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Comparative Immunogenicity and Neutralization Potency of Four Approved COVID-19 Vaccines in BALB/c Mice

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ABSTRACT

Background: Since the outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), several vaccine candidates have been developed within a short period of time. Although the potency of these vaccines was evaluated individually, their comparative potency was not comprehensively evaluated.

Objective: To compare the immunogenicity and neutralization efficacy of four approved COVID-19 vaccines in Iran, including: PastoCovac Plus, Sinopharm, SpikoGen, and Noora in BALB/c mice.

Methods: Different groups of female BALB/c mice were vaccinated with three doses of each vaccine. The serum levels of antibodies against the viral receptor binding domain (anti-RBD) and spike (anti-spike) protein as well as the vaccine formulation (anti-vaccine) were evaluated using enzyme-linked immunosorbent assay (ELISA). The neutralization efficacy of these four vaccines was assessed through four neutralization assays: conventional virus neutralization test (cVNT), pseudotype virus neutralization test (pVNT), surrogate virus neutralization test (sVNT), and inhibition flow cytometry.

Results: All four vaccines induced seroconversion in vaccinated animals. All vaccines successfully induced high levels of anti-vaccine antibody; however, PastoCovac Plus and Sinopharm vaccines induced significantly higher levels of anti-RBD antibody titer compared to Noora and SpikoGen. Moreover, the results of the antibody response were corroborated by the virus neutralization tests, which revealed very weak neutralization potency by Noora and SpikoGen in all tests.

Conclusion: Our results indicate significant immunogenicity and neutralization efficacy induced by PastoCovac Plus and Sinopharm, but not by Noora and SpikoGen. This suggests the need for additional comparative assessment of the potency and efficacy of these four vaccines in vaccinated subjects.

Keywords: COVID-19, Immunogenicity, Neutralization, SARS-CoV-2, Vaccine

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INTRODUCTION

The COVID-19 pandemic has had a significant negative influence on the social, economic, and health conditions of people around the world (1, 2). Since the outbreak of the SARS-CoV-2 infection in December 2019, a number of variants of concern (VOCs), including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2), have emerged and rapidly spread globally (3). On November 26, 2021, the fifth VOC called Omicron (B.1.1.529) was identified, causing global alarm (4). South Africa first reported the B.1.1.529 variant to the World Health Organization (WHO) on November 24, 2021 (5). Throughout the six waves of the COVID-19 pandemic in Iran, the Alpha, Beta and Delta variants were circulating during the fourth wave. The fifth wave exclusively consisted of the Delta variant, while the Omicron variant was circulating during the sixth wave (6).

The most effective strategy for controlling the COVID-19 pandemic has been through the development of effective and safe vaccines (7, 8). As a global priority, pharmaceutical companies and scientists from all over the globe have devoted their efforts to develop and distribute safe and effective vaccines against COVID-19 (9).

The spike protein acts as the main target for neutralizing antibodies as the virus enters the host cells after receptor-binding domain (RBD) of the spike protein binds to human angiotensin converting enzyme-2 (ACE2) receptor (10). Therefore, vaccines that stimulate the immune response against the spike protein can effectively prevent the virus entry to the host cells and therefore the subsequent infection (11). Having a deep understanding of the structure and function of the spike protein and RBD is essential for the development of effective COVID-19 vaccines that can generate strong immune responses and offer protection against the virus (12).

In just under two years, hundreds of vaccine candidates were in the process of being developed worldwide. Eventually, by mid-2021, emergency use authorization (EUA) had been granted to some COVID-19 vaccines in various countries (13, 14). Despite the widespread rollout of these vaccines, several questions, including comparative safety, immunogenicity, and protective efficacy of these vaccines needed to be answered.

Different effects of various COVID-19 vaccines in preventing infection, hospital admission, and death have been reported in the Iranian population (15). Addressing these questions is crucial for the public health officials, healthcare providers, and policymakers to have a thorough understanding of the strengths and weaknesses of the different COVID-19 vaccines currently used in each country (16).

Accordingly, we conducted a comparative study on immunogenicity and neutralization efficacy of the four approved COVID-19 vaccines in Iran, including the PastoCovac Plus, Sinopharm, SpikoGen, and Noora. Since the majority of individuals received heterologous prime-boost vaccine regimens, conducting a comparative study using human serum samples is difficult. Consequently, to examine the immunogenicity and neutralization efficacy of these vaccines, multiple groups of female BALB/c mice were vaccinated with three doses of each vaccine. The level of antibodies against the vaccine formulation (anti-vaccine), RBD (anti-RBD), and spike (anti-spike) proteins was determined. Additionally, the neutralization efficacy of these four vaccines was evaluated using multiple neutralization tests including: sVNT, pVNT, cVNT, and inhibition flow cytometry.

MATERIALS AND METHODS

Ethical Statement

Research Ethics Committee of Tehran University of Medical Sciences approved this study (IR.TUMS.SPH.REC.1400.334).

Table 1. Approved SARS-CoV-2 vaccines employed in this study

Vaccine	Sinopharm	PastoCovac Plus	SpikoGen	Noora
Platform	Inactivated SARS-CoV-2	Recombinant RBD protein (Dimer)	Recombinant spike protein ECD (Trimer)	Recombinant RBD protein (Monomer)
Expression system	Vero cells	CHO cells	Insect cell line	E.coli
Adjuvant	Alum	Alum	Advax-CpG55.2™	Alum
Injected dose (µg)	2	10	5	40

RBD: Receptor Binding Domain, ECD: Extra Cellular Domain, CHO: Chinese Hamster Ovary, SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2, E.coli: Escherichia coli

Animal Vaccination

Six to eight week-old female BALB/c mice (Pasteur Institute of Iran, Karaj, Iran) were divided into various groups randomly. Each group consisted of five mice. The mice in each group were vaccinated with their respective vaccines. Based on previously published preclinical studies (17-20), the doses of Sinopharm, PastoCovac Plus, SpikoGen, and Noora vaccines were determined to be 2, 10, 5, and 40 µg per injection, respectively (Table 1). All vaccines were provided by a health center affiliated to Tehran University of Medical Sciences. Intramuscular (i.m.) injections of three doses of each vaccine were administered into the hind limb muscle to each mice at 21-day intervals (Fig. 1). The control animals were mock immunized with phosphate buffered saline (PBS) (0.14M, pH 7.4) following the same schedule. The immunogenicity study was conducted twice by the SpikoGen and Noora vaccines, utilizing two different lot numbers. Prior to the first injection, the blood samples were collected via tail vein. After the final booster dose, the blood samples were collected again three weeks later, and the sera were stored at -20°C until further use.

Measurement of Anti-vaccine Antibody Level in the Sera of Vaccinated Mice

To assess the immunogenicity of the vaccines, the antibody level against each vaccine formulation in their corresponding groups was measured using ELISA. MaxiSorp flat-bottom 96-well plates (Nunc, Roskilde, Denmark) were coated with 2.5 µg/ml of each vaccine. After overnight incubation at 4°C, the plates were blocked with 3% w/v skim milk (Merck, Darmstadt, Germany) in PBST (0.05% v/v Tween-20 in PBS). Serially diluted sera from each group were added to the corresponding coated wells and incubated at 37°C for 1 h, followed by three washes with PBST. The plates were subsequently incubated with horse-radish peroxidase (HRP)-conjugated sheep anti-mouse immunoglobulin (SinaBiotech,

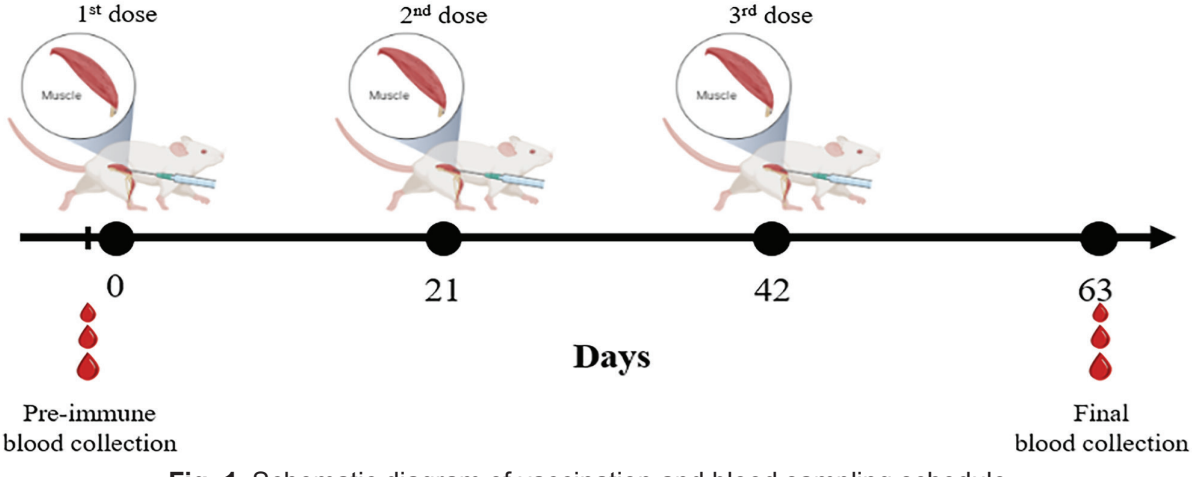

Fig. 1. Schematic diagram of vaccination and blood sampling schedule.

Tehran, Iran) at 1:2000 dilution prepared in the blocking buffer, and incubated at 37°C for 1 h. The reaction was developed by adding tetramethylbenzidine (TMB) substrate (Pishtaz Teb, Tehran, Iran), and then stopped with 2N H2SO4. Finally, the optical density (OD) of the reactions at 450/630 nm was measured using an ELISA microplate reader (BioTek, Winooki, VT, USA). Blank wells were included as the controls.

Measurement of the Levels of Anti-RBD and Anti-spike Antibodies in Sera of Vaccinated Mice

MaxiSorp flat-bottom 96 well plates (Nunc, Roskilde, Denmark) were coated with recombinant RBD (Wuhan-Hu-1; expressed in mammalian CHO-K1 cells in our laboratory, unpublished data) or full-length trimeric spike protein (Wuhan-Hu-1; expressed in mammalian HEK293T cells from BioServUK–CalibreScientific, Sheffield, United Kingdom) at a concentration of 2 µg/ml. The plates were then incubated overnight at 4°C and subsequently blocked with 3% w/v skim milk (Merck, Darmstadt, Germany) in PBST. Two-fold serially diluted sera were added to the plates and incubated at 37°C for 1 h, followed by three washes with PBST. Subsequent steps were conducted following the protocol described above. Serial dilutions of serum from a mouse hyperimmunized with RBD in combination with complete and incomplete Freund's adjuvant were used as standard sample for the assignment of arbitrary units (AU). To evaluate the level of anti-spike response, the sera of all the mice in each group were pooled and the test was conducted in triplicate.

SARS-CoV-2 Neutralization Assays

Pseudotyped Virus Neutralization Test (pVNT)

HEK293T cells expressing surface human angiotensin converting enzyme-2 (hACE2) protein (HEK-ACE2) (kind gift from Renap Therapeutics Co., Tehran, Iran) were cultured in RPMI-1640 medium (Gibco, Grand Island, NE, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine, and incubated at 37°C with 5% CO2 and 95% humidity. To evaluate the neutralizing potency of the serum samples for pseudovirus cell entry inhibition, eGFP-spike pseudotyped lentivirus, derived from the Delta variant of SARS-CoV-2 (kind gift from Renap Therapeutics Co., Tehran, Iran), was co-incubated with diluted sera. After 2 h incubation at 37°C, the mixture was added to a well of flat-bottom 96-well plate containing 14×10^3 HEK-ACE2 cells. After 48 h incubation, the culture medium was removed and the eGFP-positive cells infected by the pseudovirus were visualized using fluorescence microscopy. Multiple microscopic images were acquired from at least four different fields and the images were subsequently analyzed to quantify the number of GFP-expressing cells. The inhibition dilution 50 (ID50) values, which represent the serum dilution required to achieve 50% reduction in virus infection (indicating virus neutralization), was calculated as described by Ferrara et al. (21).

Conventional Virus Neutralization Test (cVNT)

The effectiveness of vaccines against the SARS-CoV-2 Delta variant was assessed using a conventional virus neutralization test (cVNT). The complement cascade proteins were initially inactivated by heating the samples at 56°C for 30 min in a water bath. Then, 50 µl of serially diluted serum samples were combined with 50 µl Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island, NE, USA) containing 100 Tissue Culture Infectious Dose 50 (TCID50) of SARS-CoV-2 Delta variant. After 1 h incubation at 37°C, the serum-virus mixture was added to a 96-well plate containing $1\text{-}2\times10^4$ Vero-E6 cells per well (80% confluency). The supernatant was removed after 1 h infection at 37°C, and the infected cells were washed twice with DMEM and incubated in DMEM at 37°C in a 5% CO2 incubator. After 72 h incubation,

the virus-specific cytopathic effect (CPE) was evaluated. The ID50 value, which represents the serum dilution that resulted in a 50% decrease in CPE formation, was determined. A neutralization antibody titer below 1:4 was considered negative, while a titer equal to or greater than 1:4 was considered positive for neutralizing antibodies (17, 22).

Surrogate Virus Neutralization Test (sVNT)

A surrogate virus neutralization test (sVNT) (Pishtaz Teb Co., Iran) was used to assess the efficacy of serum neutralizing antibodies, competing with HRP-conjugated ACE2 protein for binding to coated RBD protein (Delta variant). Accordingly, 50 µl of serially diluted serum samples or standard solutions were mixed with 50 µl of HRP-conjugated ACE2 in individual wells coated with RBD protein. The mixture was gently mixed for 15 seconds and then incubated for 30 min at 37ºC. Following the incubation, the microplate wells were washed, and 100 µl of TMB substrate solution was added. After 15-minute incubation at room temperature, 100 µl of stop solution was added, and the optical densities (ODs) were measured at 450/630 nm. To determine the percent inhibition value, the following formula was used: ((sample OD - negative control OD) / negative control OD)×100.

Inhibition Flow Cytometry Virus Neutralization Test

We developed an inhibition flow cytometry neutralization assay to assess the effectiveness of vaccinated sera for inhibiting the binding of RBD to ACE2 receptors on surface of HEK-ACE2 transfected cells. To perform the assay, 25 µl of serially diluted serum samples were mixed with an equal volume of Wuhan-Hu-1 strain of RBD fused to human Fc (RBD-Fc) (0.25 µg/ml) and the mixture was incubated at 37ºC for 2 h on a shaker incubator at 90 RPM. The serum/RBD-Fc mixture was then added to 1×10^5 HEK-ACE2 cells and incubated on ice for 45 min. Human IgG was used as an isotype control. After washing, 50 µl FITC-labeled sheep anti-human antibody (SinaBiotech, Tehran, Iran) at a final dilution of 1:100 was added to the cells and incubated on ice for 45 min. Following the washing with PBS-2% FBS, the cells were scanned using a Partec PAS Flow Cytometer (Partec, Nuremberg, Germany) and the data were analyzed by FlowJo V10 software.

Statistical Analysis

The data were analyzed using Graphpad Prism 9.0 software (GraphPad Software Inc., La Jolla, USA). The data are expressed as the mean±standard deviation (SD). Multiple group comparisons were performed using one-way ANOVA followed by Tukey's multiple comparison post-test. Statistical significance was indicated as follows: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. The inhibition dilution 50% (ID50) values for the sera were calculated using non-linear regression analysis with the [inhibitor] vs. normalized response -- Variable slope model. The correlation between anti-RBD titer and neutralization results obtained by flow cytometry, sVNT, pVNT, and cVNT was analyzed by the Spearman test.

RESULTS

Determination of the Antibody Response Against Vaccine Formulations, RBD and Spike Proteins

Complete seroconversion (100%) was observed in all groups of immunized mice. High levels of anti-vaccine antibody were induced by all the vaccines, while the mice vaccinated with SpikoGen displayed much higher titer of anti-vaccine antibody compared with the other vaccines (Fig. 2a).

Next, we measured the serum level of anti-RBD and anti-spike antibodies in all the groups of immunized mice. The results showed that the level of anti-RBD antibody was significantly higher in mice receiving Sinopharm and PastoCovac Plus compared with the SpikoGen and Noora vaccines (Fig. 2b).

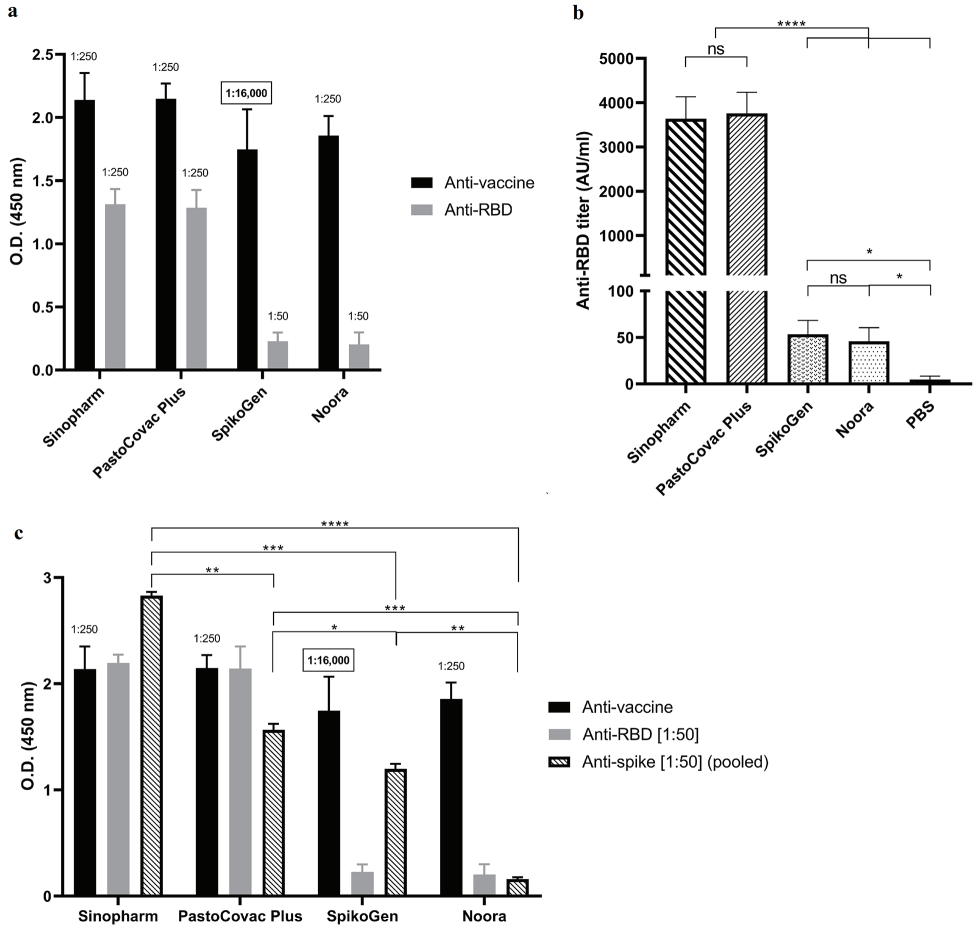

Fig. 2. Comparison of anti-vaccine, anti-RBD, and anti-spike antibody levels in mice sera vaccinated with various approved COVID-19 vaccines. Different groups of mice were vaccinated with various vaccines. The vaccination schedule consisted of three doses administered at 21-day intervals. The serum samples were collected from mice on day 21 after the final injection. (a) The optical density (O.D.) of anti-vaccine and anti-RBD antibodies in mice groups vaccinated with different vaccines was compared. The numbers on each bar graph represent the serum dilution used in the assay. (b) The titer of anti-RBD antibodies was assessed following vaccination with different vaccines. Arbitrary units (AU) were assigned to the measurements using hyperimmunized mouse serum as a standard sample. The control group received PBS injections. (c) The optical density (O.D.) of anti-RBD and anti-spike antibodies at 1:50 serum dilution was compared in the mice groups vaccinated with different vaccines. The data are presented as the mean ± SD. The statistical analysis was performed using one-way ANOVA to determine *p*-values between the different groups. ns: not significant, **p*<0.05, ***p*<0.01, ****p*<0.001, and *****p*<0.0001.

There was no statistically significant difference in the level of anti-RBD antibody response between Sinopharm and PastoCovac Plus vaccines.

In addition, the level of anti-spike response in the pooled sera of each group was evaluated. The test was conducted in triplicate to ensure accuracy and consistency. Sinopharm, PastoCovac Plus, and SpikoGen vaccines induced a high level of anti-spike antibody response compared with Noora vaccine, inducing a negligible anti-spike response (Fig. 2c).

Assessment of the Virus Neutralization Potency Induced by the Vaccines

To determine SARS-CoV-2 neutralizing efficacy in the sera of vaccinated mice, we used sVNT, pVNT, cVNT, and inhibition flow cytometry techniques. For pVNT, pseudovirus that expressed the spike protein of the Delta variant of SARS-CoV-2 was used. For cVNT, live SARS-COV2 Delta variant was used. Three weeks after the last

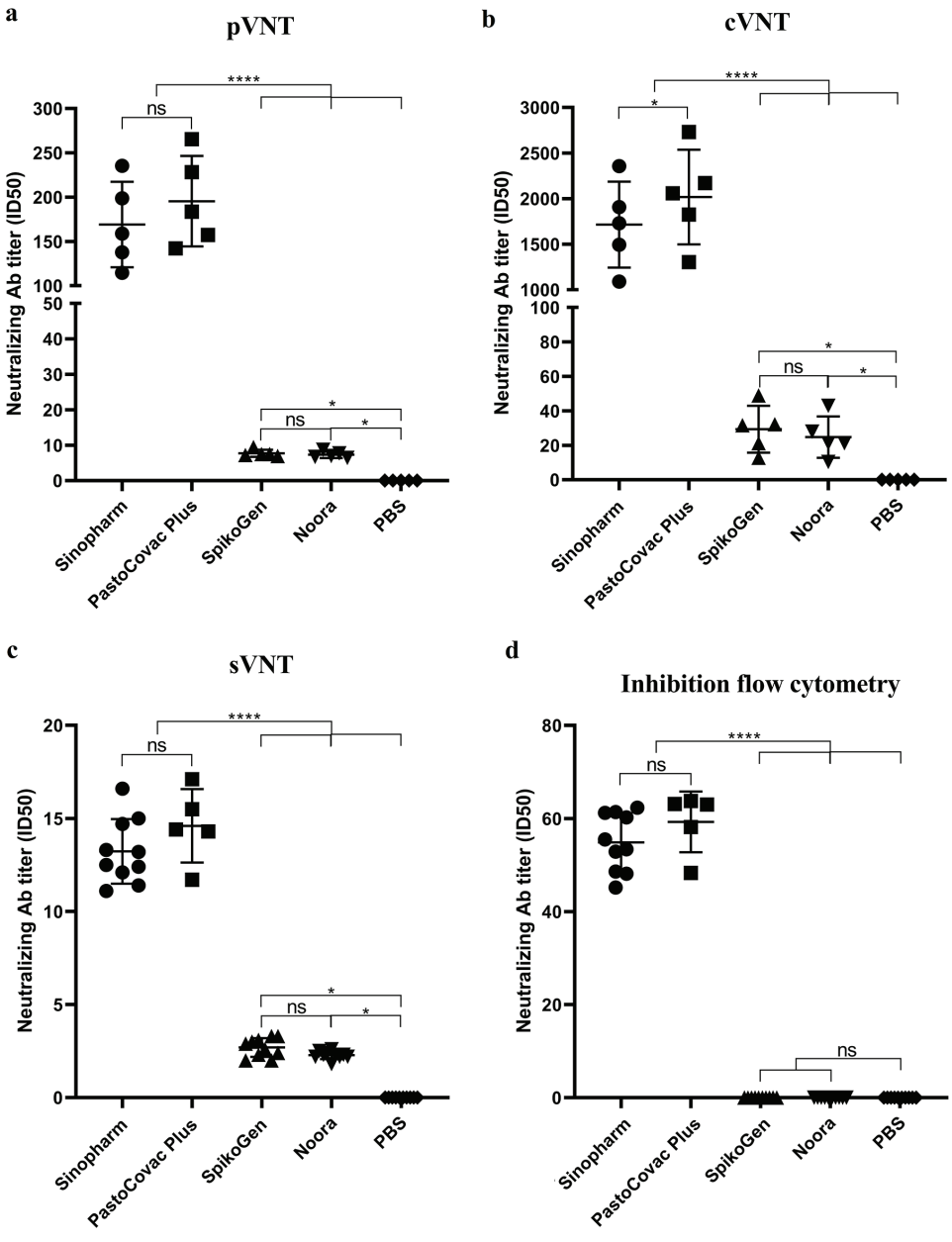

Fig. 3. Comparison of vaccine-induced neutralizing antibody responses in the serum of the mice vaccinated with various approved COVID-19 vaccines. The neutralizing antibody responses induced by different vaccines were evaluated in mice using various neutralization assays. (a) Pseudotyped Virus Neutralization Test (pVNT): eGFP-pseudotyped lentivirus containing the SARS-CoV-2 Delta variant spike protein was mixed with serially diluted sera (n=5) and added to HEK293T cells expressing ACE2. After 48 h., eGFP-positive cells infected with the pseudovirus were imaged and detected using fluorescence microscopy. (b) Conventional Virus Neutralization Test (cVNT): Heat-inactivated serum samples (n=5) were mixed with live Delta variant SARS-CoV-2 at a fixed viral dose and added to Vero-E6 cells. After the incubation, virus-specific cytopathic effects (CPE) were recorded under microscopes to determine the inhibitory serum dilution (ID50). (c) Surrogate Virus Neutralization Test (sVNT): Competitive ELISA was performed to assess the ability of RBD-specific neutralizing antibodies to inhibit the binding of RBD to ACE2. Serially diluted serum samples (n=10) or standard solutions and HRP-conjugated ACE2 were added to coated RBD in ELISA wells, and the resulting optical density (OD) was measured. (d) Inhibition flow cytometry: Serum samples (n=10) were mixed with RBD-Fc and added to HEK293T cells expressing ACE2. FITC-labeled sheep anti-human antibody was used to detect the binding of RBD-Fc to ACE2, and the data were acquired and analyzed using FlowJo V10 software. The data are presented as the mean±SD. *P*-values were determined using one-way ANOVA. ns: not significant, **p*<0.05, ***p*<0.01, ****p*<0.001, and *****p*<0.0001.

injection, the sera were collected from all the groups of immunized mice for performing the experiments.

The results of pVNT showed that Sinopharm and PastoCovac Plus similarly induced significantly higher levels of neutralizing antibody than those of SpikoGen and Noora vaccines (Fig. 3a). Accordingly, ID50 values were obtained for mice vaccinated with Sinopharm, PastoCovac Plus, SpikoGen, and Noora were 161, 189, 7 and 7, respectively.

To further test the neutralization potency of the antisera, we performed a CPE assay using live SARS-CoV-2 Delta variant infection in Vero-E6 cells. The ID50 values obtained by cVNT were 1657, 1991, 26 and 23 for the Sinopharm, PastoCovac Plus, SpikoGen, and Noora vaccinated groups, respectively (Fig. 3b).

We, additionally, performed a competition ELISA (sVNT) and also developed an inhibition flow cytometry to evaluate whether the neutralization effect of the antisera is through inhibiting the RBD binding to its receptor, ACE2. Based on the results of sVNT, the inhibitory effect of Sinopharm and PastoCovac Plus vaccinated sera was significantly higher than those in SpikoGen, and in Noora vaccinated sera (Fig. 3c). This pattern was similar with the results of anti-RBD level, cVNT and pVNT.

Similar to sVNT results, Sinopharm and PastoCovac Plus vaccinated sera significantly inhibited binding of SARS-CoV-2 RBD to ACE2 on the cell surface by flow cytometry. However, the sera of SpikoGen, and Noora vaccinated animals failed to show any inhibitory effect on binding RBD to its receptor, ACE2, on the cell surface. (Fig. 3d and Supplementary Figs. S1, S2).

Highly significant correlations (p<0.0001) were observed between anti-RBD titer and the neutralization tests (Figs. 4a-4d), and also, between all the four neutralization assays employed in this study (Figs. 4e-4j).

DISCUSSION

The development of highly effective COVID-19 vaccines has played a pivotal

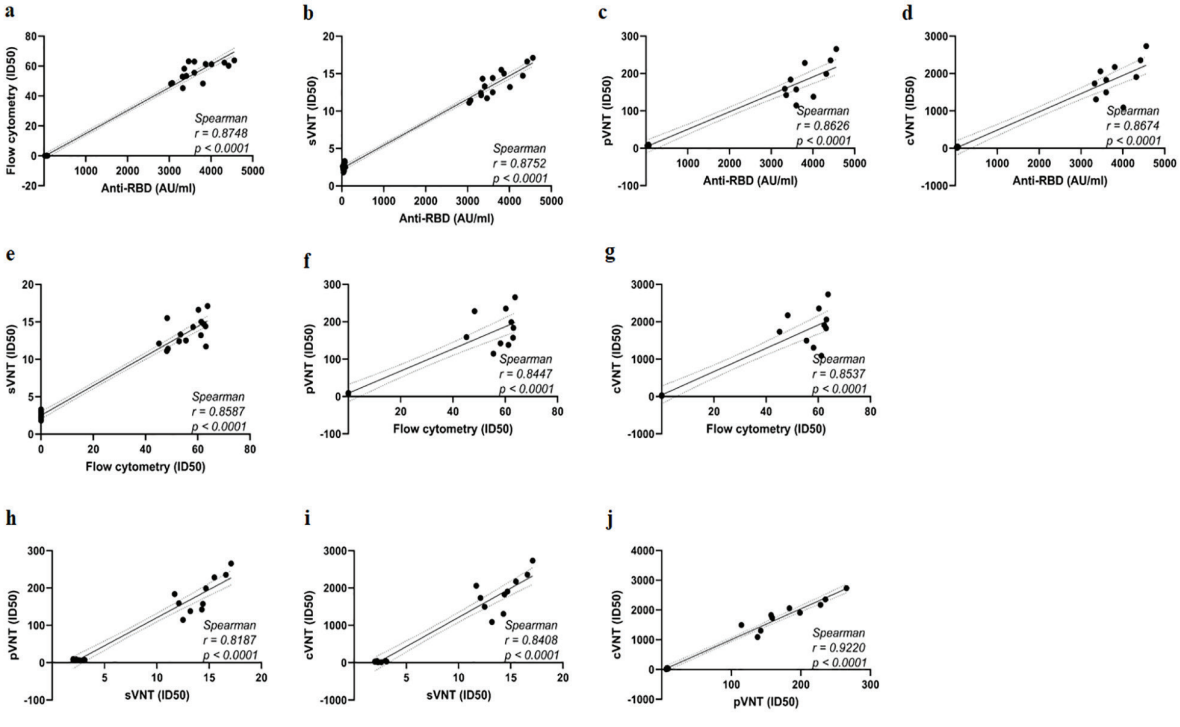

Fig. 4. The correlation of the results obtained by different assays. The correlation of anti-RBD titer and ID50 neutralization values obtained by different neutralization assays (a-d); the correlation between ID50 values obtained by inhibition flow cytometry, sVNT, pVNT, and cVNT (e-j).

role in the management of the COVID-19 pandemic (23). While COVID-19 is no longer categorized as a Public Health Emergency of International Concern, its ongoing impact on the global health system persists, primarily due to the potential emergence of new variants. Until recently, vaccine development was a long and complex process lasting for decades before clinical introduction of the product. Due to the emergency under the COVID-19 pandemic, we witnessed a race among scientists and pharmaceutical companies to develop and distribute different COVID-19 vaccines based on new and old vaccine technologies. However, there is a gap in knowledge of comparative evaluation of immunogenicity and neutralization efficacy among these different vaccine formulations. Here, we conducted a comparative study on immunogenicity and neutralization potency of four COVID-19 vaccines authorized for use in Iran, including: Sinopharm, PastoCovac Plus, SpikoGen, and Noora.

Sinopharm vaccine contains inactivated form of SARS-CoV-2 virus, PastoCovac Plus vaccine contains recombinant dimer RBD, SpikoGen vaccine contains recombinant trimeric extra cellular domain (ECD) of the spike protein, and Noora vaccine contains recombinant monomeric RBD (Table 1). All the four vaccines were developed based on the original (Wuhan-Hu-1) variant (Gen Bank accession: NC_045512). These vaccines were employed as a control in a preclinical study on the RBD-Fc fusion protein that we developed as a vaccine candidate and compared their immunogenicity and neutralizing potency with our fusion protein. The results of our RBD-Fc fusion protein have been reported in a separate article (submitted for publication).

Until 2nd December 2022, Sinopharm was approved for use in 93 countries, while PastoCovac Plus (SOBERANA Plus) received approval in two countries. There was also a joint effort for the production of SpikoGen vaccine between two companies from Australia and Iran. This vaccine, however, was licensed for use only in Iran. The Noora vaccine, like the SpikoGen vaccine, has been approved for administration to the general public, just in Iran.

Our findings showed that all the vaccines successfully induced potent humoral immune response against their corresponding vaccine formulations (Fig. 2a). Since anti-RBD antibodies are essential for neutralizing the virus and preventing the infection (24, 25), we evaluated anti-RBD and anti-spike responses, as an indication of virus neutralization potency, in the serum of vaccinated animals. Our results showed that Sinopharm and PastoCovac Plus vaccines similarly induced significantly higher levels of anti-RBD antibody than SpikoGen and Noora vaccines. Contrary to our expectations, the results of anti-RBD response after vaccination with SpikoGen and Noora were not comparable with the anti-vaccine antibody response (Fig. 2b). Despite the lower administered dose (5 μg), SpikoGen vaccine induced stronger anti-vaccine response compared with the other vaccines. The antibody response against the vaccine formulation was much higher than anti-RBD and anti-spike levels. Anti-RBD level in the sera of SpikoGen vaccinated mice was negligible, while anti-spike was higher (Fig. 2c). Considering the structure of SpikoGen vaccine, which utilizes trimeric ECD of the spike protein (26), it can explain the stronger antibody response against spike compared with RBD. It seems that most of the generated antibodies are directed against epitopes outside the RBD region. In another study that used two doses of 5 μg SpikoGen for mice vaccination (19), a similar pattern of antibody response was reported, indicating a stronger response against spike compared with RBD proteins.

The prokaryotic recombinant proteins are significantly different from those expressed in the eukaryotic expression system. The prokaryotic RBD protein is deglycosylated and mostly insoluble (refolded from inclusion bodies) (27-30), and the RBD produced in eukaryotic system elicits a significantly stronger humoral immune response compared

with the RBD expressed in E.coli (31). In our study, three doses of 40 µg Noora vaccine were used for animal vaccination. Noora vaccine induced low levels of anti-RBD and anti-spike antibodies compared with Sinopharm and PastoCovac Plus vaccines (Fig. 2c). The level of anti-RBD response after SpikoGen and Noora vaccinations was similar and significantly lower than those of Sinopharm and PastoCovac Plus vaccines (Fig. 2b). The Noora vaccine contains a monomeric recombinant RBD produced in E.coli (20). To evaluate the anti-RBD and anti-spike levels in the sera of vaccinated animals, we employed an ELISA with recombinant RBD and spike proteins produced in mammalian CHO-K1 and HEK293T cell lines, respectively, having differences in post-translational modifications with prokaryotic recombinant RBD and spike proteins (32, 33). Protein folding, conformation, distribution, stability and activity are significantly affected by post-translational modifications (34-37). One possibility is that the antibodies generated against the Noora vaccine are not capable of recognizing the epitopes of recombinant RBD and spike produced in a mammalian host, similar to the native infecting viruses. In a study examining the Noora vaccine in the preclinical phase, the dose of 120 µg was used three times for vaccinating the mice (20). Significantly higher titers of anti-RBD were reported, but the response to spike protein was not determined. This observed difference could not be due to the lower injected dose of vaccine in our study, because after performing phase 1 clinical trial, 80 µg was selected as the best dose of Noora vaccine for human (38). In the preclinical study, the prokaryotic or eukaryotic origin of the RBD protein used in the ELISA for titration of the anti-RBD response was not identified. It is possible that the same prokaryotic RBD was also used for ELISA coating, which could contribute to the higher OD values obtained in that study.

To further evaluate the effectiveness of these vaccines in neutralizing the virus, we performed several virus neutralization tests, including inhibition flow cytometry, sVNT, pVNT, and cVNT (as the gold standard of neutralization test). Our results showed that the sera from mice vaccinated with Sinopharm and PastoCovac Plus exhibited strong neutralizing activity against the Delta variant of SARS-CoV-2 pseudovirus and live virus, as evidenced by the pVNT and cVNT results, respectively (Figs. 3a, 3b). In fact, the SpikoGen and Noora vaccines showed very little neutralization potency in all four neutralization tests employed in our study. These findings were consistent with the higher levels of antibodies targeting the RBD region induced by Sinopharm and PastoCovac Plus (Fig. 2b). It should be noted that unlike the strong anti-vaccine response induced by the SpikoGen vaccine, this response did not have a positive impact on the neutralizing capacity of the vaccine. Moreover, the high neutralizing activity of antibodies induced by Sinopharm and PastoCovac Plus vaccines was further confirmed by the competition ELISA (sVNT) and inhibition flow cytometry (Figs. 3c, 3d).

The differences observed in the neutralization efficacy among these four vaccines could be attributed to different parameters, such as differences in their composition, formulation, or antigen structure. Different vaccine platforms such as inactivated virus (Sinopharm) or protein subunits expressed in different hosts (Table 1) activate the immune system through distinct mechanisms. These differences in vaccine design may affect the magnitude and quality of vaccine-induced immune protection.

It should be noted that the inhibition flow cytometry virus neutralization test, which was optimized in our lab, was developed only for evaluating Wuhan-Hu-1 strain. Unfortunately, we did not have access to the RBD-Fc fusion protein specifically for the Delta variant, which restricted our ability to evaluate the Delta variant in the inhibition flow cytometry assay.

The sensitivity of different neutralization tests against the virus varies (39), resulting in

differences in the obtained ID50 values among different tests for each vaccine. However, in all the groups of the vaccinated mice, the ID50 values obtained from different tests exhibited a relatively consistent ratio of change. In our study, the sera from mice vaccinated with SpikoGen, were able to neutralize 50% of the infection caused by the Delta variant pseudotyped SARS-CoV-2 at 1:7 dilution. In the published preclinical results of this vaccine (19), sera from BALB/c and C57BL/6 mice immunized with this vaccine, were able to induce 50% neutralization of Delta variant pseudotyped SARS-CoV-2 at >1:256 and >1:512 serum dilutions, respectively.

Our results on the neutralizing potency of the Noora vaccine against the Delta variant of SARS-CoV-2 showed that in the pVNT and cVNT, the serum dilutions of approximately 1:7 and 1:23, respectively, were able to neutralize 50% of the infection. In the published preclinical data of this vaccine (20), 1:10 dilution of immunized mice sera was found to inhibit >60% of pseudovirus infection in the pVNT test, without specifying the pseudovirus variant or the infection detection system. It should be noted that in that preclinical study, the dose of 120 µg was used for the mice immunization.

The observed differences in immunogenicity and neutralization potency among the four vaccines emphasize the significance of comparative studies for currently used vaccines in Iran to address their relative advantages and disadvantages. Our findings have important implications for vaccine selection strategies. Vaccines that are more likely to induce higher neutralizing immune response, such as Sinopharm and PastoCovac Plus are more effective in virus neutralization and achieving optimal protection against SARS-CoV-2. Our findings showed that these two vaccines displayed greater immunogenicity and induced significantly higher virus neutralizing responses compared with SpikoGen and Noora vaccines. Based on the results of sVNT and inhibition flow cytometry, this higher neutralizing capacity could be due to the inhibitory effect on RBD binding to ACE2 receptors.

It is important to note that the findings of our study were obtained in the BALB/c mice model and may not be fully applicable to human responses. Animal models are valuable tools for initial evaluation of vaccines, but further research in human populations is needed to confirm and extend these findings. Moreover, the study primarily examined the immunogenicity and neutralizing effectiveness of the vaccines and did not evaluate other important factors such as the duration of immune response, protection against different variants of SARS-CoV-2, cell-mediated immune responses, protection against severe disease and hospitalization. Future studies should take these factors into consideration to gain a more comprehensive understanding of the relative effectiveness of these vaccines.

Ineffectiveness of SpikoGen and Noora vaccines, could also be considered as the result of unfavorable storage condition of these vaccines lot. To rule out this possibility, another lot of both vaccines was obtained from a health center affiliated to Tehran University of Medical Sciences and administered to separate groups of mice. The results of serum anti-RBD, anti-spike and anti-vaccine antibodies, as well as the titer of neutralizing antibodies were similar to those obtained for the first vaccines lot with no significant differences (data not presented).

In lieu of directly inhibiting the binding of RBD to ACE-2 receptor, Noora and SpikoGen vaccines may employ other mechanisms to effectively prevent infection in vaccinated individuals. Among these mechanisms, two notable ones are antibody-dependent cell-mediated cytotoxicity (ADCC) and the induction of antiviral mediators such as type 1 interferons (alpha and beta), which we did not investigate in the present study. ADCC is a process in which specific immune cells, such as natural killer (NK) cells, recognize and attach to virus-infected cells marked by

antibodies. Once attached, these immune cells release substances that can destroy the infected cells, thereby aiding in the clearance of the virus from the body. This mechanism enhances the immune response and contributes to the prevention of infection in vaccinated individuals.

CONCLUSION

Our study evaluated the comparative immunogenicity and neutralization efficacy of the four approved COVID-19 vaccines (against Delta variant) in Iran. Sinopharm and PastoCovac Plus vaccines exhibited greater immunogenicity and induced significantly stronger neutralizing antibody responses compared with SpikoGen and Noora vaccines. Further investigation is necessary to validate these findings in human populations and explore additional aspects of the vaccine performance, such as evaluating the neutralization efficacy of these vaccines against other circulating SARS-CoV-2 variants in Iran and other regions of the world.

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AUTHORS' CONTRIBUTION

FS designed, funded, supervised the study, curated and validated the data. ND performed all the experiments, analyzed the data and wrote the manuscript. FGS, MJT, AHZ and MMA curated and validated the data. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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