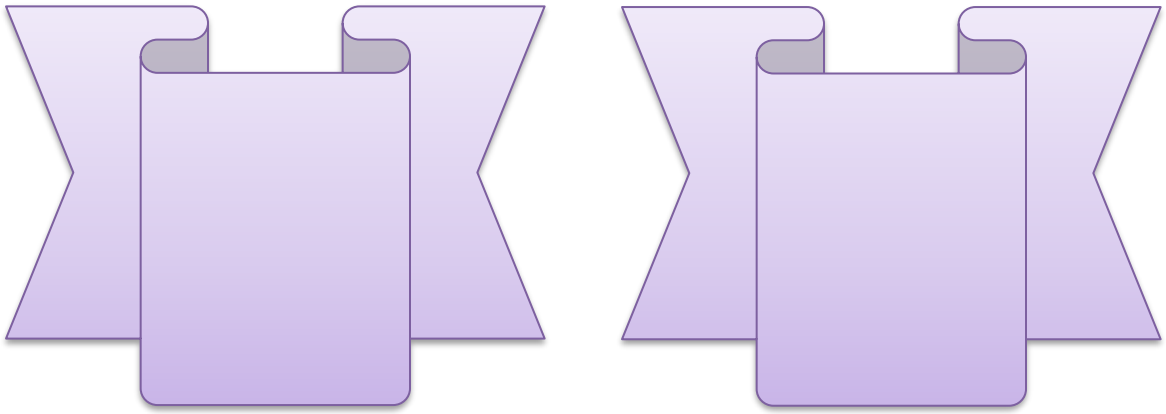

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Original Article

Development of Lipid Nanoparticles [LNP]-RNA Vaccine Against Neisseria Meningitidis CPS Gene Locus Facilitating the Synthesis of The Capsular Polysaccharide in Egypt

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ABSTRACT

Background: Neisseria meningitidis meningitis is a dangerous, widespread illness in Egypt nowadays. Immunity lasts a long time. Worldwide, meningitis and meningococcemia, which are caused by Neisseria meningitidis, are the leading infectious causes of mortality in children.

Aim of the work: The creation of a lipid nanoparticle [LNP]-RNA vaccine in Egypt against the Neisseria meningitidis cps gene locus has made it easier to produce capsular polysaccharide.

Patients and Methods: This was an Experimental screening examination. In this investigation, we created an LNP-RNA vaccination against the cps gene locus, which facilitates the pathogenic bacterium's ability to synthesize capsular polysaccharide. Lipid nanoparticles with a particle size of 76 nanometers served as the vaccine delivery method

Results: The vaccine's effectiveness was 93%. Compared to other conventional vaccinations, it had fewer side effects and higher biological activity. Due to the development of protective neutralizing antibodies similar to those elicited by earlier conventional vaccinations, the immunity was long-lasting.

Conclusion: In the current trial, the vaccination was successful in preventing that bacterial illness. It must be regularly updated to avoid the issue of a high mutation rate.

Keywords: Neisseria meningitidis; Vaccine; mRNA; Capsular polysaccharide.

INTRODUCTION

Both meningitis and meningococcemia, which are caused by meningococci, are severe infections that are the greatest cause of infant mortality globally [1]. Large polysaccharide capsules [2], oxidase-positive nephrodicoccus [3], and Gram-negative nephrodicoccus are all characteristics [4]. They are capsules of pyogenic bacteria [5]. A habitat is the human upper respiratory system [6]. Infection is spread by respiratory droplets [7]. The organism enters the body after colonizing it through the circulation, the meninges, and the upper respiratory tract. Individuals with meningococcemia experience septic shock as a result of endotoxin found in cell walls. Infection in the respiratory tract causes the production of IgA protease; however, no exotoxins are created [8]. Phagocytic cells are slowed down by the bacterial capsule. Late complement component insufficiency is a risk factor for recurrent meningococcal infections [9].

Laboratory diagnosis includes the formation of oxidase-positive colonies on chocolate agar, maltose fermentation, and Gram stain culture and staining [10]. Penicillin G is used as a therapy since there is no evident resistance [11]. There are now two types of vaccinations. The capsular polysaccharide of A, C, Y, and W-135 is the only component of one vaccine form, but W-135's polysaccharide is coupled to a carrier protein in the other vaccine form [diphtheria antitoxin]. Because there are just a few drugs that are effective against meningococcal infections, antibiotic prophylaxis by immunizing agents is critical. Bacterial meningococcal infections can be avoided by using either vaccines that provide active protection or prepared antibodies that provide passive immunity [12]. Titers, which are limited by the maximum sample dilution, are the result of multiple immunological investigations carried out during the administration of previous vaccines against this virus.

A patient with a titer of 1/64 has more antibodies, or a wider titer, than a patient with a titer of ¼ [13]. Linked Immunosorbent Assay [ELISA] technology may detect antigens or antibodies in non-exempt samples. It is based on covalently binding an enzyme to a recognized antigen or antibody, digesting the enzyme-bound material by time-consuming sampling, and detecting enzyme activity concurrently with the addition of enzyme substrate. The new procedures are nearly as delicate as radioimmunoassays [RIAs] and do not require any specific instruments or radiolabels [14]. The Fluorescence-Activated Cell Sorting [Flow Cytometry] approach is brilliantly employed to reference the various types and amounts of immunologically activated cells in body fluid. If a helper T cell count must be tracked, the patient's cells are labelled with monoclonal antibodies specific to the cell in question, such as the CD4 protein. Fluoresce-in and rhodamine are two fluorescent dyes that are used to detect monoclonal antibodies. Individual cells are passed via a laser beam, and an equipment called a fluorescence-activated cell sorter is used to count the quantity of fluorescing cells [FACS] [15].

Types of vaccinations and their effectiveness: Conjugated vaccines are typically preferred over unconjugated vaccines. Unconjugated immunizations are effective at preventing meningitis epidemics and lowering carrier rates, especially among military personnel. Travelers to endemic areas are required to get inoculated. It is recommended that university students who live in dorms obtain their vaccines. There is no recommended booster dose for any form of immunization after the age of 16. Conjugate vaccines are recommended for children aged 11 to 12, and they reduce the risk of meningococcal disease in teens and young adults. A booster dose is recommended for persons who had the combination immunization before the age of 16. According to reports of immunization side effects, some cases of Guillain-Barré syndrome have been related to the Menactra vaccination. It has not been shown that vaccination causes Guillain-Barré syndrome. A factor H binding protein-based group B meningococcal vaccine was approved in 2014. It induces the formation of antibodies against the binding protein, preventing the bacteria from adhering to factor H. Since factor H reduces the complement component C3b, complement activity, a critical component of host defence, is increased. In E. coli, recombinant DNA technology is employed to produce factor H binding proteins for use in vaccines. This immunization is appropriate for those aged 10 to 25 [16]. The current study aimed to produce a meningococcal vaccine utilizing lipid nanoparticles and RNA to treat these devastating bacterial infectious diseases. This article describes the development of a meningococcal RNA vaccine based on the co-expression of cps gene locus proteins in host cells.

PATIENTS AND METHODS

Ethical statement: In the current investigation, all pertinent national, institutional, and/or international standards for using both people and animals were postdated. According to the recommendations of the Weatherall report, all procedures used in the study—including those involving people and animals—were approved by the local authorities, the Ethical Committee for Human and Animal Handling at Cairo University [ECAHCU], and the Pharmacy faculty at the University of Cairo, Egypt, with approval number P-17-5-2021. Every attempt was made to minimize the number of people and animals used in the study as well as their suffering.

Source of animal models: One hundred male transgenic mice with human brain implants weighing 45-50 g were procured and approved for license from the college of pharmacy's pharmacology and toxicology department at Cairo University in Egypt.

Inclusion criteria for animal models: Using transgenic mice humanized by brain human cells to increase expression of bacterial proteins and elicit strong hum-oral and cell-mediated immunity, meningitis infectious disease can be induced in adult male mice weighing 45–50 g after inoculation with Neisseria meningitidis serotypes A, C, Y, and W135 at an infectious dose of 1*102TCID50 for each serotype. The onset of symptoms took place between 5-7 days after the start of the incubation period.

Exclusion criteria are Female mice which are pregnant and young mice.

Samples collection: In Egypt, 100 meningitis patients with Neisseria meningitidis infection had blood samples taken from them.

Material: The Algomhoria Pharmaceutical Company in Cairo, Egypt, and the Alnasr Pharmaceutical Company in Abo zabal Alkhanka, Qalyobia, Egypt provided all of the chemical and biochemical ingredients that were used in this study.

Equipment: Table [1]: List of instruments

Instrument	Model and manufacturer
Autoclaves	Tomy, japan
Aerobic incubator	Sanyo, Japan
Digital balance	Mettler Toledo, Switzerland
Oven	Binder, Germany
Deep freezer -80	Artikel
Refrigerator 5	Whirpool
PH meter electrode	Mettler-toledo, UK
Deep freezer -20	whirlpool
Gyratory shaker	Corning gyratory shaker, Japan
190-1100nm Ultraviolet-visible spectrophotometer	UV1600PC,China
Light[optical] microscope	Amscope 120X-1200X,China

Place and date of the study: Between January 2020 and March 2022, this study was conducted at Cairo University's pharmacy faculty in Egypt.

Type of study: Investigatory testing study

Procedures: Construction of RNA transcripts of cps gene locus facilitating the synthesis of the bacterial capsule of Neisseria meningitidis by bio-informatics:

By using bioinformatics, we were able to determine the putative open reading frames for the coherent, conserved structural, and functional proteins of the cps gene locus that facilitate the production of the capsular polysaccharide of Neisseria meningitidis.

Downstream and upstream process

By using an RNA extraction approach, we were able to synthesize and purify the coherent structural and functional mRNA product of the cps gene locus before encasing it in lipid nanoparticle bubbles. Lipid nanoparticles with a 76-nanometer particle size served as the vaccine delivery method. Interest-related genes [cDNA] were cloned using PCR, and they were subsequently introduced into PUC18 using the restriction endonucleases Bam HI and Sphl for the digestion of the plasmid and ligase enzyme

for joining. The restriction enzymes EcoRI, Eam11041, and Lugul completed linearization of the pDNA template [obtained from ThermoFisher scientific company, USA]. In vitro transcription of the linearized pDNA template into mRNA was carried out using T7 recombinant RNA polymerase, nucleoside triphosphates, RNAse inhibitor, and 5X transcription buffer [obtained from Thermo-Fisher Scientific company, USA]. By trans-criptionally adding a cap analogue, such as the dinucleotide m7G[5,]-PPP-[5,] G, mRNA transcripts were capped [called regular cap analogue obtained from ThermoFisher scientific company, USA]. Additionally, Poly [A] polymerase changed mRNA transcripts by adding mRNA tails [obtained from ThermoFisher scientific company, USA]. After that, DNase I and protein kinase K were added for the post-in vitro transcription cleaning [obtained from Thermo-Fisher scientific firm, USA]. mRNA extraction was used for purification. Acid guanidinium thiocyanate-phenol-chloroform extraction, an organic extraction technique, was used to complete the mRNA purification process. It was a method of liquid extraction used to separate RNA. It provides a high level of RNA recovery and purity. Chloroform solutions are composed of a combination of 96% chloroform and 4% isoamyl alcohol [which secured the inactivation of RNases and minimized foaming] that is combined with an equivalent amount of phenol to create a 25:24:1 solution. The PH was maintained at around 5, which benefits the preservation of mRNA in the aqueous phase during mRNA purification. This method relies on the separation of an aqueous sample and a solution of water-saturated phenol and chloroform into an upper aqueous phase and a lower organic phase using centrifuge [chiefly phenol]. To aid in the denaturation of RNases that break down mRNA, guanidinium thiocyanate, a chaotropic substance, was added to the organic phase. The RNA was partitioned in an aqueous phase, while the protein is distributed into an organic phase. DNA partitioned into the organic phase under acidic conditions [PH 5]; while mRNA stiffed in the aqueous phase. By precipitating 2-propanol with ethanol, RNA was extracted from the aqueous phase. The pellet was then quickly air-dried and liquefied in Ribonuclease-free water. At 260 and 280 nanometers, the absorbance of mRNA transcripts was determined. The purity of mRNA transcripts was assessed using the ratio of absorbance at 260 and 280 nm. Using the Northern blot method, the quantities and sizes of recombinant mRNA transcripts were further examined. mRNA transcripts were separated according to their sizes on an agarose gel, denaturated, and then transported to a nitrocellulose membrane, where they were hybridised with radioactive probes. The dimethyl dioctadecyl ammonium bromide [DDAB] [a quaternary ammonium lipid that forms complexes with mRNA and invokes innate immunity] lipid nano-particles formed bubbles encapsulating recombinant mRNA transcripts, which were used to encapsulate the purified RNA. The vaccine delivery method made of lipid nanoparticles included particles as small as 76 nm.

Formulation: mRNA and lipid nano-particles A sterile suspension of Neisseria meningitidis vaccine was available for intra-muscular injection. Each 1 ml dosage contained 30 mcg of the Neisseria meningitides cps gene locus mRNA, 50 mcg of dimethyl dioctadecyl ammonium bromide lipid [DDAB], and 0.5 mg of aluminium hydroxide. Each dosage also contained 0.618 mg of sodium dihydrogen phosphate dihydrate and 4.4 mg of sodium chloride.

Assessment of immunogenicity: By administering the pure RNA to transgenic mice by intraperitoneal injection, we evaluated immunogenicity in animal models.

In vitro evaluation of vaccine on transgenic animals[mice]

[i] Transgenic mice were those whose genes had been modified using tissue culture methods and recombinant DNA technologies. A DNA sequence [a trans-gene] that was introduced into a cell's genome by human intervention was incorporated into the genome of a transgenic animal. [ii] The vaccination was given to 100 transgenic mice. They got two dosages, separated by 28 days. Half of the second booster dosage was given in the first dose. In order to boost the production of harmful bacterial proteins, transgenic animal models were used [17].

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

Protection assays were used to evaluate the efficacy of vaccinations.

[i] Active: After receiving the experimental vaccination, groups of transgenic mice were given explosive doses of bacteria [8 g/ml of each of the meningococcal A, C, W, and Y strains]. To evaluate the vaccine's efficacy as a preventative measure, the lowest number of germs that would be 50% lethal to animals [i.e., LD50] was seen and compared to LD50 in unvaccinated animals. [ii] Passive: Normal mice received varying doses of serum from vaccinated people, which was then combined with the infectious agent. It was mandated as a reference point for the efficacy of the immunizing agent to use the widest dilution of serum effective for protecting 50% of animals [i.e. ED50%].

Randomized human clinical trials for valuation of test vaccine

Stage 1: A small group of healthy human volunteers were utilized in a phase 1 study to meticulously evaluate the pharmacokinetics and dose-response relationship of the new medicine [e.g., 20–100]. The phase 1 trials of extremely risky drugs and cancer treatment are an exception; in these studies, the medicines were administered to consenting patients who were afflicted with the target disease. Phase 1 studies assessed the drug's immediate effects at a variety of doses, beginning at a level that had no noticeable effects and increasing to one that either caused a significant physiologic response or just had a very slight detrimental effect.

Stage 2: A drug was tested in a phase 2 study on a sufficient number of sick people [for instance, 100–200] who had the target illness. A placebo or a drug used as a positive control was part of a single-blind or double-blind design. Throughout the experiment, which was carried out under stringent supervision and usually in a hospital research center, patients were continually observed. The goal was to determine whether the medication creates a therapeutic response at a level that sick patients may tolerate; while, still having the necessary effectiveness. In-depth research was done on the medication's pharmacokinetics and pharmacodynamics in this patient population.

Stage 3: In a phase 3 study, the medicine was often administered to a significant number of patients [1000–6000 or more, at various sites], as well as a large number of physicians, in the manner recommended for its ultimate universal use [e.g., in outpatients]. These studies frequently included placebo and positive controls, as well as a double-blind crossover design. The goals were to more closely examine the new medication's assortment of positive effects in the context of the proposed clinical use, to contrast it with placebo [a negative control] and more established treatments [a positive control], and to look for any toxicities that might be present infrequently enough to escape detection in phase 2 studies. These investigations were often quite expensive and required the gathering of enormous volumes of data. Sadly, the current was used in relatively few phase 3 studies.

Stage 4: The post-marketing surveillance phase, or phase 4, of the assessment process was where it was anticipated that extremely uncommon toxicity might be discovered and notified in time to prevent catastrophic therapeutic disasters. The manufacturer was required to submit periodic reports to the FDA detailing any observed adverse drug reactions. Phase 4 has traditionally been subject to less stringent FDA regulation than the previous three phases. There was presently a lot of focus on enhancing phase 4 surveillance, since so many drugs have only been commercialized when they have been demonstrated to cause unacceptable damage.

Human evaluation of mRNA Neisseria meningitidis vaccine via human randomized clinical trials phases 1/2

In present investigation, 3 sets of human volunteers were used. Each group had 100 participants:

Group 1 [the negative control group] received an intramuscular injection of the placebo.

Injections of Neisseria meningitidis standard vaccination were given to Group 2 [the positive control group].

Group 3 [the test group] received intramuscular injections of the test Neisseria meningitidis vaccine.

After two weeks, the three groups were exposed to varying concentrations of the pathogenic bacteria to induce the development of protective neutralizing antibodies [this was ethical and was sanctioned to assess the efficacy of the test vaccine]. The three groups received

intradermal booster dosages after 21 days. Over the course of two years, the test vaccine's level of protection was assessed. While the protective cell-mediated immunity was assessed using a flow cytometry technique, the protective antibodies were discovered using an enzyme-linked immunosorbent [ELISA] assay.

ELISA procedure for detection of the neutralizing antibodies to mRNA Neisseria meningitidis vaccine

Antigen was attached to the bottom of the well. Patient serum contained antibody that was bound to antigen. IgG from the patient was linked to a human IgG antibody that was enzyme-conjugated [Horseradish per-oxidase enzyme]. A substrate for the enzyme was introduced, and as the enzyme interacted with it, the substrate changed color. By including the enzyme's substrate and calculating the color reaction in a UV spectrophotometer operating at 450 nm wavelength, enzyme activity was determined.

Flow cytometry for detection and counting CD+4and CD+8 T lymphocytes specific to mRNA Neisseria meningitidis vaccine

Equipment: United States, Invitrogen Attune Cytpix flow cytometer.

A fluorescent Rhodamine-tagged mono-clonal antibody interacts with a cell. A sensor counted the cells as they travelled down the tube thanks to the dye fluorescing due to UV radiation. Using an Invitrogen Attune Cytpix flow cytometer, CD+4 and CD+8 T cells with the mRNA cps vaccination specificity were identified and analyzed [obtained from the USA]. A monoclonal antibody was utilized in this experiment to tag the patient's cells. These antibodies were made against proteins that are unique to cells [e.g. CD4 protein when measuring the number of T helper cells]. Monoclonal antibodies were labelled using fluorescent dyes like rhodamine and fluorescein. Individual cells were exposed to the laser beam, which caused them to light. The fluorescence was assessed using a tool known as a Fluorescence Activated Cell Sorter [FACS].

Statistical analysis

Triplets were used for all cultures. They used mean and standard deviation to present their findings. In order to undertake statistical analysis, one-way analysis of variance [p value. 05] and statistical analysis using Excel spreadsheet software were also employed. This was accomplished using the f-test.

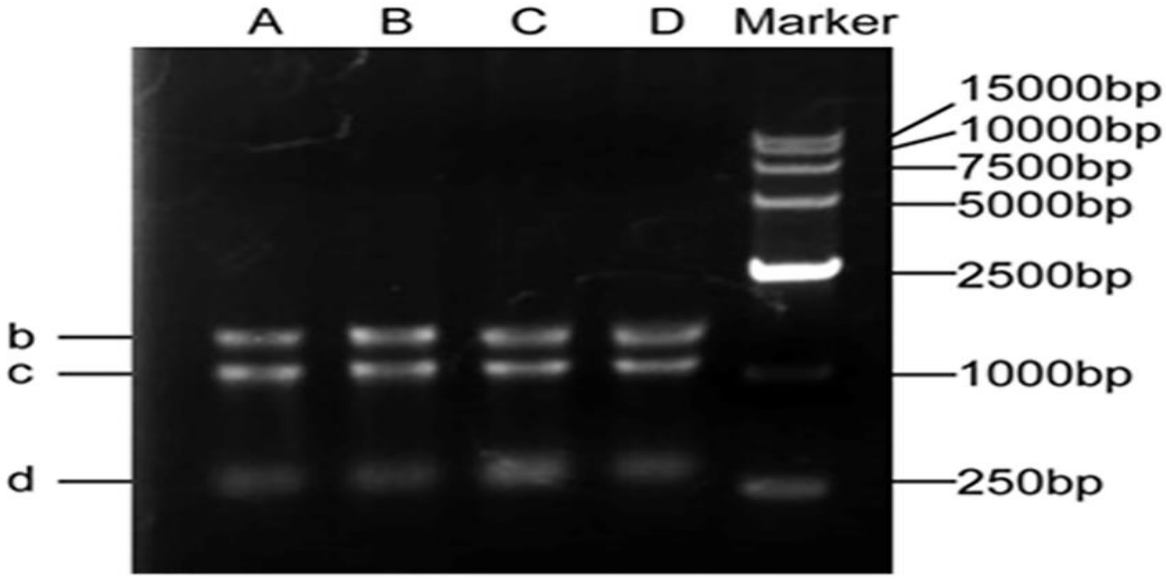
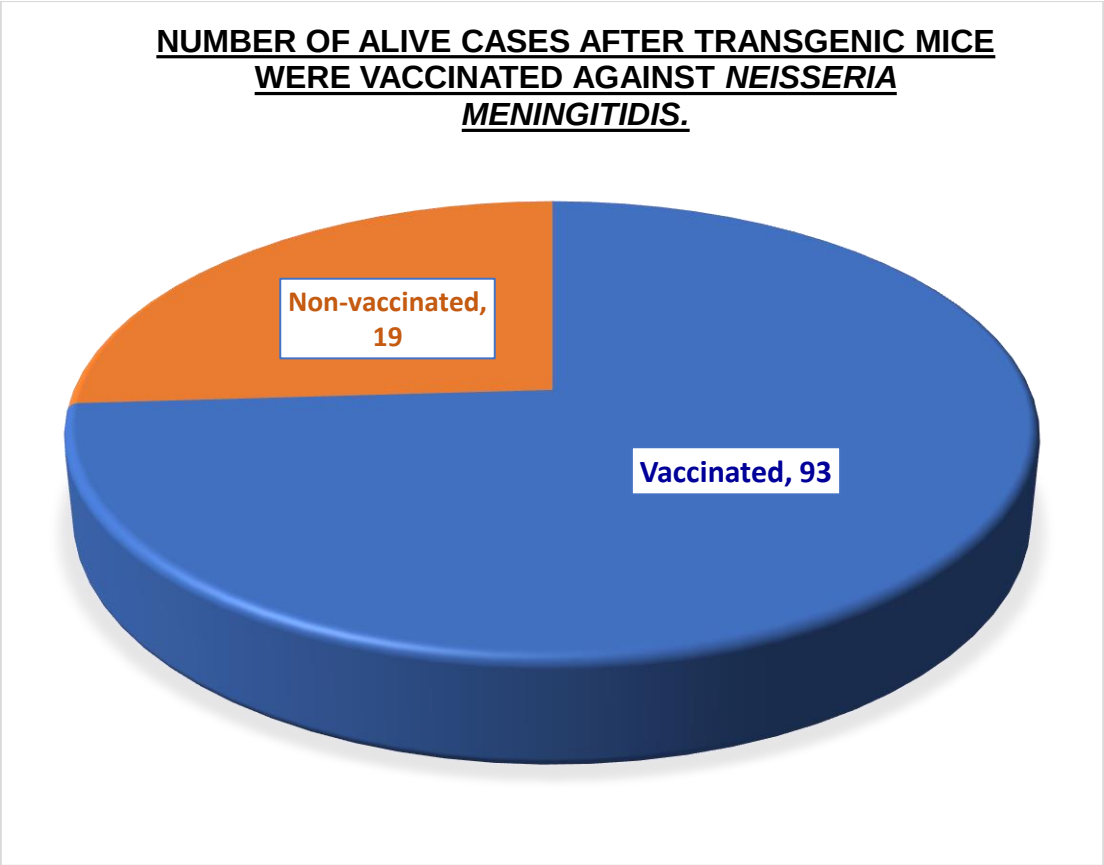
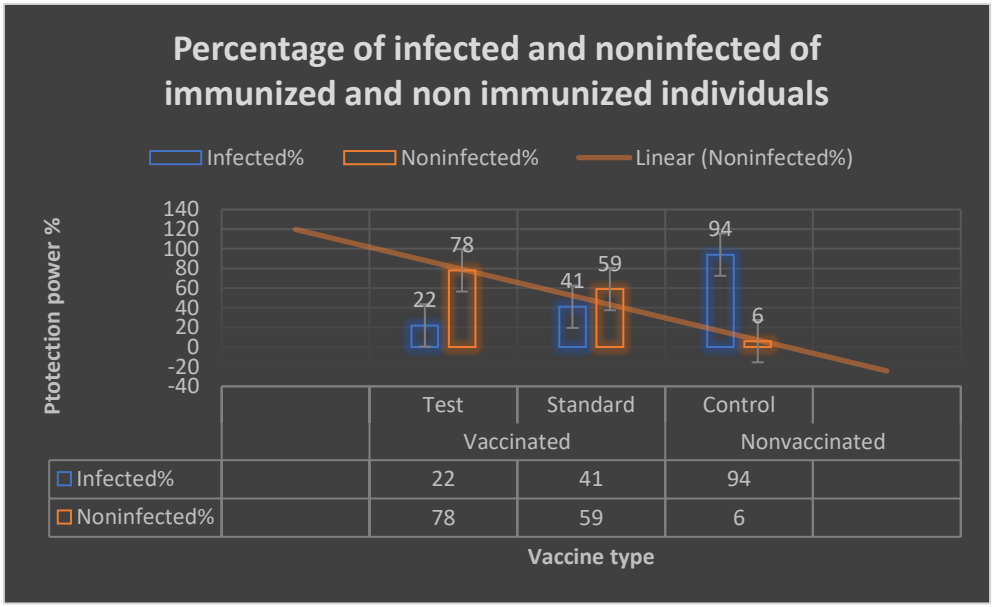


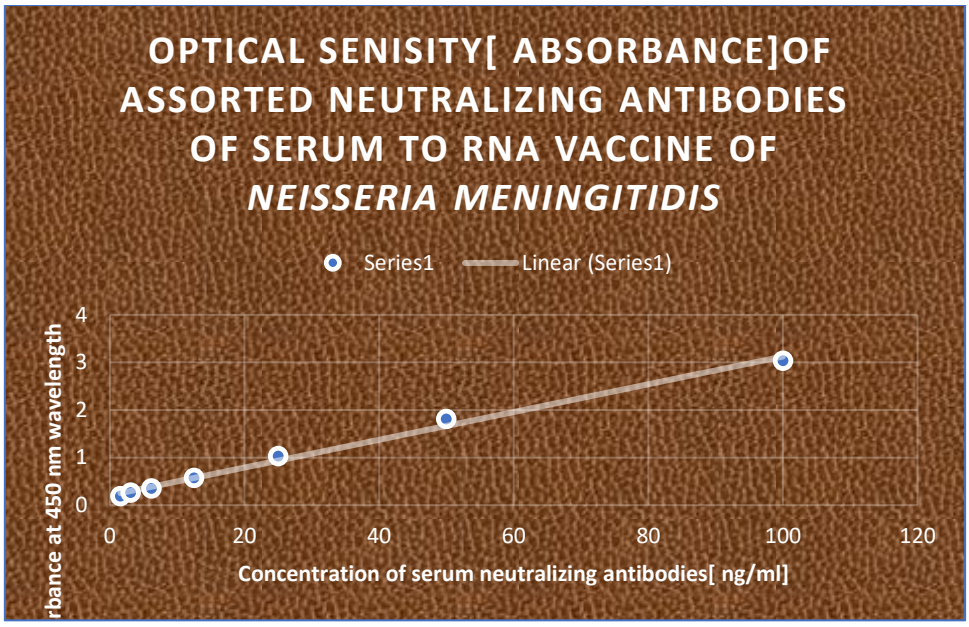
Figure [1]: Different amounts and sizes of mRNA transcripts measured via the Northen technique



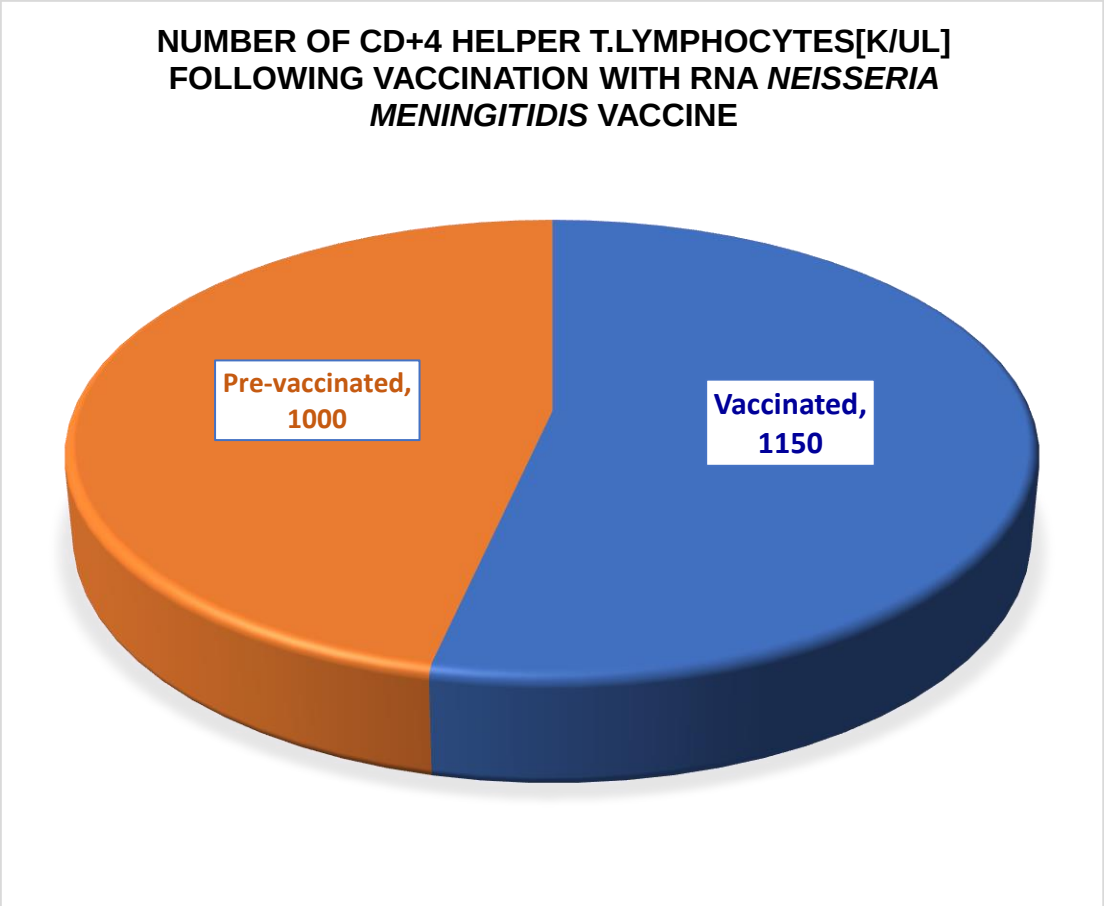
Graph [1]: The protective covering power of the immunizing agent against Neisseria meningitidis during animal testing was 93%. There were live animal models with a P value = 0.004.



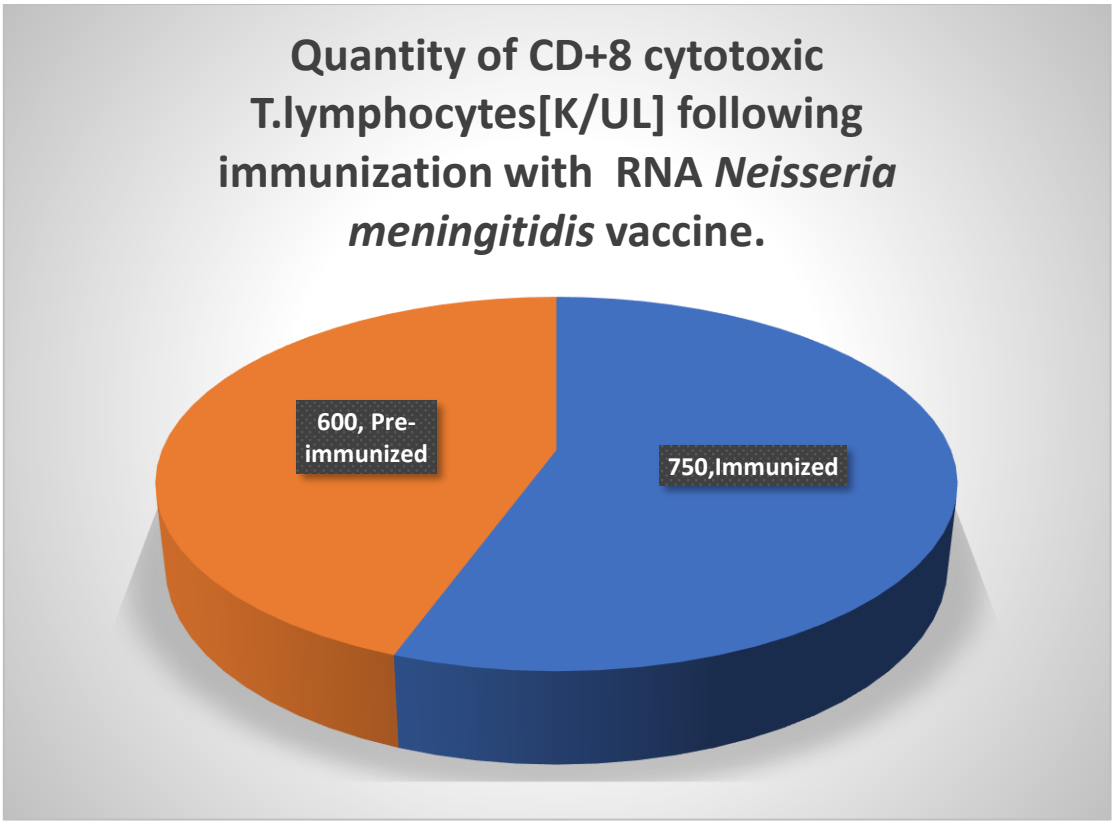
Graph [2]: It displays that the protective covering power of test mRNA Neisseria meningitidis immunizing agent in human clinical trials stages 1/2 was 79%.



Graph [3]: It shows the absorbance of various concentrations of serum neutralizing antibodies to vaccine of Neisseria meningitidis through ELISA



Graph [4]: It symbolizes increasing count of the helper CD+4 T lymphocytes subsequently the immunization with mRNA Neisseria meningitides prophylactic vaccine. This reflected an increment in statistical significance difference. The p-value was 0.001



Graph [5]: It represents increasing the count of the cytotoxic CD+8 T lymphocytes following the immunization with mRNA Neisseria meningitidis prophylactic immunizing agent. This mirrored growth in statistical significance difference. The p-value was 0.002.

RESULTS

Neisseria meningitidis' LD50% was found to be higher than 50 mcg/ml. The RNA Neisseria meningitidis vaccine's ED50% was discovered to be 15 mcg/ml. The RNA Neisseria meningitidis vaccine contained 30 mcg of mRNA from the cps gene locus per milliliter. Preclinical testing of the vaccine showed 93% effectiveness whereas phases 1 and 2 of clinical trials showed 79% efficacy.

Table [2]: It shows the absorbance of various serum-neutralizing antibodies to the RNA Neisseria meningitidis vaccination using ELISA

Concentration[ng/ml]	Optical density
1.44	0.185
3.11	0.257
6.20	0.341
12.88	0.564
26	1.028
51	1.801
99.9	3.035

The significance of this test: The data in table 2 indicated that the test vaccine evoked a strong humoral immunity in randomized human clinical trials phases1/2.

Table [3]: It illustrates the effectiveness of the test RNA Neisseria meningitidis vaccine during phases 1 and 2 of clinical trials

	Test	standard	Control
% infected	21	43	90
%non-infected	79	57	10

The data in table 3 were significant because it indicated that the protection power of the test vaccine was 79% during randomized human clinical trials phases 1/2.

Table [4]: It represents vaccination against Neisseria meningitidis

Description	Vaccinated	Pres-vaccinated	P- Value
CD+4 COUNT	1150	1000	**0.001**
CD+8 COUNT	750	600	**0.002**
Total	1850	1600	**0.001**

The helper T lymphocyte count was 1150; while the cytotoxic T lymphocyte count was 750. These data indicated that the test vaccine elicited moderate cell-mediated immunity. The p-value was < 0.05

Table [5]: It represents the vaccination of transgenic mice against Neisseria meningitidis

Description	Vaccinated	Non-vaccinated
Alive	67	21
Dead	33	79
Total	100	100

DISCUSSION

In the present study, a novel lipid nanoparticle mRNA vaccine against different serotypes of N. meningitidis [A, B, C, Y, and W135] was developed. Its efficacy was about 80%. It showed superior efficacy and safety profile compared to other current standard vaccines against Neisseria meningitis. A previous study conducted in Australia [17] examined the efficacy and safety profiles of various vaccines against Meningococcal disease such as the Menactra vaccine. They found that previous vaccines were efficacious and moderately safe. Vaccines against meningococcal groups A, C, Y, and W-135 contain polysaccharide capsules as immunogens. The group B meningococcal vaccine contains a factor H binding protein as an immunogen. There are three forms of polysaccharide vaccines in use worldwide, all of which contain groups A, C, Y, and W-135 capsular polysaccharides as immunogens. Combination vaccines come in two forms: Menactra contains four polysaccharides as carrier proteins conjugated to diphtheria toxoid, and Membeo contains four polysaccharides as carrier proteins conjugated to a non-toxic variant of diphtheria toxin. Menomune, a non-conjugated vaccine, contains only four polysaccharides [not bound to a carrier protein]. Conjugate vaccines produce higher antibody titers in children than nonconjugated vaccines. The vaccine induces similar antibody titers in adults. Notably, there are no vaccines containing group B polysaccharides as they are not immunogenic in humans. A fourth vaccine, MenAfriVac, was developed for use in African meningitis zones and is a conjugate vaccine containing only Group A polysaccharides [18]. In 2015, a second group B meningococcal vaccine containing four surface proteins [fHbp, NadA, NHBA, and PorA] was approved. It was [19]. In human clinical trials 90 susceptible were infected in the negative control group; 43 were infected in the positive control[standard] group and 21 were infected in the test group. It showed fewer side effects and superior biological activity than other standard vaccines. Its efficacy was long-lasting; on their hand due to the high rate of mutations, the vaccine needed to be updated periodically.

Indicating the high purity of recombinant mRNA transcripts that were purified using the organic extraction approach, the ratio of mRNA transcript absorbance at 260 and 280 nm using a UV spectrophotometer was almost 2. For those older than two years of age, the RNA vaccination is advised. The recommended dosage for this vaccination is a 25–30 mcg/ml intramuscular injection, followed by a booster dose administered intradermally after 21 days. In presence of 0.5 mg aluminum hydroxide as an adjuvant, the immunogenicity increased from 79% to 84%. Those who have sensitivities to any of the test vaccine's ingredients should not get it. Simple analgesics like paracetamol and ibuprofen helped to ease the slight discomfort at the site of intramuscular and intradermal injections as well as a brief period of mild fever. The present immunizing agent incorporates mRNA of the cps gene locus responsible for the formation of the capsule which is the main mode of prevention. RNA vaccines are recommended to be stored at -70 $^{\circ}$C. In a comparison with a previous study [vaccination with weakened Neisseria meningitidis strains defends against challenge with live meningococci] conducted in the USA, the conjugate vaccines are superior to the unconjugated type. The unconjugated vaccine is

efficient in reducing the carrier rate and in averting meningitis epidemics, curiously in a military organization. The conjugate vaccines will cut down the relative frequency of meningococcal disease in adolescents and two-years-old. Our vaccine is considered like conjugate vaccine due to evoking the production of foreign protein in vivo which moderately stimulates cell-mediated immunity. The function of immunity in preventing infection: This vaccination significantly increased humoral immunity and just slightly increased cell-mediated immunity: The primary bodily defense against the virus was humoral immunity. IgM and IgG were the primary neutralizing antibodies in blood against the cps cluster proteins necessary for Neisseria meningitidis to construct its capsule. Due to the IgA not being absorbed by the usual route of infection, few IgA antibodies were generated. Cell-mediated immunity was only weakly triggered by this vaccination. Merits: I It was impossible to revert to virulence.

Drawbacks: [i] Evacuation of vaccine and transmittance to liable contacts was not realizable; hence it did not contribute to development of herd insusceptibility versus this bacterial infection. [ii] Action lasted less time than a live, attenuated vaccine. [iii] To prevent spoiling and contamination, it had to be kept at -70 ˚C in a refrigerator. Bioinformatic analysis of cps gene locus of meningococci: They encode proteins that were highly conserved proteins among all Meningococci. Cps proteins are 485 amino acids and were characterized by being homologous membrane regulatory proteins with a leading extracellular part. The homologous proteins' extracellular domains have been crystallized and were thought to be accountable for pyrophosphate linkage hydrolysis between the cps and the membrane lipid anchor and subsequent cps affection to the peptidoglycan. This explained their role in facilitating the biosynthesis of the virulent capsule which hinders phagocytosis [20]. The findings presented in table [4] and graphs [4 and 5] indicated that the count of T lymphocytes [helper and cytotoxic] increased after the vaccination reflecting the enhancement of the cell-mediated immunity; while in the study of **Pizza *et al.*** [14], no detectable rise in the count of both types of T lymphocytes was observed. In preclinical trials, the protection power reached 93% as shown in table [3] and graph [2] while it was approximately 90% in else study conducted by **McMillan *et al.*** [17]'s study. It was interesting that the findings during clinical trials phase 1/2 confirmed that the protection power was about 79% as demonstrated in a graph [1] and table [5]; while the efficacy of the current vaccine MenAfriVac was greater than 80% in clinical trials phase 3 as shown by Christodoulides and Heckels' study [21]. In **Garland** [22]'s study there was a moderate enhancement of the humoral immunity elicited via the previous vaccines against Neisseria meningitidis; while in the existing study a remarkable increment in the protective neutralizing antibodies to Neisseria meningitidis was noticed consequently on a strong humoral immunity evoked by test mRNA vaccine.

Conclusion: As a preventative measure against the bacterial infectious illness meningitis brought on by these harmful bacteria, the LNP-mRNA vaccination of genes responsible for the creation of a capsule of Neisseria meningitidis [A, B, C, Y, and W135 serotypes] proved successful. It could be distributed to people of various ages.

Acknowledgment: An Egyptian ministry of scientific research and higher education-approved patent investigation with registration number 1356/2021.

Conflict of interest: No conflict of curiosity exists.

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Data availability: The Cairo University's pharmacy faculty in Egypt produced the raw data. Traced data supporting the study's conclusions are available upon request from the corresponding author.

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