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Original article

# Exploitation of hepatitis C virus like particles vaccine against genotype 4 by bioinformatics

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**ABSTRACT**

**The aim of the study:** To counteract Hepatitis C viral lethality, an insect recombinant DNA vaccine of Hepatitis C virus genotype 4 subtype 4a was developed using E1, E2, and Capsid protein structural proteins. **Methodology**: In the current study, HCV-like particles vaccinations composed of coherent structural proteins (E1, E2, and Capsid protein) of HCV were created in an expression host insect cell line employing a Baculovirus expression vector. Recombinant Baculovirus VLPs were produced by modified bac-to-bac site-specific transposition and purified via affinity chromatography. The current vaccine's immunogenicity was assessed in preclinical animal testing on transgenic mice, followed by clinical trials stages 1/2. **Results:** The current vaccine achieved 71% efficacy in animal models and 63% efficacy throughout stages 1-2 of human clinical trials. They have little biological influence and have few negative implications. The effect lingered for a time. An HCV-like particle immunization improved both humoral and cell-mediated protection against hepatitis C virus infection. Advantages: It is not feasible to revert to virulence.Cons: Because the vaccine virus cannot be expelled or transferred to people who have not received the immunization, it does not contribute to herd immunity against this viral infection. **Conclusions:** The immunization proved effective in avoiding HCV virus infection in the current study. To counteract the HCV virus's rapid mutation rate, it must be updated on a frequent basis.

## Introduction

Hepatitis C virus (HCV) is a deadly and devastating viral infection that affects more than 2% of the world's population today. Most of the time, just the liver cells are infected. Acute hepatitis C may emerge 2 weeks to 6 months after the hepatitis C virus enters the bloodstream. A high rate of mutation in Hepatitis C virus leads in long-term protection and easy re-infection. Approximately 80% of freshly infected subordinates have chronic pathogenic symptoms [1]. A considerable risk of liver-colored malignant neoplasms is associated with developed cirrhosis in 10-20% of chronically infected persons (1-5% of chronically infected patients aged 20-30 years and older). Many modelling studies show that direct-acting antivirals (DAAs) are insufficient to eliminate HCV. Because few treatments are effective against infectious pathogen diseases, the use of immunizations to prevent viral infections is critical. Viral disease can be avoided by employing

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either immunizations that induce active immunity or producing prepared antibodies that provide passive protection [2].

The relationship between hepatitis C and hepatocellular carcinoma is a distinguishing aspect of the disease. HCV is the most frequent blood-borne pathogen in Egypt. An acute hepatitis C infection is characterised by jaundice, light urine; white faeces, nausea, and pain in the upper right region of the abdomen. These symptoms and signs will fade in 2 to 12 weeks [3]. Hepatitis C virus (HCV) is an enveloped, positive-polarity, single-stranded RNA virus that is the sole member of Hepacivirus genus in Flaviviridae family. Virion polymerase is not present. With HCV, there are several serotypes. HCV binds to the surface of liver-colored host cells via low-density lipoprotein receptors (LDLR) and glycosaminoglycans (GAGs) [4]. HCV RNA levels in infected patients were shown to decrease dramatically when anti-sense nucleotide that bind to miR-122 and restrict its function were employed in clinical trials in 2013[5]. The majority of illnesses are transmitted by blood. Both transmission from mother to child and sexually transmitted diseases are common [6]. Pathogenesis: Cytotoxic T lymphocytes unintentionally induce hepatocellular damage. The multiplication of HCV does not in and of itself kill cells. There are no cytopathic effects as a result. More than 50% of HCV infections are caused by long-term carrier diseases. Advertisements for chronic carriers mention hepatocellular cancer and chronic liver disease [7]. Laboratory diagnosis: HCV antibodies are found by serological research. The presence of an active infection can be determined by a PCR settled "viral load" test [8]. Detection of HCV antibodies in transfused blood can prevent post-transfusion hepatitis. There are no hyperimmune globulins available. No vaccination exists. Management: Peg interferon-alpha therapy for hepatitis C reduces the number of people who develop into chronic carriers [9].

NS5A inhibitors, such as ledipasvir, RNA polymerase inhibitors, such as sofosbuvir, and protease inhibitors, such as paritaprevir, are used in conjunction to treat chronic hepatitis C [10]. The human clinical studies for the HCV-VLPS vaccination are divided into four phases. Tier 1 research meticulously investigates the dose-response relationships and pharmacokinetics of novel immunizations in a restricted number of typical human volunteers (e.g. 20-10). In stage 1 research, the acute effects of the causative agent are fully explored throughout a wide range of dosages, beginning with doses that have no observable effects and advancing to those that generate any physiological response or very minor toxicological consequences [11]. Stage 2: At this level, studies analyse a large number of drugs. For example, 100-200 human volunteers suffering from topographic point disease. To encapsulate a placebo or positive control immunization, a single-blind or double-blind design is utilized.

The purpose is to determine whether the chemical has the necessary effectiveness (i.e., whether it has a sufficient preventative impact) at impaired tolerance dosages. At this step, extensive information is gathered while accounting for the vaccine's pharmacodynamics and pharmacokinetics [12]. Stage 3: Utilizing the vaccine in a manner intended for future widespread use, Phase 3 trials frequently involve a large number of natural human volunteers (e.g., 1000-6000 or more in many locations) (e.g., outpatient)[13]. These experiments, which typically use a double-blind crossover design, incorporate a positive control as well as a placebo [14]. The goals are to explore the spectrum of healthy effects of the new vaccination in light of the anticipated clinical application, compare it to both positive and negative controls, and monitor morbidity, which seldom occurs. Post-marketing monitoring is the fourth stage of review [15]. Morbidity is rarely felt and is rumored to be severe enough to exclude generating a medical care problem [16]. The present study's purpose was to create an HCV-like particle immunization to counteract potentially fatal viral transmission sickness.

**Material and methods**

The Algomhoria Pharmaceutical Company in Cairo, Egypt was used for the purchasing of all chemical components.

**Ethical statement:**

In the current investigation, we complied with all applicable institutional, international, and/or national regulations regarding the use of both humans and animals. According to the recommendations of the Weathrall 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), at the Pharmacy Faculty, University of Cairo, Egypt, with approval number P-8-2-2020. All possible measures were taken to reduce the number of individuals and animals used in research and their suffering.

**The type of the study:**

Exploratory testing research. Source of animal models: They were collected and given the green light for legalization by the college of pharmacy's pharmacology and toxicology department at Cairo University in Egypt.

**Inclusion criteria for animal models were:**

Male mice that are 50 mg in weight. Mice exposed to hepatitis C, such as transgenic mice injected with human liver cells, may get the disease.

**Exclusion criteria were:**

Pregnant female mice and young mice were not included. Place and date of the study: Between February 2020 and November 2022, the faculty of pharmacy at Cairo University conducted this study.
**Collection of the samples:**

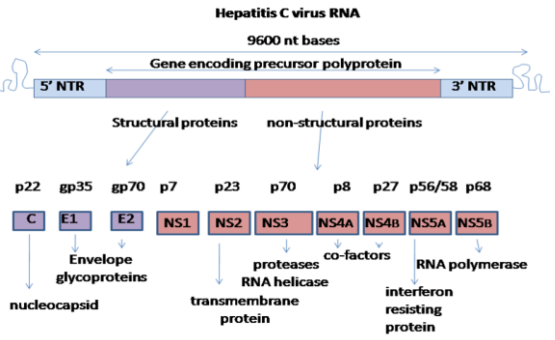
100 HCV-infected individuals' blood samples were obtained from various medical facilities in Egypt.

**Outlines of methods:**

The current study began with the identification of probable open reading frames for HCV's cohesive structural and functional proteins utilizing bioinformatics. After the preceding step, the HCV structural and functional proteins were expressed and purified. Immunogenicity was assessed using transgenic mice as animal models by intraperitoneal injection of purified proteins. Human clinical studies in phases I and II were completed.

**Preparation of HCV-VLPs vaccine by bioinformatics:**

To begin, neutralizing antibodies to the structural part of the HCV genome were induced by targeting the envelope glycoproteins proteins E1/E2 and nucleocapsid protein.




**Production, purification and formulation of HCV-VLPs vaccine**:

Three primary steps make up the generic manufacturing process for the HCV-VLPs vaccine:

**Upstream processing(production):**

**Cell lines:** Spodoptera frugiperda (Sf21) cells were donated by Thermo Fisher Scientific in the United States. Insect-XPRESSTM medium with 10% foetal bovine serum (FBS) and L-glutamine was employed to keep cells alive in shaking conical flasks at 27 0C. Recombinant Baculovirus VLPs are created using modified bac-to-bac site-specific transposition.

Polyhedrine is the activator. Histidine tag (6x) linked to the C-terminus of the target recombinant protein. The cell line is Sf21 subclone. The donor plasmid is called PFASTBAC TOPO. For construction, Tn7 transposition was employed. These biochemicals, vectors, and cell lines were all purchased in the United States from Thermo Fisher Scientific. Expression enhancement: Incubation temperature: 28 0C. PH: 6.3. 372 mOsm/kg is the osmolality value. Shearing pressures were mitigated by the use of 10% foetal bovine serum in addition to shear force inhibitors such as Pluronic F-68 (obtained from Invitrogen life technologies firm, USA).

**Aeration:** A large bioreactor capable of transferring adequate amounts of dissolved oxygen at 35% air saturation. Procedures: The cDNA of foreign genes involved in the coding for (E1,E2, and C)proteins was sub-cloned using PCR-set procedures. Using site-specific transposition with Tn7, we introduced foreign genes into bacmid DNA passed on by Escherichia coli. The genes of involvement were cloned by breaking down the genes of involvement using the restriction enzymes ECORII and Bam HI endonuclease, then inserting and ligating the genes into the PFASTBAC TOPO donor plasmid with ligase. The donor plasmid was injected into competent DH10BAC E.coi cells that already possessed the helper plasmid and the bacmid with a mini-Tn7 target site. On the third day, transposition and antibiotic selection using ampicillin sodium (blue/white) screening occurred. On the fourth day, the recombinant bacmid DNA was transfected into the Sf21 insect host cell line while containing 1 ml of cell infectin reagent (obtained from Thermo Fisher Scientific, USA).

**Downstream processing(purification):**

Polyhistidine proteins with molecular tags were added to recombinant unfused proteins (HCV-VLPs). After precipitating (salting out) 100 ml of the superannuate with 53 ml of a 4.1 M ammonium sulphate saturated solution at 25 0C for 3 minutes and centrifuging at 4000 rpm for 3 minutes, which could be quickly refined from the superannuate by nickel columns using immobilised metal affinity

chromatography (the metal ligand was a nickel metal ion, while the target bio-molecule was polyh Before the final formulation, the preparations were disinfected by passing them through sterile-grade 0.22 micron filters (Whatman-1541-042 filter paper, USA).

**Formulation:** The HCV-VLPs immunization was given intramuscularly in the form of a sterile suspension. Each 1 ml dose comprised 0.6 mg of aluminium hydroxide, 45 micrograms of 3-O-desacyl-4-monophosphoryl lipid A (MPL), 5 micrograms of E1, 10 micrograms of E2, and 10 micro-grams of C HCV genotype 4 proteins. Each dose also contained 4.4 mg of sodium chloride and 0.618 mg of sodium dihydrogen phosphate dihydrate.

**In vitro evaluation of vaccine on transgenic animals(mice):**

Transgenic mice are animals whose genes have been altered using tissue culture and recombinant DNA methods. A transgenic animal is one that has had a gene or DNA sequence (a trans-gene) inserted into its genome by human intervention. The immunization was given to 16 100 transgenic mice. They were separated by 21 days between the two doses they received. The first dosage was precisely the same as the second booster dosage. The use of transgenic mice injected with human hepatocytes was intended to increase the production of HCV viral proteins and efficiently elicit humoral and cell-mediated immunity [17].

**Screening and bio-assay of the biological activity:**

**Protection assays were exploited to measure the efficiency of vaccinations:** After successfully giving the experimental vaccine to groups of transgenic mice, escalating dosages of infectious agents (10 RNA(+)-100 RNA(+) virions) were administered to them. To estimate the vaccine's protective capacity, the lowest number of germs that will render 50% of animals fatal (i.e., LD50) was determined and compared to LD 50 in non-vaccinated animals [18].

Passive: Normal animals were administered the infectious factor after receiving progressively higher quantities of serum from vaccinated mice.

The highest serum dilution effective in protecting 50% of mice (ED50%) was defined as the benchmark efficacy of a vaccine [19].

**Randomized Human Clinical Trials Evaluating Investigatory Vaccines:**

Level 1: Phase 1 studies use a small group of healthy volunteers to rigorously assess the pharmacokinetics and dose-response characteristics of novel medicines (eg, 20-100). Phase 1 studies of extremely risky medicines and cancer treatment are exceptions. The medicine is given to people with the target illness in these studies. Phase 1 studies look at the immediate effects of medicines at various dosages. This progression begins with no observable impact and progresses to levels that elicit either huge physiological reactions or very minor deleterious consequences. Level 2: Phase 2 trials investigate medications in a sufficient number of people (e.g., 100-200) suffering from the ailment of interest. A placebo or a positive control medication is used in single-blind or double-blind designs. Patients are regularly watched under strict supervision, frequently as part of studies done in hospital research facilities. The purpose is to assess whether a medicine has the required potency (i.e., whether it provides an appropriate therapeutic response) at levels that sick people can tolerate. Drug pharmacokinetics and pharmacodynamics have been widely investigated in this patient group. Level 3: Large numbers of patients (>1000-6000 at numerous sites) and medication delivery in the way advised for future general use are common in phase 3 studies (e.g. outpatient). There are a big number of physicians engaged. Such studies frequently employ a double-blind crossover design with a placebo and a positive control. The goal is to investigate the new drug's spectrum of favourable benefits in the context of its intended therapeutic application, compare it to a placebo (negative control) and past therapies (positive control), and discover toxicities. This may occur seldom enough in phase 2 studies to be disregarded. These investigations are often costly and need the collecting of massive volumes of data. However, there are currently just a few Phase 3 studies. Level 4: The post-marketing surveillance phase of the assessment process is where it is hoped that very uncommon toxicities would be detected and reported in a timely way to avert severe therapeutic catastrophes. The company must disclose all documented medication adverse effects to the FDA on a regular basis. Historically, Phase 4 has been subject to less strict FDA oversight than the previous three phases. With so many medications becoming unacceptably dangerous only after they are launched, there is a renewed focus on strengthening Phase 4 surveillance.

**Randomized human clinical stages 1/ 2 assessment:**

Three groups of human volunteers are being used in the current study. Each group had 100 people:

The placebo was administered intramuscularly to Group 1 (the negative control group).

The second group (the positive control group) got intramuscular injections of the standard HCV-VLPs immunization.

The test HCV-VLPs vaccine was administered intramuscularly to Group 3 (the test group). Following two weeks, the three groups were subjected to increasing quantities of harmful bacteria in order to induce the formation of protective neutralizing antibodies. After 21 days, the three groups received intradermal booster doses. The amount of protection provided by the test vaccination was evaluated over a two-year period. Although flow cytometry was used to assess protective cell-mediated immunity, an enzyme-linked immunosorbent assay (ELISA) was used to identify protective antibodies.

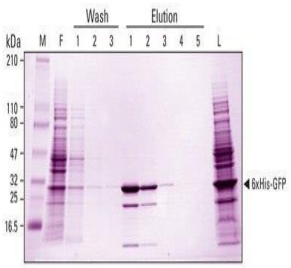
**ELISA procedure for detection of the neutralizing antibodies to HCV-VLPs:**

The antigen was affixed to the bottom of the well. Antigen and antibody were found together in the patient's serum. IgG levels in patients Human IgG was coupled with an antibody, and the enzyme conjugated antibody was linked to human IgG. (Horseradish peroxidase enzyme). The substrate for the enzyme was added, and the enzyme's reaction altered the colour. By introducing the enzyme's substrate and monitoring the colour response at 450 nm in a UV spectrophotometer, the enzyme's activity was estimated.

**Flow cytometry for discovery and enumeration of CD+4and CD+8 T lymphocyces specific to HCV-VLPs:**

**Equipment:** Attune Cytpix flow cytometer from Invitrogen. A cell interacts with a fluorescent Rhodamine dye-tagged monoclonal antibody. As the cell descended the tube, a sensor measured its fluorescence, which was reduced by UV radiation.

**Figure 1.** It demonstrates purification of fused recombinant HCV-VLPs via Nickel columns using immobilized metal affinity chromatography on Nickle affinity resins.Purity of recombinant proteins was approximately 85%. The maximum yield of recombinant proteins was 60-65mg/l.




**Statistical analysis**

Every culture was carried out in triplicate. They used standard deviation and means 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. F-test was utilized during present study.

**Figure 2.** It represents protection power of test HCV-VLPs vaccinewhich was estimated as 63%.

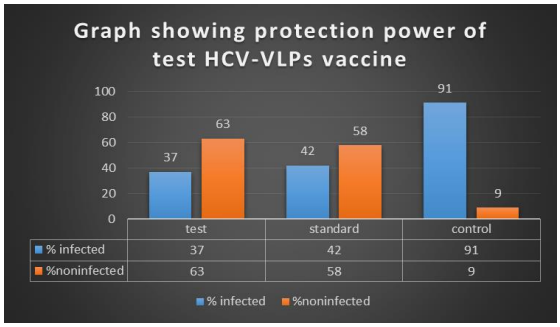



**Figure 3.** It shows NO. OF CD+4 helper T.lymphocytes(k/ul) AFTER VACCINATION with HCV-VLPS vaccine.

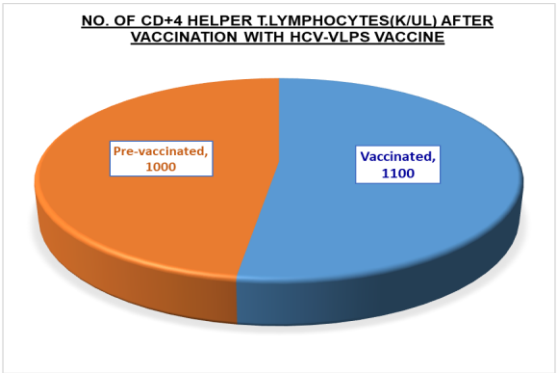


**Figure 4.** It represents number of CD+8 cytotoxic T.lymphocytes(K/UL) after vaccination with HCV-VLPS vaccine.

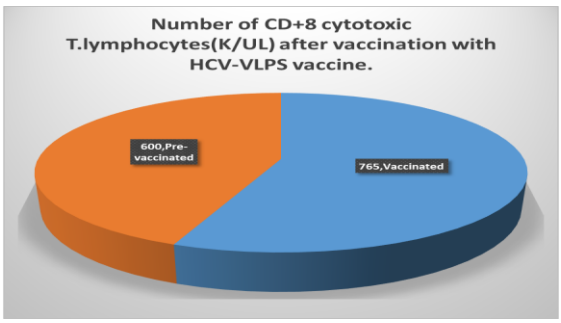



**Figure 5**. It demonstrates NO. of alive cases after vaccination of transgenic mice with HCV-VLPS vaccine.

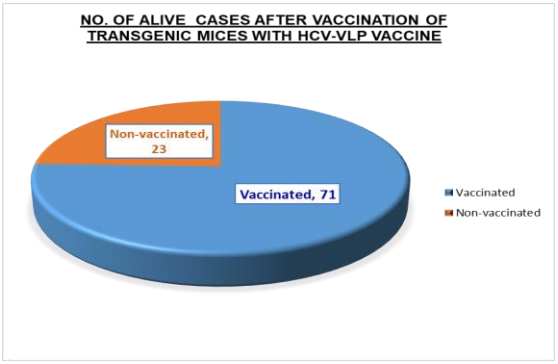
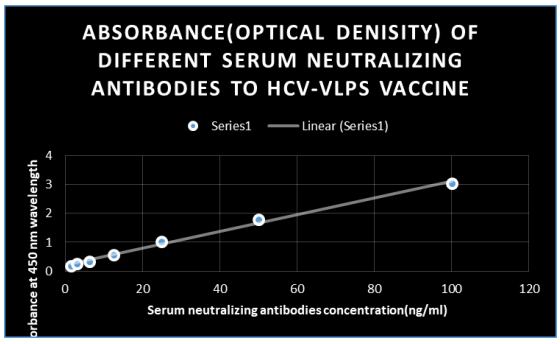



**Figure 6.** It displays Absorbance(optical density) of different serum neutralizing antibodies to HCV-VLPS vaccine.




## Results

The non-adjuvanted HCV VLP vaccine yielded 71% efficacy in preclinical (animal) studies, but 63% in human clinical evaluations. The LD50% of HCV virus was found to be over 60 mcg/ml. The ED50% for the HCV VLP vaccine was 15 µg/ml. The HCV VLP vaccine formulation was 25 µg/ml (5E1, 10E2, 10C HCV genotype 4 protein). Figure 1 shows how immobilized metal affinity chromatography on nickel affinity resins was utilized to purify fused recombinant HCV-VLPs. Recombinant proteins had an average purity of 85%. Recombinant protein output peaked at 60–65 mg/l. Transgenic mice were immunized against HCV genotype 4 in Table 1. The protection level of the test HCV-VLPs vaccination during phases 1 and 2 of clinical trials is shown in Table 2. The immunization against HCV genotype 4 is shown in Table 3. The estimated 63% protective power of the test HCV-VLPs vaccination is shown in Figure 2. Figure 3 displays the number of CD+4 helper T cells (k/ul) following HCV-VLPS vaccination. Following immunization with the HCV-VLPS vaccine, the number of CD+8 cytotoxic T cells (K/UL) is shown in Figure 4. Figure 5 shows the number of instances that were still alive after transgenic mice were given the HCV-VLPS vaccine. Figure 6 shows the serum neutralizing antibodies to the HCV-VLPS vaccination that vary in absorbance (optical density). The absorbance of various serum neutralizing antibodies to the HCV-VLPs vaccination using ELISA is shown in Table 3. During human clinical trial stages 1/2, vaccinated individuals had a CD+4 count of 1100, whereas unvaccinated candidates had a count of 1000. In clinical trial stages 1/2, the CD+8 count was 765 following vaccination with HCV-VLPS vaccine; however, it was 600 in pre-vaccinated people. During human clinical trials phases 1/2, total T lymphocytes counted 1865 for vaccinated applicants and 1600 for unprotected individuals.

**Table 1**: Vaccination of transgenic mice against HCV genotype 4.

| Description | Vaccinated | Non-vaccinated |
|---|---|---|
| Alive | 71 | 23 |
| Dead | 29 | 77 |
| Total | 100 | 100 |

**Table 2.** It represents protection power of test HCV-VLPs vaccine during clinical trials phases1/2

| | vaccinated | Non vaccinated | |
|---|---|---|---|
| Description | test | standard | control |
| % infected | 37 | 42 | 91 |
| %noninfected | 63 | 58 | 9 |

**Table 3.** It represents the absorbance of different serum neutralizing antibodies to HCV-VLPs vaccine via ELISA.

| Concentration (ng/ml) | Absorbance (optical density) |
|---|---|
| 1.60 | 0.177 |
| 3.04 | 0.268 |
| 6.21 | 0.325 |
| 12.4 | 0.572 |
| 26 | 1.025 |
| 50 | 1.708 |
| 98 | 3.022 |

**Discussion**

The family of flaviviruses includes HCV. It is an encapsulated virion with a single-stranded, positive-polarity RNA genome. Virion polymerase is absent. Based on variations in the genes that encode one of the proteins, HCV has at least six genotypes and several subgenotypes. its two glycoprotein envelopes. The envelope glycoprotein develops a "hypervariable" area as a result of this genetic diversity. The high rate of mutation in the envelope gene and the lack of a proofreading feature in the RNA polymerase encoded by virion are the two factors responsible for the genetic heterogeneity. Hepatocytes are the primary target of HCV infection, although there is no proof that the virus has any cytopathic effects on the liver cells. Instead, it is likely that the hepatocytes' demise is brought by T cell immune assault. Hepatocellular carcinoma is significantly associated with HCV infection, however there is no proof that the viral genome contains an oncogene or that a copy of the viral genome has been inserted into the DNA of the cancer cells. Alcoholism significantly raises the incidence of hepatocellular carcinoma in people with HCV infection. Though HCV antibodies are produced, over 75% of individuals have a chronic infection and continue to generate virus for at least a year. About 10% of these individuals had cirrhosis and chronic active hepatitis. In the current work, bioinformatics was used to create the HCV-VLPs vaccine. The vaccination had a modest level of bioactivity and minimal adverse reactions. The vaccination has to be updated frequently because of its long-lasting effectiveness and the HCV virus's rapid rate of mutation. In human clinical trials, 42 members of the positive control group (standard) and 37 members of the test group contracted the infection, compared to 91 members of the negative control group. The HCV virus-like particles were around 60 nm in size. The coherent structural proteins E1, E2, and C of HCV form a monolayer inside the encapsulated VLPs produced by the HCV VLPS vaccination type. The HCV-VLP vaccination is advised for anyone above the age of two. This vaccine's recommended dosage is 20–25 mcg/ml intramuscularly, followed 21 days later by an intradermal boost. Immunogenicity rose from 63% to 66% when aluminium hydroxide was included in the adjuvant dose of 0.5 mg. People who are allergic to the components in the test vaccines should not get them. Mild discomfort at the injection site (intramuscularly and intradermally) and a low-grade fever lasting several days were observed as side effects. These symptoms might be treated with over-the-counter pain relievers such paracetamol and ibuprofen. It is advised to keep HCV VLPs (E1, E2, and C genotype 4 proteins) vaccination between 2 and 8 degrees Celsius. In contrast to another study ( Shata et al., 2014) carried out in the USA, the prepared vaccine in that study was found to be more effective against HCV genotype 3 than other genotypes, including genotype 4 predominating in Egypt, due to evoking strong humoral cell mediated immunity[20]; while in the present study, the HCV-VLPS vaccine was excellent against genotype 4 due to moderate stimulation of humoral immunity and strong evoking of cell mediated immunity. The role of immunity in preventing infection: These HCV-VLPS vaccinations highly enhanced cell-mediated immunity while only slightly stimulating humoral immunity.

The body's main infection defence has always been cell-mediated immunity. The predominant defence against HCV infection was provided by cytotoxic CD+8 T cells and helper CD+4 T lymphocytes. The humoral immunity against viral infection was only mildly stimulated by these vaccinations. IgM, IgG1, and IgG2 antibodies, which target the coherent structural proteins E2 and C of HCV genotype 4, were the main neutralizing antibodies in blood. Few IgA antibodies were generated against the vaccinations since they were not delivered orally or through the respiratory system. Despite the absence of measurable antibodies against the E1 envelope transmembrane protein, E1 was crucial to the HCV VLP vaccine's ability to self-assemble. High quantities of neutralizing antibodies to the endogenous structural proteins E2 and C were detected during the ELISA together with high absorbance (optical density). The typical CD+4 helper T cell count in immunocompetent patients was roughly 1000 ku/L, but following

immunization, it exceeded 1000 k/uL. Similar to this, following immunization, the average concentration of CD+8 cytotoxic T cells rose from 600 k/uL to over 750 k/uL. Advantages: It is impossible to revert to virulence. Cons: Since the vaccination virus cannot spread to people who are not immune and is not shed, it does not help the population build up a protective immunity to HCV infection. Summary of bionformactics analysis of E1, E2, and C proteins: C is an integral protein, while E1 and E2 are both type 1 transmembrane glycoproteins. All take part in membrane fusion and receptor binding. In HCV infection, the E2 and C proteins are both immunodominant antigens, and antibodies that prevent these proteins from interacting with their plasma membrane receptors are known as neutralizing antibodies. According to the Alex S et al, 2021 study, only one vaccine candidate successfully passed to phase II clinical efficacy testing in humans. Despite substantial preclinical evidence supporting its potential efficacy, this recently completed trial, which used recombinant viral vectors to stimulate pan-genotype T cell responses against HCV nonstructural proteins, failed to prevent chronic infection in a cohort of high-risk injection drug users[ 21]. The present study immunisation, on the other hand, exhibited protection against the onset of chronic infection in a group of high-risk injectable drug users.

**Conclusion**

Recombinant and HCV-derived VLPs are promising possibilities for HCV vaccination. The HCV VLP immunization effectively prevented genotype 4 and related variants of Hepatitis C virus. It is available to adults over the age of two. Researchers recommend researching controversial new ideas for developing new immunizations to counteract each new virus strain that emerges.

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**Conflict of interest:** There is no conflict of interest.

**Fund:** This study was carried out in a research project number 46361/2020 funded by STDF.

**Data availability:** Raw data were generated at faculty of pharmacy, Cairo university, Egypt. Derived data supporting the findings of this study are available from the corresponding author Dr.Mohammed Kassab up on request.

**Refrences**

**1. Ahmed Amal et al ( 2015).** Hepatitis C virus: A global view. World journal of hepatology. 7(26): 2676-2680.

**2. Parveen K (2020):** Kumarm, Clarks clinical medicine. Ninth edition,Elsevier Edinburgh London. 13(5):367-391.

**3. Caroline S, Zeind G (2020):** Applied therapeutics, the clinical use of drugs. Eleventh edition, Wolters Kluwer, London. 18(5):902-918.

**4. Trevor A, Katzung B, Kruidering M (2021):** Katzung Trevor pharmacology examination board review.Thirteen edition, Mc Graw Hill Education, New York. 4(3):744-759.

**5. Bardal S,Waechter J, Martin D (2020):** Applied pharmacology. Fourth edition, Elsevier Edinburgh, London. 23(2):217-233.

**6. Olson J (2020):** Clinical pharmacology made ridiculously simple.Seventh edition, MedMaster, Miami, United States of America. 5(3):118-127.

**7. Levinson Warren (2021):** Review of medical microbiology and immunology. fifteen edition,Mc Graw Hill Education, New York. 14(7):207-215.

**8. Swanson Larry N, Souney F,Muntnick H, Shargel L (2019):** Comprehensive Pharmacy Review for NAPLEX. Tenth edition,Wolters Kluwer, London. 3(2):541-549.

**9. Fisher B, Champe P, Harvey R (2021):** Lippincott illustrated reviews microbiology. Sixth edition,Wolters Kluwer, London. 21(4):202-223.

**10. Dipro C,Schwinghammer T,Dipro J,Well B (2021):** pharmacotherapy handbook. Eleventh edition,Mc Graw Hill Education, New York. 7(2):608-621.

**11. Golderg S (2020):** Clinical physiology made ridiculously simple. Sixth edition, Med Master, Miami, United States of America. 6(8):1024-1061.

**12. Wilson N (2019):** Biochemistry and genetics.Eighth edition, Mc Graw Hill Education, New York. 15(4):336-352.

**13. Metting J (2019):** Physiology. Sixteen edition, Mc Graw Hill Education, New York. 9(1):1204-1221.

**14. Qianqian Zaho, Kun He , Huanajie LI (2022),** production and immunogenicity of different prophylactic vaccines for hepatitis C virus(review), experimental and therapeutic medicine. 2(2):117-126.

**15. Behzad dehghani, Tayebeh Hashempour, Zahra Hasanshabi, javad Moayedi (2019),** bioinformatics analysis domain 1of HCV core protein, international journal of peptide research and therapeutics.5(4):289-299.

**16. Saghi N et al (2021):** Virus like particles: preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers, Journal of nanobiotechnology. 3(8): 557-569.

**17. Ran-Ran Zhang et al (2016).** Generation of a humanized mouse liver using human hepatic stem cells. J Vis. 2016; (114): 54167.

**18. Jone Karmel et al (2021).** Journal of the lancet gastroenterology and hepatology. Volume 6 issue 4, P253, April 01, 2021.

**19. Malihe Naderi et al (2014).** Hepatitis C virus and vaccine development. Int J Mol cell Med. 2014 Autumn; 3(4):207-215.

**20. Shata M et al (2014).** Exposure to low infective doses of HCV induces cellular immune responses without consistently detectable viremia or seroconversion in chimpanzees. 314(2): 601–616.

**21. Alex S et al (2021).** Hepatitis C virus vaccine research: Time to put up or shut up. Journal of viruses. Published online 2021 Aug; 13(8): 1560. doi: 10.3390/v13081596.

Kassab, MMS. The exploitation of Hepatitis C virus-like particles vaccine against genotype 4 by bioinformatics. International Journal of Health Sciences (Egypt), 2023; 1(3): 75-83.