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Research Article

Preclinical Evaluation of EGYVAX; an Inactivated anti-SARS-CoV-2 Vaccine Candidate

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Abstract

Background: The SARS-CoV-2 pandemic showed the urgent need for building the capacity for both development and production of low-technology-based vaccines particularly in low-and middle-income countries. **Aim:** to evaluate both safety and immunogenicity of EGYVAX, an Egyptian anti-SARS-CoV-2 vaccine candidate. **Material & methods:** Ten rhesus macaque monkeys were randomly allocated into five groups received the EGYVAX vaccine at rising doses. Animals were assessed daily for both clinical signs and mortality. Cellular, biochemical, and molecular analyses were conducted. **Results:** Neither morbidity nor mortality were seen in the immunized monkeys, except for vomiting in one of the immunized animal groups, other clinical signs, behavior, and mental status were comparable to those of the control groups. **Conclusion:** The obtained results revealed both safety and immunogenicity of the evaluated anti-SARS-CoV-2 vaccine candidate in rhesus macaques, which is a step forward towards the production of protective vaccines against any future pandemic.

Keywords: SARS-CoV-2; Inactivated vaccine; Morbidity; Mortality; Safety; Immunogenicity

Introduction

The SARS-CoV-2 pandemic, which has affected over 200 million people and killed 4.3 million people globally as of August 2021, has been difficult to control because of its heavy economic, healthcare, and social costs [1]. Thus, the pandemic has created an urgent need for rapid vaccine development based on scientific discoveries and our understanding of the virus biology, variation, and transmission potential [2]. Despite the time required to develop safe and effective vaccines (typically 1–2 years), vaccination remains the most effective and cost-efficient strategy for achieving "herd immunity" [3]. According to Anderson et al., vaccination of up to 90% of the population may be necessary to achieve COVID-19 herd immunity [4]. While each COVID-19 vaccine has its unique benefits and drawbacks, these vaccines must be widely accessible and affordable, especially in developing nations, to contain the pandemic [5].

The human body's initial response to infection by the SARS-CoV-2 virus is to secrete cytokine IL-6, which is believed to modulate both innate and adaptive immunity. The virus causes a capillary leak, endothelial cell destruction, and macrophage activation. The continuous generation of IL-6 leads to cytokine storm, ultimately resulting in the clinical and pathological features of acute respiratory distress syndrome (ARDS) [6]. SARS-CoV-2 also triggers an immune response, including B cell activation to produce antibodies against specific antigens. Neutralizing antibodies, the most common strategy for treating viral infections, should be used to minimize SARS-CoV-2 transmission. This strategy requires vaccination to strengthen immune defenses [7].

A successful vaccine should induce a strong Th1 and a weak Th2 response. This is because a robust Th1 cell response can trigger protective humoral and cell-mediated immunity and reduce the risk of disease enhancement, while excessive Th2 activity can cause uncontrolled tissue damage and negate the Th1-mediated microbicidal effect [8]. The Th1/Th2 balance in COVID-19 is a prognostic indicator of the disease outcome, with 78% of patients who died having an overactive Th2 response. This suggests that the type of Th response is essential for disease resolution. A well-regulated Th1-mediated immune response can effectively clear the virus, but a poorly organized response can lead to an exacerbated Th2-mediated cytokine storm, which is strongly associated with poor prognosis. Ideally, for optimal protection against SARS-CoV-2 exposure, high neutralizing antibodies titer, a robust cytotoxic CD8+ T cell response, and a Th1-biased CD4+ effector response are essential [9].

Live vaccinations may pose a risk to individuals with immunosuppression or weakened immune systems. Inactivated viruses may be safer, but they often provide less long-lacting protection and require repeated booster doses [10]. Inactivated viral vaccines are both rapid and reliable to raise protective immunity, and have been widely used to prevent emerging infectious diseases. However, caution is warranted in developing COVID-19 vaccines, as SARS-CoV-2 infection can exhibit antibody-dependent enhancement, highlighting the importance of safety assessment.

In a previous study, we described the development of the inactivated SARS-CoV-2 vaccine candidate EGYVAX, including sample collection, virus isolation, propagation, virus inactivation, and vaccine production, as well as safety and immunogenicity evaluation of two doses of the vaccine in mice [11]. Here, we report the results of the preclinical dose-finding studies of EGYVAX, demonstrating its safety and immunogenicity at different vaccine doses in rhesus macaques. This paves the way to initiate the first-in-human clinical trial for a SARS-CoV-2 vaccine candidate in Egypt.

Material and Methods

The preclinical studies of the inactivated EGYVAX vaccine were conducted in accordance with the Food and Drug Administration (FDA) guidelines for developing and licensing SARS-CoV-2 vaccines [12], which are based on the World Health Organization (WHO) guidelines for the preclinical development of vaccines [13] including the required study designs, number of doses, related investigations, and procedures.

Experimental animals

Ten rhesus macaque monkeys with an equal ratio of males and females, aged 7–14 years and weighing 9–16 kg, were obtained from Giza and Alex Zoo. The animals were acclimatized for 7 days before dosing. The monkeys were housed in standard macaque cages at a temperature of 25°C (±3°C), relative humidity of 30% to 70%, and a 12-hour dark-light cycle. They were fed a standard macaque diet and given tap water at the Egyptian Army Veterinary Hospital from March 23, 2021, to May 04, 2021. Animals were identified by their cage number for the duration of the experiment. The observation period was 42 days. We used a sample size of 10 animals based on availability and literature precedent [14].

Six experimental macaque monkeys (n = 6) were randomly assigned to receive three doses (0.5 ml) of inactivated vaccine containing 35, 70, and 140 µg total protein, administered by intramuscular (IM) injection on days 0, 14, and 28 (initial and two booster doses considering the N+1 rule). Four control animals were injected

with 0.5 ml saline at the same time points. Two control animals previously immunized with the vaccine 7 months apart were used as positive controls.

Animals were randomly allocated into five groups (n=10; 5 males & 5 females) each of 1 male and 1 female. Groups 1-3 received 35, 70 and 140 µg protein of, the inactivated virus, EGYVAX, while group 4 which served as negative control received saline IM doses and group 5 which served as positive control were previously immunized with EGYVAX and received saline IM doses.

Group	No. of animals	Male/ Female	Protein concentration of the EGYVAX (µg/ 0.5 ml)	Dosing Interval (Days)
Dose level 1	2	1♂/1♀	35	0,14, 28
Dose level 2	2	1♂/1♀	70	0,14, 28
Dose level 3	2	1♂/1♀	140	0,14, 28
Positive control*	2	1♂/1♀	0.5 ml saline	0,14, 28
Negative control**	2	1♂/1♀	0.5 ml saline	0,14, 28
*Positive control are animals previously injected with high dose of the vaccine (250 and 556 µg total protein) 7 months before initiation of this study. **Negative control are animals with no prior vaccination and located in Giza Zoo.				

Table 1: Dosing scheme of the study.

Animals were assessed daily for both clinical signs and mortality. Cellular, biochemical, and molecular analyses were conducted, including complete blood count (CBC), serum chemistry, serum neutralizing antibody (NAB) titers, immunophenotyping, chest X-ray, and RT-PCR for detecting viral RNA.

1- General clinical signs & Mortality:

Morbidity and mortality were monitored twice daily. Clinical observations were performed using a general welfare assessment score sheet adapted from Wolfensohn & Honess, 2005 [15]. Animal appearance and behaviour were monitored daily. Body weight, food, and fluid intake were monitored every week.

2- Hematology and serum biochemical analyses. Blood samples were collected from the brachial or femoral veins:

CBC and serum chemistry were assessed on days 0, 7, 14, 28, 35, and 42 following blood sampling. Serum NAB titers were measured at days 0, 7, 14, 28, 35, and 42 following blood sampling. The CD4/CD8 ratio was assessed at days 0, 7, 14, 28, and 42 following blood sampling. Th1/Th2 antibody levels were determined at days 0, 7, 14, 28, and 42 following blood sampling.

3- Other investigations:

X-ray examination was carried out on days 0, 14, and 42. Oropharyngeal swab specimens from vaccinated and control macaques were collected at days 0, 7, 14, and 42 and tested for viral RNA by RT-PCR.

The schedule of examinations is summarized in Table 2 as follows:

	Day 0	Day 7	Day 14	Day 28	Day 35	Day 42
Clinical observations						
Appearance*	x	x	x	x	x	x
Food and water intake	x	x	x	x	x	x
Natural behaviour*	x	x	x	x	x	x
Provoked behaviour*	x	x	x	x	x	x
Lab investigations						
Serum chemistry tests	x	x	x	x	x	x
CBC	x	x	x	x	x	x
Serum neutralizing antibody titre	x	x	x	x	x	x
CD4/CD8	x	x	x	x		x
Th1/Th2 antibody panel	x	x	x	x		x
Other investigations						
X-ray examination	x		x			x
PCR detection of COVID-19 RNA	x	x	x			x
*Tests to be performed on daily basis.						

Table 2: Schedule of examinations during the study.

4- Assessment of immunogenicity using viral microneutralization (VMN) test:

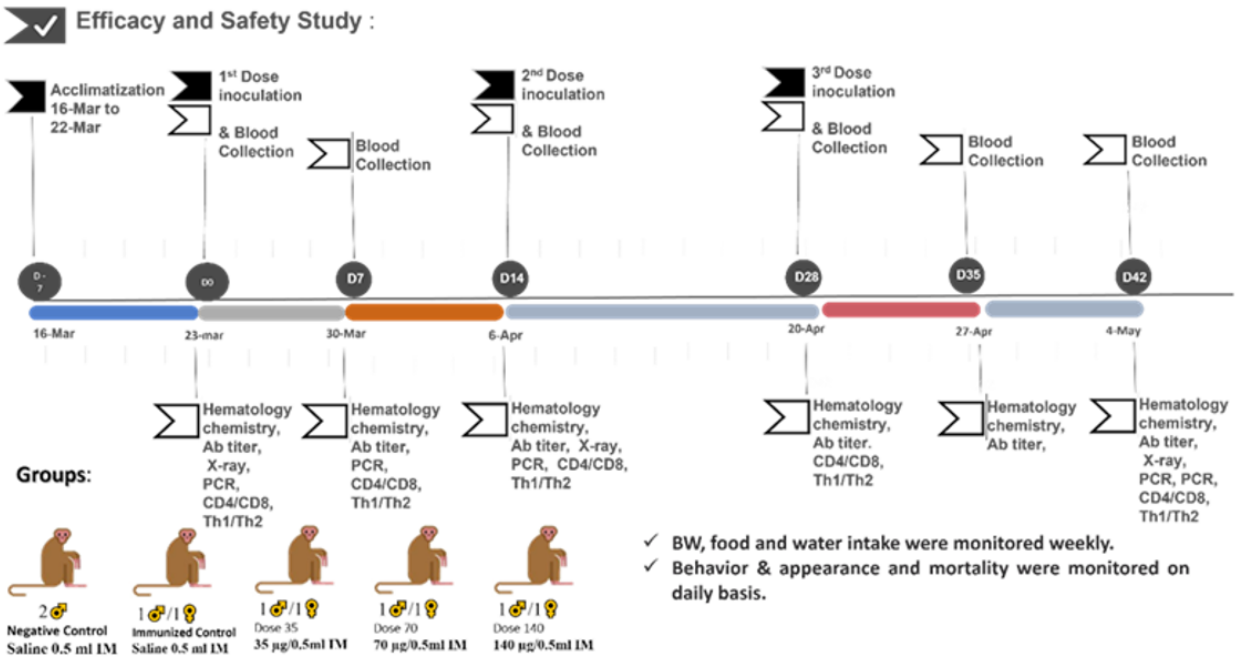
The viral microneutralization (VMN) test described in Alessandro et al. was used to assess the neutralizing activity of antibodies against SARS-CoV-2 in VERO cells, which are permissive for SARS-CoV-2 infection. Serum samples were heat-inactivated for 30 min at 56°C and serially diluted twofold starting from 1:10. An equal volume containing 100 TCID50 of SARS-CoV-2 was added to each dilution, and the serum-virus mixture was incubated for 1 h at 37°C in a humidified atmosphere with 5% CO2. After incubation, 100 µl serum-virus mixture was added in duplicate to a cell plate containing a semi-confluent VERO monolayer, and the plates were then incubated for 4 days at 37°C in a humidified incubator with 5% CO2. The antibody titer of each serum sample was defined as the highest serum dilution that completely protected the cells from cytopathic effect (CPE) in at least half or at all wells, respectively [16].

5- Hematologic and blood chemistry analysis:

CBC was performed on blood samples collected in sterile ethylene diamine tetra acetic acid-containing vacutainer tubes using an automated cell counter (IDEXX Laboratories, USA). Additionally, blood samples were collected in plain tubes and sera were separated for automated analysis of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase (ALP), urea, and creatinine with the IDEXX analyzer system, and inflammatory markers (i.e., TNF-α, IL-6, and INF-ɣ) using specific ELISA kits (Origene, USA).

Statistical analysis

The Instat version 3 and GraphPad Prism version 5 (GraphPad Software Inc., USA) software packages were used for data analysis. Data were expressed as mean ± SD. Comparisons between different means were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to identify statistically significant differences between individual groups. Comparisons between different means of antibody titers at different time intervals were performed using two-way ANOVA. Statistical significance was defined when $p<0.05$. Data were tested for normality.



Scheme 1: A general outline of the efficacy and safety study conducted from March 23, 2021, to May 04, 2021.

Results

In this study, four animals of each gender were subjected to immunization with escalating vaccine doses per animal. The kinetics of gender-specific total and differential counts of white blood cells, erythrocytes, and platelets were monitored at regular intervals post-immunization. In addition, T-cell responses and virus-neutralizing antibody titers were documented. Furthermore, procalcitonin, liver enzymes, and kidney function were recorded. Both data analysis and plots were generated using GraphPad PRISM version 5 software. The results were expressed as means ± standard deviations.

By presenting data for the highest dose of the vaccine (140 µg), the authors aim to highlight the significant results obtained. Although the other doses showed minimal post-immunization parameters, this particular dose gave the most appreciable results. The data presented is a testament to the efficacy of the vaccine and reinforces the importance of considering higher doses to achieve optimal results.

In general, monkeys showed no morbidity or mortality among all groups throughout the experiment. No abnormal clinical signs were observed in any macaque following immunization with the three doses.

Hematological and biochemical laboratory values were relatively normal at all the study endpoints compared to the control group. In several occasions, females showed higher both total and differential white blood cells counts than males in response to vaccination. This was hard to explain in the light of the used small sample size and needs to be confirmed on a bigger sample size (Fig. 1). Reticulocytes counts were higher among males than females at 7-42 days post-immunization. The peak reticulocytes percentage was seen for both genders at 7 days post-immunization (Fig. 2).

All platelets related parameters showed gradual increase post immunization and were relatively higher among females compared to males. The female-bias was hard to explain in the light of the used small sample size and needs to be confirmed on a bigger sample size (Fig. 3).

Where Th1/Th2 ratios were higher among males at early time points the opposite was seen at 42 days post vaccination. At 7 days post vaccination males showed slightly higher CD4/CD8 ratio than females but the opposite was seen at later time points. All test groups developed detectable neutralizing antibodies at all study intervals. The virus neutralizing antibodies titers were generally higher among females than males (Fig. 4).

There was a general female-bias in the levels of procalcitonin, alanine transaminase, alkaline phosphatase and bilirubin/creatinine ratio at all time points post vaccination and the opposite was recorded for the creatinine levels. This was hard to explain in the light of the used small sample size and needs to be confirmed on a bigger sample size (Fig. 5).

RT-PCR testing confirmed the absence of virus genome amplification, indicating vaccine safety. X-ray evaluation showed no abnormalities for either test group.

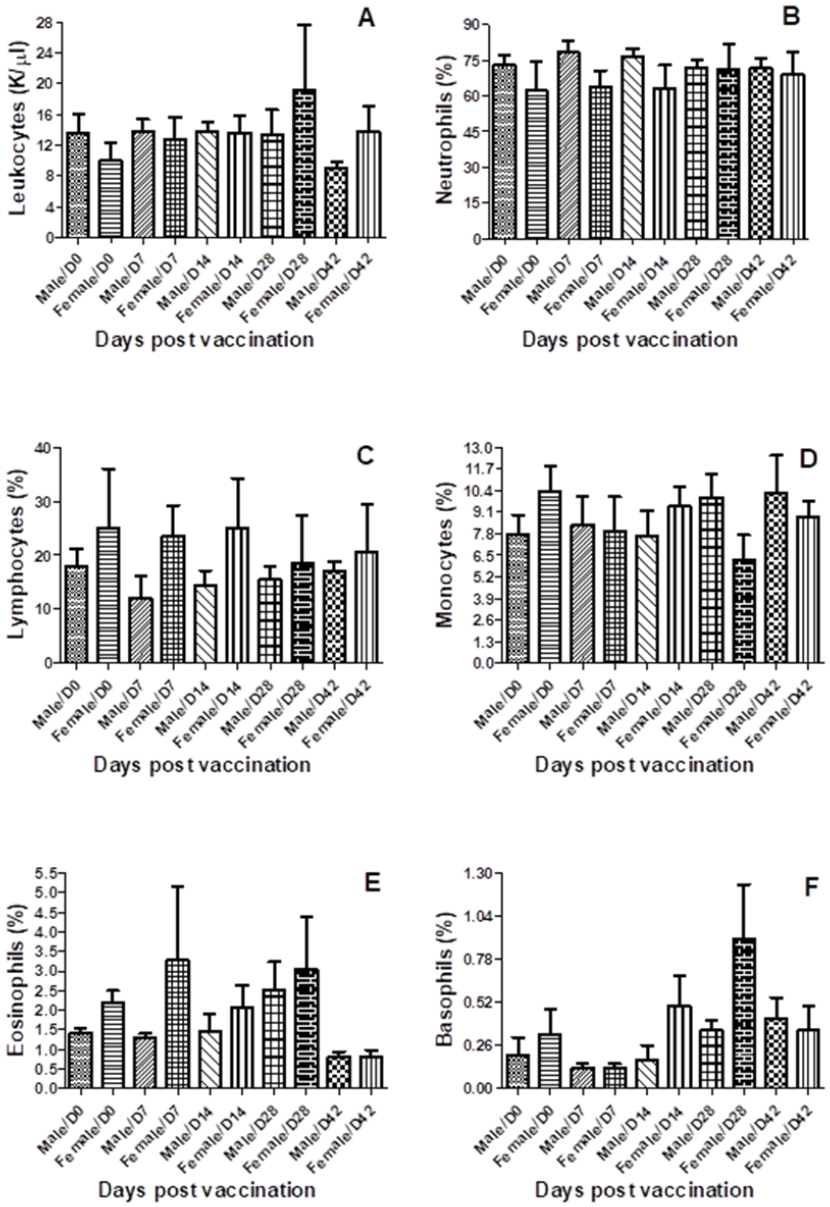


Figure 1: Kinetics of the gender-specific total and differential white blood cells counts in rhesus macaque monkeys in response to immunization with EGYVAX. A total of 4 animals from each gender were immunized by 140 µg of the vaccine per animal. Both the total and differential counts were kinetically monitored at regular time intervals post immunization. Both data analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations. In several occasions, females showed higher both total and differential counts than males in response to vaccination. This was hard to explain in the light of the used small sample size and needs to be confirmed on a bigger sample size.

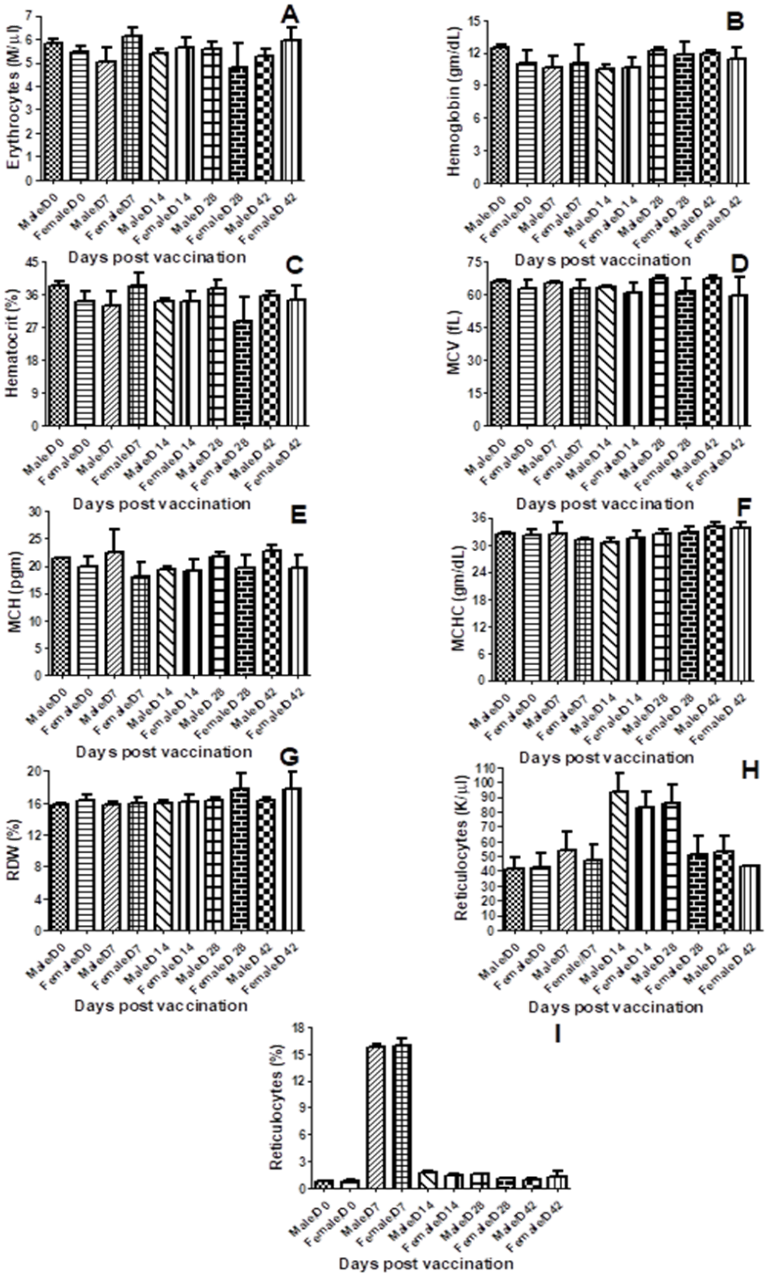


Figure 2: Kinetics of the gender-specific erythrocyte counts and related parameters in rhesus macaque monkeys in response to immunization with EGYVAX. A total of 4 animals from each gender were immunized by 140 µg of the vaccine per animal. Both the total and differential counts were kinetically monitored at regular time intervals post immunization. Both data analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations. Reticulocytes counts were higher among males than females at 7-42 days post immunization. The peak reticulocytes % was seen for both genders at 7 days post immunization.

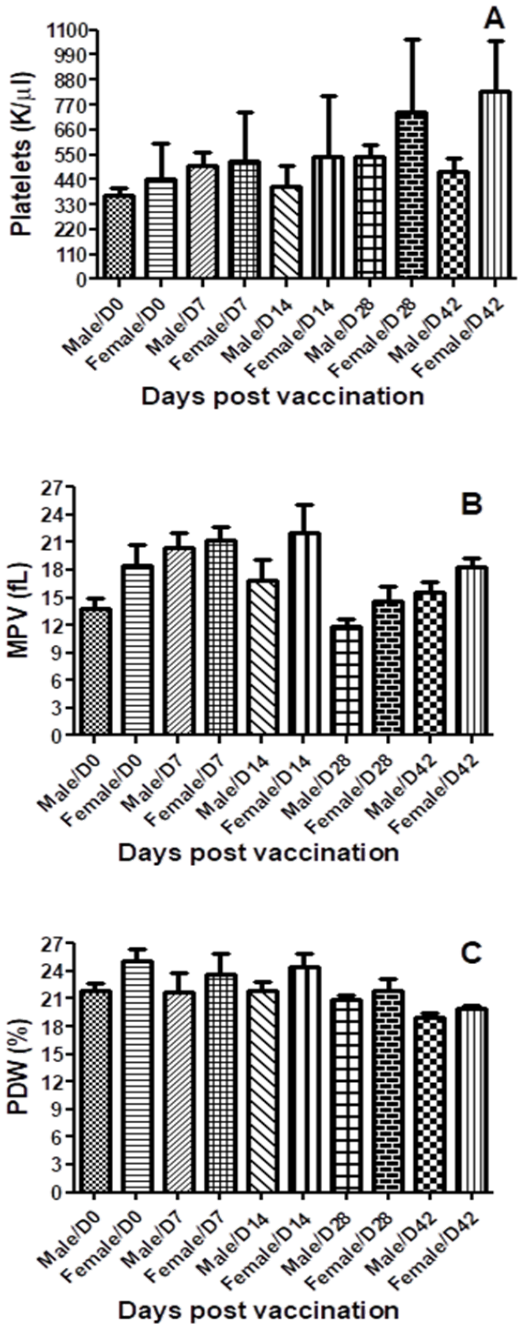


Figure 3: Kinetics of the gender-specific platelets counts and related parameters in rhesus macaque monkeys in response to immunization with EGYVAX. A total of 4 animals from each gender were immunized by 140 µg of the vaccine per animal. Both the total and differential counts were kinetically monitored at regular time intervals post immunization. Both data analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations. All platelets related parameters showed gradual increase post immunization and were relatively higher among females compared to males. The female-bias was hard to explain in the light of the used small sample size and needs to be confirmed on a bigger sample size.

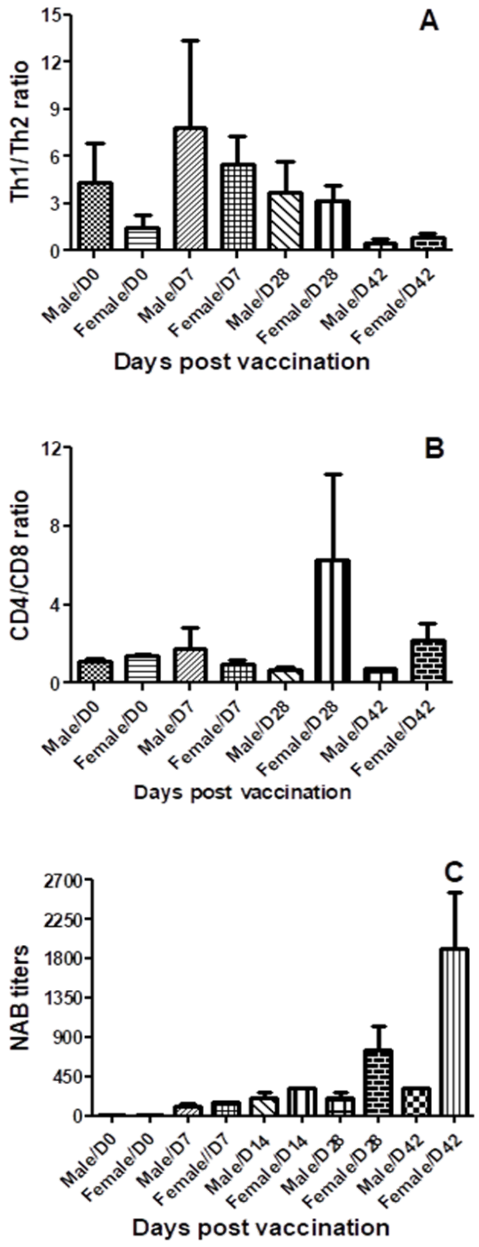


Figure 4: Kinetics of the gender-specific T-cell responses and virus neutralizing antibodies titers in rhesus macaque monkeys in response to immunization with EGYVAX. Both data analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations. Where Th1/Th2 ratios were higher among males at early time points the opposite was seen at 42 days post vaccination. At 7 days post vaccination males showed slightly higher CD4/CD8 ratio than females but the opposite was seen at later time points. The virus neutralizing antibodies titers were generally higher among females than males.

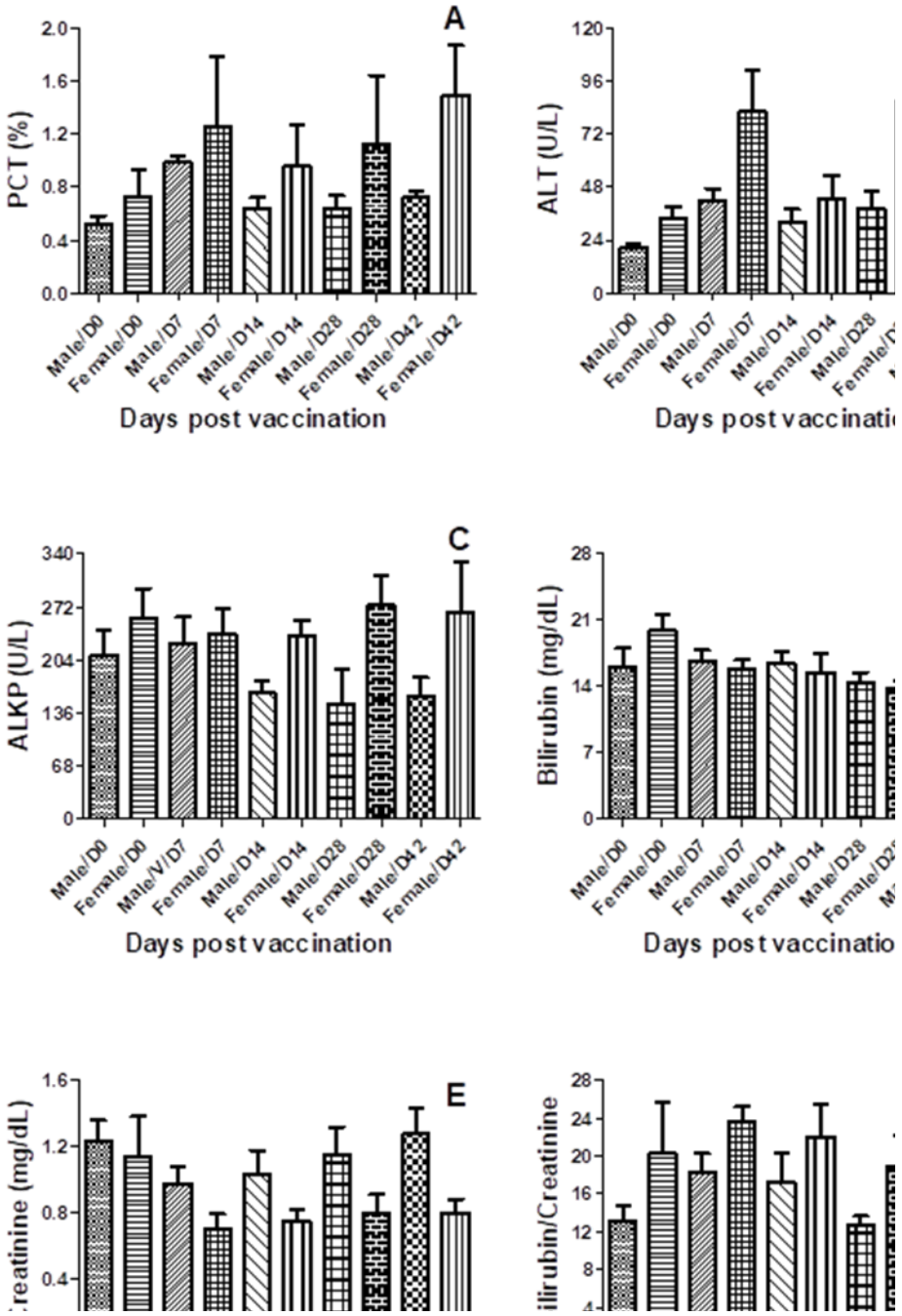


Figure 5: Kinetics of the gender-specific procalcitonin, liver enzymes and kidney function in rhesus macaque monkeys in response to immunization with EGYVAX. Both data analysis and plots were done using the GraphPad PRISM version 5 software. Results were expressed as means ± standard deviations. There was a general female-bias in the levels of procalcitonin, alanine transaminase, alkaline phosphatase and bilirubin/creatinine ratio at all time points post vaccination and the opposite was recorded for the creatinine levels. This was hard to explain in the light of the used small sample size and needs to be confirmed on a bigger sample size.

Discussion

During the early stages of the COVID-19 pandemic, Egypt relied on imported vaccines from other countries. However, the need to produce local vaccines for sustainable access became evident. These vaccines will be used routinely to achieve herd immunity since vaccine hesitancy could prolong the pandemic and increase morbidity and mortality [17]. The WHO estimates that immunization programs save 2–3 million lives each year by preventing 20 different diseases, including polio, diphtheria, tetanus, smallpox, pertussis, influenza, and measles [18].

Moreover, replication mutations could lead to the emergence of new variants, causing additional pandemic waves [19]. The immunogenicity and effectiveness of the prepared vaccine candidate were previously assessed in vitro using a neutralization assay and in vivo using experimental mouse models [20].

In the present study, macaques non human primates for which the observed findings are more relevant to humans [21], were used to assess the efficacy and immunogenicity of the vaccine candidate EGYVAX. Notably, neither morbidity nor mortality was reported in vaccinated monkeys. Other clinical signs, behavior, and mental status were comparable to those of the control groups, except vomiting, observed in a subset of vaccinated monkeys at sporadic intervals. Vaccines are the most effective means to prevent morbidity and mortality and are one of the most significant advances in medicine over the last centuries. However, vaccines are used for preventing infections in healthy people, unlike antimicrobial drugs, which are used to treat infected people. Therefore, adverse effects associated with vaccination are of utmost importance [22].

We observed that hematological and biochemical laboratory values at all research endpoints were comparable to those of the control group. The respiratory tract, gastrointestinal tract, central nervous system, liver, kidneys, and heart are the primary organs that may be affected by COVID-19 onset [23]. Whole-virus inactivated or live attenuated vaccines are conventional vaccine production platforms. The primary concern with this platform is the potential for incomplete viral inactivation, which could cause post-vaccination health complications in vulnerable people. Consequently, each batch must be validated to ensure complete inactivation of all recruited pathogens [5].

In addition, to the above, all male and group 3 female test monkeys vaccinated at a dose of 140 ug total protein on day 28 exhibited CD8 dominance, while all female and group 3 male test monkeys treated with 140 ug on day 7 showed CD4 dominance. CD8 T cells can attack pathogens by releasing cytokines and fight tumors by directly eliminating altered cells [24]; while CD4 T cells aid in coordinating the immune response by stimulating other immune cells to fight infection, such as macrophages, B lymphocytes, and CD8 cells [25]. The CD4+/CD8+ ratio is a clinical measure to assess immunity. A low CD8 T cell count has been associated with disease severity and mortality in severe COVID-19 patients [26].

All test groups showed a Th1 dominant-immune response on day 28, 14 days after the initial booster dose. Adult participants aged 50 and above exhibited similar vaccination-induced responses, with a higher Th1/Th2 ratio after two doses of an adjuvanted SARS-CoV-2 recombinant protein vaccine candidate [27]. A Th1/Th2 cell imbalance has been well-documented in groups affected by COVID-19 at high risk of infection and death [28].

Our results revealed that all test groups developed detectable neutralizing antibody titers at all study intervals, with groups 1 (35 ug) and 2 (70 ug) having higher titers than other groups. Interestingly, females showed higher neutralizing antibody titers than males at all vaccine concentrations. Neutralizing antibodies are essential immunological markers that reflect the elicitation of defense mechanisms to prevent and control viral infections and disease progression [29]. Due to the significance of the spike protein in viral entry to host cells, most vaccine candidates, including adenovirus-, mRNA-, and DNA-based options, generate neutralizing antibodies against the spike protein to prevent viral infectivity. Inactivated virus vaccines contain all of the viral antigens and thereby immune responses induced in vaccinated individuals are expected to target several viral antigenics determinants [30]. Recent studies have shown a correlation between clinical protection and vaccine-induced neutralizing antibody levels. Data from preclinical nonhuman primates challenge trials investigating the relationships between neutralizing antibody levels and anti-SARS-Cov-2 vaccine effectiveness suggest that these vaccines can induce neutralizing antibody production in nonhuman primates [31].

In conclusion, our results demonstrate both the safety and immunogenicity of the vaccine candidate EGYVAX in rhesus macaque monkeys specifically when used in the highest dose, which is a promising step towards clinical trials and licensing for human use. The emergence of new variants with high both, transmission and immune evasion capabilities significantly reduces the efficacy of available licensed vaccines. Therefore, rapid efficacy assessment of new COVID-19 vaccines based on different technological platforms is essential to address this challenge [32].

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Ethical Considerations: The experimental protocol was approved by the Institutional Animal Care and Use Committee in Cairo

University **(**Approval ID: CU/III/F/21/21).

Conflict of Interest: Authors hereby declare no conflict of interest.

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