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RESEARCH ARTICLE

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Safety and immunogenicity of a recombinant receptor-binding domain-based protein subunit vaccine (Noora vaccine™) against COVID-19 in adults: A randomized, double-blind, placebo-controlled, Phase 1 trial

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Abstract

The development of a safe and effective vaccine is essential to protect populations against coronavirus disease 2019 (COVID-19). There are several vaccine candidates under investigation with different mechanisms of action. In the present study, we have evaluated the safety and immunogenicity of a recombinant receptor-binding domain (RBD)-based protein subunit vaccine (Noora vaccine) against COVID-19 in adults. This Phase 1 trial is a randomized, double-blind, placebo-controlled study to evaluate the safety and immunogenicity of the recombinant RBD-based protein subunit vaccine (Noora vaccine) against COVID-19 in healthy adults volunteers. Eligible participants were included in this study after evaluating their health status and considering the exclusion criteria. They were then randomized into three groups and received three doses of vaccine (80 µg, 120 µg, and placebo) on Days 0, 21, and 35. Primary outcomes including solicited, unsolicited, and medically attended adverse events were recorded during this study. Secondary outcomes including the humoral and cellular immunity (including anti-RBD IgG antibody and neutralizing antibody) were measured on Days 0, 21, 28, 35, 42, and 49 by using the ELISA kit and the Virus Neutralization Test (VNT) was performed on day 49. Totally 70 cases were included in this Phase 1 trial and 60 of them completed the study.

Safety assessments showed no severe adverse events. Local pain at the vaccine injection site occurred in 80% of the vaccinated volunteers. Induration and redness at the injection site were the other adverse reactions of this vaccine. There was no significant difference between the studied groups regarding adverse reactions. Anti-RBD IgG antibody and neutralizing antibody assessment showed significant seroconversion in comparison to the placebo group (80%, and 100% respectively, $p < 0.001$). The cellular immunity panel also showed mild to moderate induction of TH1 responses and the VNT showed 78% of seroprotection. The results of this Phase 1 trial showed acceptable safety without serious adverse events and significant seroconversions in the humoral and cellular immunity panel. The dose of 80 μg is an appropriate dose for injection in the next phases of the trial.

KEYWORDS
COVID-19, immunogenicity, Noora vaccine, protein subunit, safety, SARS-CoV-2

1 | INTRODUCTION

The present pandemic of coronavirus disease 2019 (COVID-19) which has been caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has extremely impaired the lives of most people.[1] This pandemic has led to a significant public health crisis around the world. According to a report of the World Health Organization (WHO), up to July 2022, COVID-19 has affected 221 countries and territories with about 575 million confirmed cases and six million deaths.[2,3] COVID-19 is a severe infectious disease that mainly affects the respiratory system.[4,5] It is associated with lethal complications such as severe acute respiratory distress syndrome, pneumonia, cardiac problems, coagulation impairment, organ failure, and death.[6–8] Based on the abovementioned, it is necessary to design effective vaccines to protect populations against COVID-19.[9] An optimal vaccine against COVID-19 must have acceptable safety and protective efficacy to use in large populations. Hence, it is beneficial to develop COVID-19 vaccines with different platforms.[10] At the moment, 117 COVID-19 vaccine platforms are under clinical development and 194 COVID-19 vaccines are in the preclinical stage. These vaccines have used different platforms including inactivated virus, adenovirus vector, messenger RNA (mRNA)-based, and protein subunit vaccines.[11] All platforms of the COVID-19 vaccines have their distinct strengths and weaknesses.[12] Among the abovementioned platforms, the protein subunit platform is mostly used within 42% of the trials indicating that this platform is a feasible technology for the COVID-19 vaccine development. Fifteen protein subunit-based vaccine candidates have entered Phase 3 or Phase 2/3 trials. In addition, there are 23 candidates in Phase 1 or Phase 1/2. They elicit the human immune system against spike (which is known as an RBD protein) to neutralize the virus.[13,14] The RBD engages the angiotensin-converting enzyme 2, a receptor for cell entry, and is an interesting vaccine platform to elicit immune responses to block the receptor binding.[15] The RBD has dominant epitopes in the S protein that effectively induces neutralizing antibodies.[16,17] During a previous preclinical study, an RBD-based protein recombinant vaccine candidate was developed which showed promising results regarding immunogenicity and safety in mice, rabbits, and primates.[18] This phase 1 trial aimed to evaluate the safety and immunogenicity of a recombinant RBD-based protein subunit vaccine (Noora vaccine) against COVID-19 in an Iranian adult population.

2 | MATERIALS AND METHODS

2.1 | Trial design and participants

This study was a randomized, double-blind, placebo-controlled, Phase 1 trial to assess the safety and immunogenicity of the recombinant RBD-based protein subunit vaccine (Noora vaccine) against COVID-19 in healthy Iranian adult volunteers. The trial was launched at the Baqiyatallah University of Medical Sciences (BUMS), Tehran, Iran. Healthy volunteers were aged between 18 and 50 years. The health status of volunteers was evaluated during the screening period at the beginning of the study, by taking the medical history, clinical laboratory findings (hematology, coagulation panel, biochemistry, inflammatory panel, and urine analysis), vital signs (respiratory rate, heart rate, axillary temperature, blood pressure, and peripheral oxygen saturation), and physical examinations. Participants with a history of being infected with the COVID-19 (confirmed with real-time polymerase chain reaction assay or serological assay or clinical symptoms) were excluded at the beginning of the study. COVID-19 compatible clinical symptoms were defined as fever (axillary temperature more than 37°C), cough, myalgia, rhinorrhea, sore throat, headache, diarrhea, chest pain, dyspnea, anosmia, and ageusia. Participants with a history of close contact with COVID-19 confirmed cases during the past 2 weeks were also excluded. The pregnancy

status was checked with the assay of blood β-HCG level for women participants in the screening phase and during the study. Other exclusion criteria included a history of hypersensitivity reactions to any ingredient in the vaccine, history of seizures or psychiatric illness, congenital malformations, congenital or acquired immune diseases, growth disorders, malnourishment, kidney impairment, liver impairment, serious chronic disease, uncontrolled hypertension, heart failure, asthma, diabetes, morbid obesity, splenectomy, history of malignancy, coagulation impairment, tuberculosis, acute or chronic viral diseases, history of receiving any blood products or immunoglobulins in the previous 3 months, history of receiving any vaccines in the previous or future 3 months, health care providers, and a failure to comply with the study schedule. Participants with a history of the below conditions were also excluded before the injection of the second or third dose of vaccine: positive pregnancy test, high-grade fever (axillary temperature more than 39°C for more than 3 days), receiving steroids or immunoglobulins, and the development of severe adverse reactions after the first or second dose. The participants were included after providing signed informed consent forms. The ethical committee of the Baqiyatallah University of Medical Sciences and the Iranian Ministry of Health and Medical Education approved the protocol of this trial with the number: IR.NREC.1400.004, and was conducted according to the Declaration of Helsinki. The study was also carried out following good clinical practice. The protocol of the trial was registered on the Iranian Clinical Trial Registry Databases (IRCT20210620051639N1).

2.2 | Randomization and blinding

As a safety measure, 14 volunteers were initially randomly allocated in a 3:3:1 ratio to the 80 μg, 120 μg, and placebo groups, respectively. They were observed for reactogenicity for 12 h in the trial center. After the approval of the data monitoring and safety board (the DSMB, consists of members from the Iranian food and drug organization), the remaining participants were randomly allocated (3:3:1) into three groups to receive three doses of the vaccine (80 μg or 120 μg doses) or placebo on Day 0, 21, and 35. Vaccine regimens and trial timeline has been illustrated in Figure 1.

A random sequence of length 70 (the size of the sample size) was generated by the online system (SealedEnvelope.com). For this purpose, 10 random blocks with size seven were produced. Three cases with low doses (80 μg), three cases with high doses (120 μg), and one case with placebo. The generated codes were labeled on the vaccine vials before the start of the study and were assigned to the candidates during the study by the study software. The appearance, color, and viscosity were indistinguishable across all vials. Each vial was labeled with a unique code. Investigators and statisticians were

(A) **Vaccine regimens**

Groups	Randomised	Sentinel	Day 0	Day 21	Day 35
A	30	6	80 μg	80 μg	80 μg
B	30	6	120 μg	120 μg	120 μg
C	10	2	Placebo	Placebo	Placebo

(B) **Timeline**

Intervention/follow-up	Screening	Day 0	7	14	21	28	35	42	49
Signing the consent form	×								
Taking medical history	×								
Pregnancy test (females)	×	×			×		×		
RT-PCR test and COVID-19 serology	×								
Randomisation		×							
Injection		×			×		×		
Blood sampling (safety)	×		×			×		×	
Blood sampling (immunogenicity)		×	×			×	×	×	×
Reactogenicity and vital signs		×			×		×		
Solicited adverse events		←	→		←	→		←	→
Unsolicited adverse events		←	→		←	→	←	→	
Medically attended adverse events		←	→		←	→	←	→	
Serious adverse events		←	→		←	→	←	→	

FIGURE 1 The vaccination regimen (A) and timeline of the trial (B).

not involved in the other parts of this clinical trial and were not allowed to reveal the blinding codes to any personnel participating in the clinical trial. Volunteers, investigators, directors, employees, and the sponsor were blinded to groups' allocation. Only an independent member of the Contract Research Organization (CRO), was aware of labeling the vaccine vials and randomization codes.

2.3 | Trial vaccine, adjuvant, and placebo

The vaccine was developed by BMSU. The vaccine was developed according to good manufacturing practice guidelines by Plasma Darman Sarv Sepid Co. The recombinant vaccine encodes the SARS_CoV_2 RBD antigen (residues 319–543, accession number NC_045512).

To express the RBD protein, the *E. coli* BL21 DE3 containing the pET-28 SUMO-RBD vector was cultured in Luria-Bertani (LB) and inducted with 1 mM isopropyl ß-D-1-thiogalactopyranoside (IPTG). After cell lysis, the supernatant was purified by SUMO-tagged proteins under denaturing conditions. The column-bound protein was eluted using the elution buffer (Qiagen). The denaturant agent (8 M urea) was removed from the purified proteins by stepwise dialysis and the SUMO-tag was cleaved by SUMO protease. Finally, the recombinant proteins were confirmed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis. The final Noora vaccine formulation contains 80 µg or 120 µg RBD protein plus 380 µg Alum adjuvant in phosphate-buffered saline per each 0.5 ml vial.

The placebo was only filled with Alum plus buffer. Vaccines were stored in a refrigerator with a temperature of 2°C–8°C before administration. The vaccine or placebo was injected into the deltoid muscle of each volunteer.

2.4 | Safety assessments (primary outcome)

Volunteers were observed for 60 min after each injection in the observing room of the clinical trial center for any probable reactogenicity and immediate adverse events. After each vaccination, any adverse events were recorded through daily telenursing visits. The nurses responsible, called the participants daily for up to 7 days and any local (pain, redness, and induration) and systemic (fatigue, myalgia, headache, flushing, drowsiness, sore throat, aphthous, and chest discomfort) adverse events were recorded as solicited adverse events.

Laboratory safety tests including hematology, coagulation panel, biochemistry, inflammatory panel, and urine analysis were obtained to evaluate any toxicity after each vaccination. Unsolicited adverse events were also documented during the study. Serious adverse events and those events which needed medical interventions were also recorded. The safety results were graded according to the latest Food and Drug Administration (FDA) toxicity grading scale.[19] Any serious adverse events which may occur after a year of the first dose injection, and also follow-up are continuing.

2.5 | Immunogenicity assessments (secondary outcome)

Blood samples were obtained to assess the humoral and cellular immunity. Anti-RBD IgG antibody and neutralizing antibody were measured by ELISA kits (PishtazTeb Co.) on Days 0, 21, 28, 35, 42, and 49. In addition, VNT was performed on Day 49. Briefly, to analyze the VNT, we treated Vero cells with serial dilutions of both the virus and pseudo-virus, and the IC50 dilution was determined. The convalescent human sera as well as vaccinated and placebo groups were incubated with 100 TCID50 and exposed to Vero cell culture. In addition, the neutralizing antibody was measured by Abnova ELISA kit (no. KA6111; Abnova Corporation) according to the supplier's protocol.

The seroconversion was also defined as the rise of RBD IgG and neutralizing antibodies based on the ELISA kit cutoff (>5 µg/ml and >2.5 µg/ml, respectively). Cellular immunity was measured by measuring the interleukin (IL) 4, IL-10, IL-12, and interferon-gamma (IFN-γ) cytokines released from peripheral blood monocyte on Day 49.

ELISA was performed to assess the IL-4, IL-10, IL-12, and IFN-γ according to the manufacturer's instructions (R&D System). In brief, on Day 49, peripheral blood mononuclear cells from vaccinated and placebo volunteers were isolated and exposed to 20 µg/ml Noora vaccine in the cell culture. After 48 h, the supernatant culture was collected and used for cytokines assay.

2.5.1 | Flow cytometry analysis

On day 49, peripheral blood mononuclear cells from vaccinated and placebo volunteers were isolated and exposed to 20 µg/ml Noora vaccine in the cell culture. According to the same channel of CFSE and IFN-γ-FITC antibody in the Flow cytometry test, the PBMC samples were cultured in two parts. In one part the cell was labeled with CFSE for proliferation detection and in another part did not use CFSE and these wells were used for $CD4^+$, $CD8^+$, and IFN-γ finding in the Flow cytometry test. The cells were cultured completely in the same condition, media volume, cell number, mitogen, and vaccine concentrations were similar together in both parts. After 48 h, the $CD3^+$, $CD4^+$, $CD8^+$, and IFN-γ T cells (BD company, 337184 and 346048) were counted using the fluorescence-activated cell sorting (FACS) calibur flow cytometer (Becton Dickinson). Flowjo7 software was used to analyze the results.

2.6 | Statistical analysis

The safety results were analyzed with descriptive statistics and comparison was done using the χ^2 test and the Fisher's exact probability method. The immunogenicity outcomes were reported with seroconversion rates and Geometric Mean Titers (GMTs). Cellular immunogenicity was also calculated with GMTs. The SPSS

software version 24 (IBM Corporation) and Prism software version 9.2.0 (GraphPad) were used for statistical analysis.

3 | RESULTS

In this Phase 1 trial, a total of 70 cases were included and finally, 60 cases completed the study. During the study, 10 cases (one positive pregnancy test, three symptomatic cases, and seven positive RT-PCT cases) were excluded. The CONSORT diagram of the trial is shown in Figure 2.

The age of the participants was 31.78 ± 7.35 years old indicating no statistically significant differences across the three groups ($p = 0.60$) of which 39 (65%) cases were male. The gender of participants was also not statistically different across the three groups ($p = 0.97$). Table 1 shows the demographic data of participants.

In this trial, all cases in three groups experienced at least one adverse event through 1 month after injection, and no significant difference was seen across all three groups. Five out of eight (62.5%) in the placebo group, 23 out of 27 (85.19%) in the 80 μg group, and 19 out of 25 (76%) in the 120 μg group of participants reported at least one immediate adverse event after the first injection indicating no significant differences across all groups ($p = 0.366$). After the second injection, five out of eight (62.5%) in the placebo group, 22 out of 27 (81.48%) in the 80 μg group, and 17 out of 25 (68%) in the 120 μg group of participants reported at least one immediate adverse event indicating no significant differences across all groups ($p = 0.414$). Finally, after the third injection, six out of eight (62.5%) participants in the placebo group, 22 out of 27 (81.48%) in the 80 μg group, and 22 out of 25 (88%) in the 120 μg group of participants reported at least one immediate adverse event indicating no significant differences across all groups ($p = 0.650$). All events were associated with local pain at the injection site. In addition, no serious adverse event was seen (Figure 3).

The local and systemic solicited adverse events were recorded 7 days after each injection. The pain at the injection site was the most frequent local adverse event. After the first dose, 20 cases (74.1%) in the 80 μg dose group, 20 cases (80%) in the 120 μg dose group, and four cases (50%) in the placebo group experienced pain at the injection site. However, the frequency of pain significantly diminished to 31.3% in the second and third injections ($p = 0.02$). Myalgia and fatigue were among the other systemic solicited adverse events with a lower frequency (<20%). The frequency of other systemic solicited adverse events was lower than 10%. The frequency of these adverse events was not different across the studied groups ($p = 0.72$).

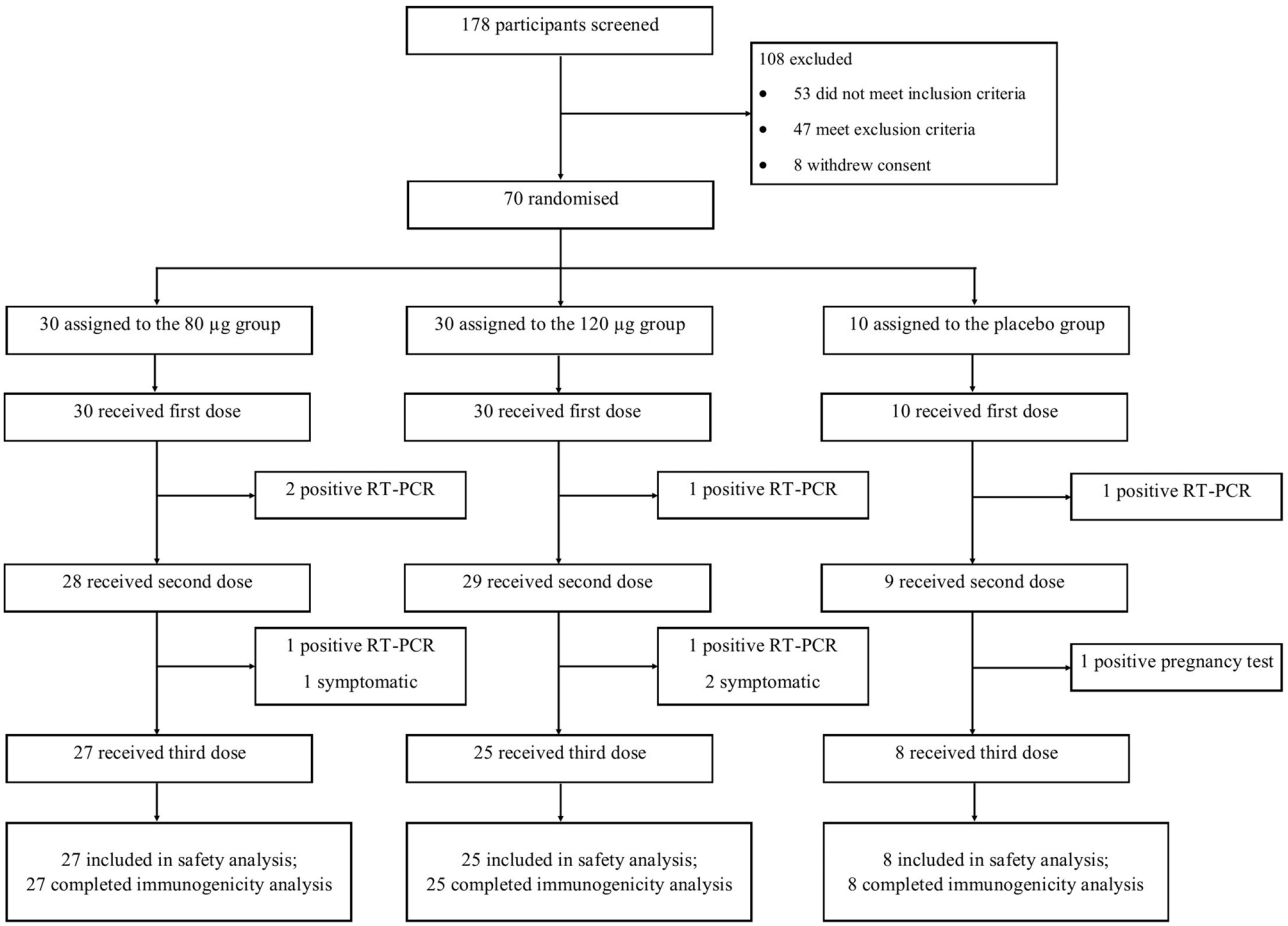

FIGURE 2 The Consolidated Standards of Reporting Trials (CONSORT) diagram of the trial.

TABLE 1 Baseline demographic characteristics of participants

		Groups			
		80 μg (*n* = 27)	120 μg (*n* = 25)	Placebo (*n* = 8)	*p*-value
Age (years)	Mean (SD)	31.68 (7.60)	32.63 (7.89)	29.62 (4.5)	0.606*
	Median (IQR)	33 (24.5–36.5)	31.5 (26–38)	28.5 (27.5–30)	
Sex	Male	18 (64.3%)	16 (66.7%)	5 (62.5%)	0.972**
	Female	10 (35.7%)	8 (33.3%)	3 (37.5%)	

Abbreviations: IQR, interquartile range; SD, standard deviation.

*χ^2 test.

**Kruskal–Wallis test.

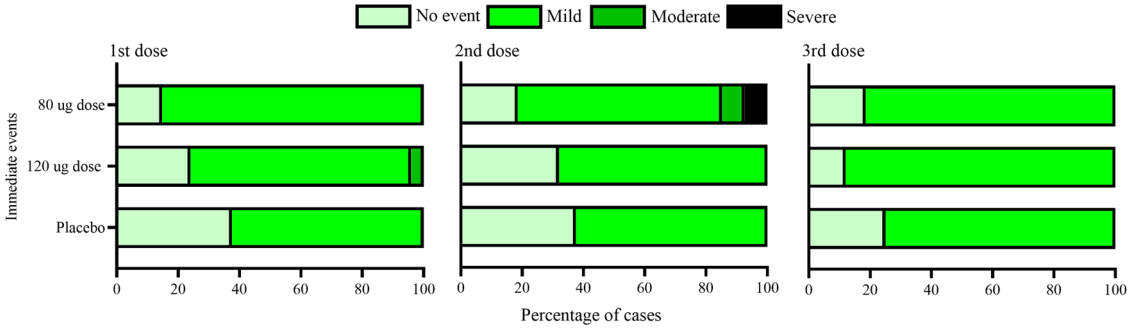

FIGURE 3 The frequency of immediate adverse events across the three groups during the study (after each injection). Local pain at the injection site was the only reported adverse effect. No significant difference was seen across groups. After first dose, 62.5% of the placebo group, 85.19% of the 80 μg group, and 76% of the 120 μg group reported local pain at the injection site ($p = 0.366$). After second dose, local pain at the injection site was reported in 62.5% of the placebo group, 81.48% of the 80 μg group, and 68% of the 120 μg group ($p = 0.414$). After 3rd dose, local pain at the injection site was reported in 62.5% of the placebo group, 81.48% of the 80 μg group, and 88% of the 120 μg group ($p = 0.640$). The bar charts represented the percentage of cases that developed adverse events.

A severe systemic adverse event was reported in only one case in the 120 μg dose group who experienced a severe headache after receiving the second injection. The occurrence of adverse events also was not statistically different between 80 μg and 120 μg doses ($p > 0.05$). The solicited local and systemic adverse events are shown in Figure 4.

The results of immunogenicity showed anti-RBD IgG increased gradually from day 0 to day 49 and maximum anti-RBD IgG titer was obtained on day 49 in both 80 μg and 120 μg groups in comparison with the placebo group ($p < 0.001$ and $p = 0.002$, respectively). At the same time, there was no significant difference between the 80 μg and 120 μg groups ($p = 0.74$). Twenty-four (88%) cases in the 80 μg group, 21 (84%) cases in the 120 μg group, and only one case in the placebo group showed seroconversion for anti-RBD IgG ($p < 0.001$). Surprisingly, the raising of neutralizing antibody titer was seen in all groups with no statistical differences ($p = 0.29$). Figure 5 has illustrated the results of immunogenicity.

The results of flow cytometry analysis are also illustrated in Figure 6. Numbers within the plots indicate the relative percentage of the IFN-γ producing T cells population in the samples. The cellular immunity was evaluated by assessing the frequency of RBD-specific $CD4^+$ and $CD8^+$ T cells, intracellular IFN-γ staining as well as measuring the releasing rate of IL4, IL-10, IL-12, and IFN-γ at Day 49.

Results of RBD-specific $CD4^+$ and $CD8^+$ T cells also showed a significant increase in both 80 μg ($CD4^+$: $p = 0.03$; $CD8^+$: $p = 0.04$) and 120 μg dose groups ($CD4^+$: $p = 0.04$; $CD8^+$: $p = 0.04$). The intracellular IFN-γ also showed similar results which increased significantly in both 80 μg ($CD4^+$: $p = 0.04$; $CD8^+$: $p = 0.006$) and 120 μg dose groups ($CD4^+$: $p = 0.03$; $CD8^+$: $p = 0.07$). There was no increase in the placebo group for those parameters. Results of the RBD-specific $CD4^+$ and $CD8^+$ T cells are shown in Figure 7.

Although the results showed that the IL-4 level decreased in all groups in comparison with their controls, this reduction was not statistically significant ($p = 0.12$ for 80 μg dose group, $p = 0.83$ for 120 μg dose group, and $p = 0.58$ for placebo).

The IL-12 level increased significantly in both 80 μg and 120 μg dose groups ($p = 0.04$ and $p = 0.01$, respectively), but there was no increase in the placebo group ($p = 0.62$). In addition, the level of IFN-γ increased significantly in both 80 μg and 120 μg dose groups ($p = 0.04$ and $p < 0.001$, respectively) but there was no increase in the placebo group ($p = 0.42$). The level of IL-10 also increased significantly in both 80 μg and 120 μg dose groups ($p = 0.02$ and $p = 0.007$, respectively) but there was no increase in the placebo

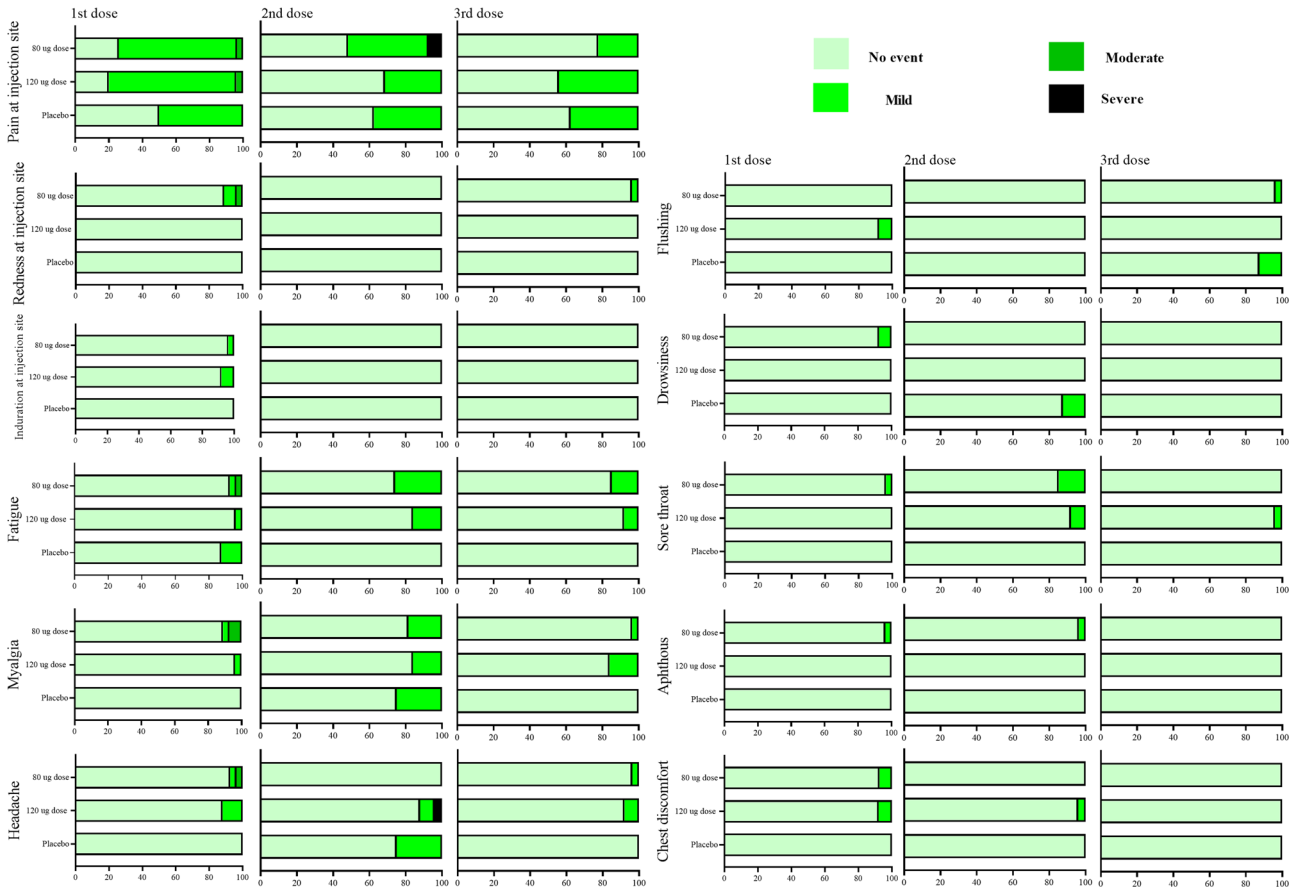

FIGURE 4 The solicited adverse events during the study. Pain at the injection site was the most frequent local adverse event (74.1% of the 80 μg group and 80% of the 120 μg group). Redness and induration of the injection site also occurred in less than 10% of participants. Myalgia, fatigue, headache, flushing, drowsiness, sore throat, aphthous, and chest discompose were among the reported systemic adverse events. The frequency of these adverse events was not different across the studied groups and they occurred in less than 10% of participants ($p = 0.72$). The result represented the percentage of cases that developed adverse events. The bar charts represented the percentage of cases that developed adverse events.

group ($p = 0.48$) (Figure 8). The results of VNT showed that among the 28 volunteer sera, 23 sera (78%) of vaccinated cases in both groups (80 μg and 120 μg doses) were able to reutilize the wild and pseudo-type virus. No significant differences were observed between the positive sera ($p = 0.62$) (Figure 9). The immunogenicity results also showed both 80 μg and 120 μg elicited the humoral and cellular immunities. Hence, there are no differences between these two doses.

4 | DISCUSSION

In this study, we evaluated the safety and immunogenicity of a recombinant RBD-based protein subunit vaccine (Noora vaccine) against COVID-19 in healthy adult volunteers by a randomized, double-blind, placebo-controlled, Phase 1 trial. This trial was carried out to evaluate an optimized vaccine candidate in response to the catastrophic pandemic of COVID-19 in Iran. Noora vaccine is an RBD-based subunit protein that showed promising results in a previous preclinical study. The subunit protein vaccine is a feasible and safe platform for developing vaccines.[20] Worldwide, several trials have used subunit protein platforms for COVID-19 vaccines, such as Zhifei, NovaVax, and CoVaXX. These vaccines showed an acceptable safety profile and also considerable immune induction.[21,22] In comparison with other platforms, protein subunit vaccines are safer than mRNA vaccines.[23,24] The main outcomes of this trial showed no serious adverse events during the follow-up period. Vaccination with the 80 μg or 120 μg doses was well tolerated by the vaccinated groups. The frequency of adverse events between the vaccines and placebo groups was not different in all groups. Most local adverse events occurred with mild to moderate severity. Pain at the injection site, redness, and induration were the most dominant adverse events. Severe local pain was also reported with only one case that needed acetaminophen administration. Notably, the pain resolved after medical intervention and did not appear in the next doses. These adverse events are expected for protein subunit vaccines because they use Alum adjuvant in their formulation.[25] It is expected that these adverse events resolve after a few days of vaccination. These

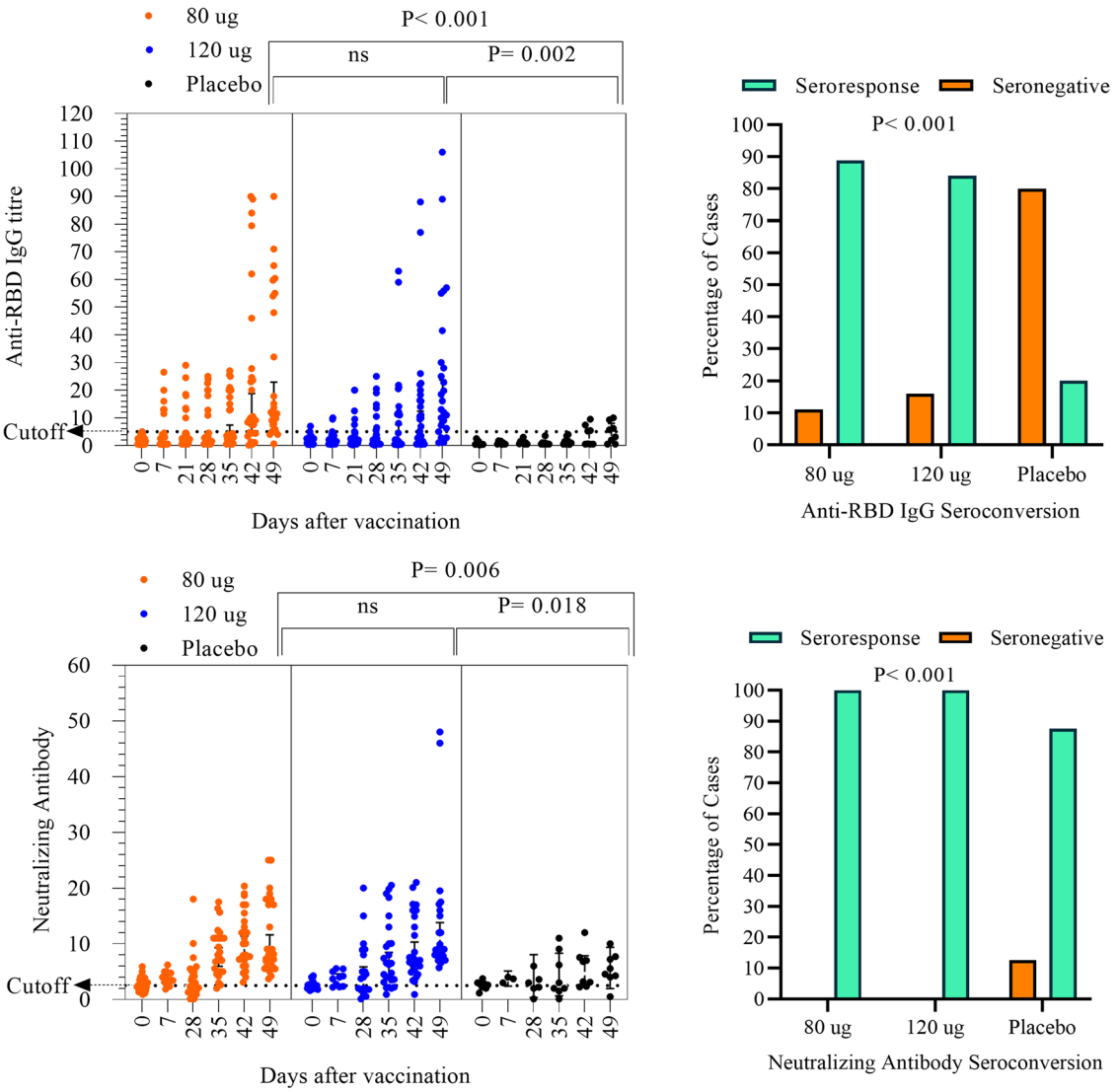

FIGURE 5 The immunogenicity results of the studied groups and seroconversion status. Anti-RBD IgG increased significantly in both 80 µg and 120 µg groups in comparison with the placebo group ($p < 0.001$ and $p = 0.002$, respectively). No significant difference between the 80 µg and 120 µg groups ($p = 0.74$). 88% and 84% of cases in the 80 µg and 120 µg group showed seroconversion for anti-RBD IgG ($p < 0.001$). Neutralizing antibody titer also increased but no significant difference was seen across the studied groups ($p = 0.29$). The seroconversion was also defined as the rise of RBD IgG and neutralizing antibodies based on the ELISA kit cutoff (>5 µg/ml and >2.5 µg/ml, respectively). ELISA, enzyme-linked immunosorbent assay; RBD, receptor-binding domain

local adverse events were similar to other vaccine candidates such as Zhifei and NovoVax. Zhifei reported less than 10% of severe adverse events among vaccinated cases and NovoVax reported no severe local adverse events.[22,26] They mostly experienced mild to moderate local adverse events. Regarding systemic adverse events, the Noora vaccine showed acceptable safety results and no serious adverse event occurred. Most systemic adverse events were mild to moderate and only one case experienced a severe headache after the second injection of the 120 µg vaccine. In comparison with the former COVID-19 vaccines such as adenovirus vectored vaccines or mRNA-based, the incidences of fever and fatigue were lower in the Noora vaccine.[27,28] Fever was reported in 16% of BNT162b2 receipts and fatigue was reported in 59%.[29] ChAdOx1 nCoV-19 vaccine receipts also experienced headache (68%) and fever (71%).[30] In comparison with NVX-CoV2373, another protein subunit vaccine, the incidence of fatigue, myalgia, and headache were also lower in the Noora vaccine.[22] However, safety results in the clinical trials may be exaggerated through individual factors, such as the personal feelings of volunteers. Hence, the severity of pain, headache, or other subjective variables may differ among participants.

Humoral and cellular immune responses against RBD protein showed significant increases in immune responses after vaccination. Up to now, different vaccine candidates have been developed. Among them, RBD base platforms have shown notable

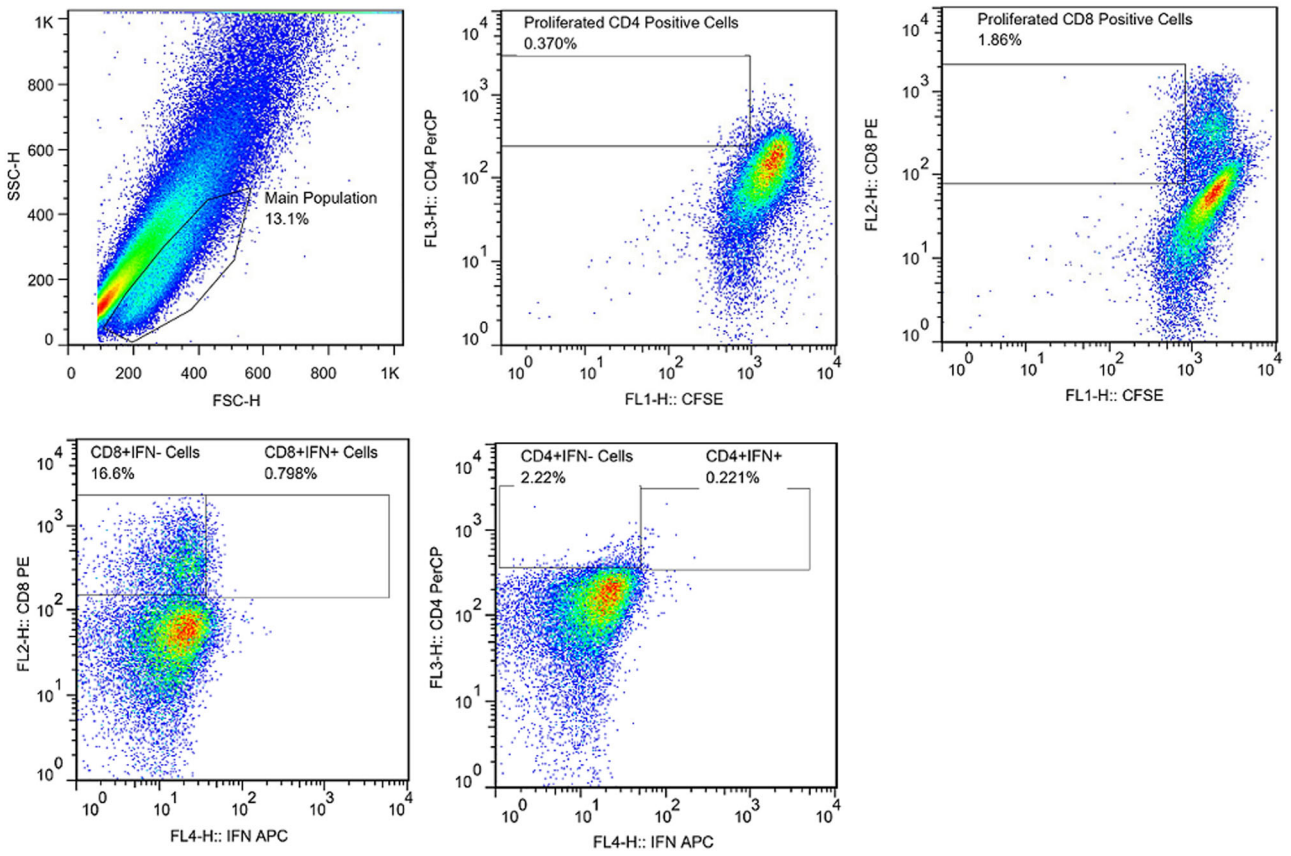

FIGURE 6 The flow cytometry analysis for gating IFN-γ-produceing CD4 + and CD8 + T cells. On Day 49, peripheral blood mononuclear cells from vaccinated and placebo volunteers were isolated and exposed to 20 μg/ml Noora vaccine in the cell culture. After 48h, the cells were stained for IFN-γ producing $CD4^+$ and $CD8^+$ T cells. On gated $CD4^+$ and $CD8^+$ T cells, IFN-γ-producing T cells were identified based on the presence or absence of IFN-γ. Numbers within the plots indicate the relative percentage of the IFN-γ producing T cells population in the control samples. IFN-γ, interferon gamma

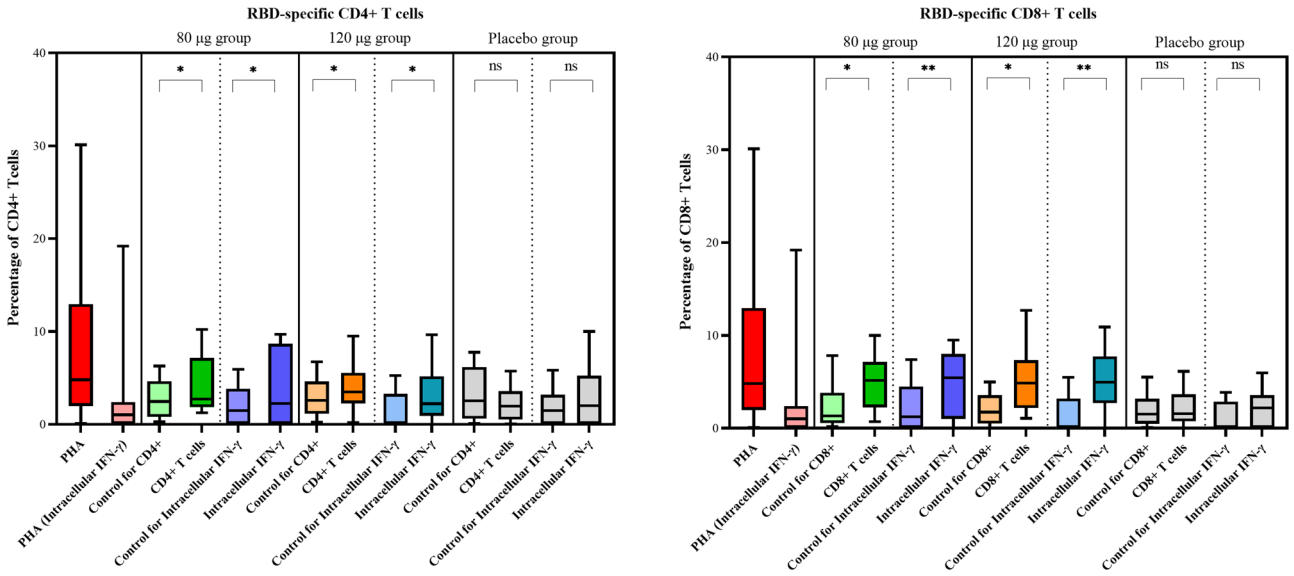

FIGURE 7 The analysis of IFN-γ producing CD4 + and CD8 + T cells populations in the vaccinated and placebo groups. On Day 49, in the vaccinated group, RBD-specific CD4 + and CD8 + T cell was proliferated and increased significantly in comparison to control which were not exposed to RBD antigen (CTR) (80 μg group; CD4 + : $p = 0.03$ and CD8 + : $p = 0.04$, 120 μg group; CD4 + : $p = 0.04$ and CD8 + : $p = 0.04$). The placebo group did not show a significant increase. Also, the RBD-specific CD4 + T cells and RBD-specific CD8 + T cells significantly produced IFN-γ in comparison to the control which was not exposed to RBD antigen (CTR). (80 μg group; $CD4^+$: $p = 0.04$ and $CD8^+$: $p = 0.006$, 120 μg group; $CD4^+$: $p = 0.03$ and $CD8^+$: $p = 0.07$). The placebo group did not show a significant increase in the IFN-γ producing CD4 + and CD8 + T cells. CTR, Control; IFN-γ, interferon gamma; PHA, phytohemagglutinin; RBD, receptor-binding domain

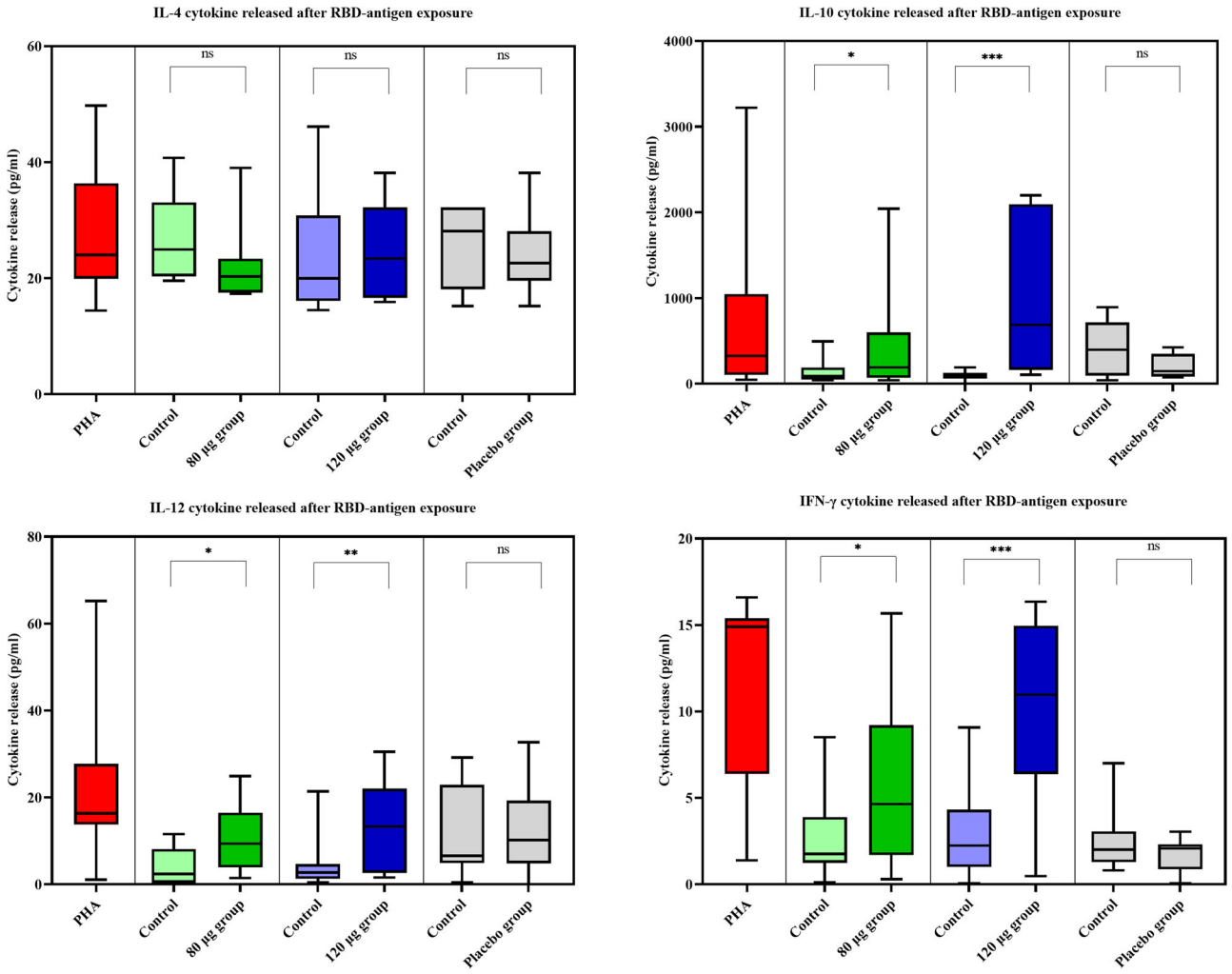

FIGURE 8 The analysis of the TH1/TH2 cytokines panel in the vaccinated and placebo group. After RBD antigen exposure, the supernatants of cultured PMBC were evaluated by assessing the IL4, IL-10, IL-12, and IFN-γ at day 49. IL-4 cytokine was not changed after RBD exposure in the vaccinated and placebo group ($p = 0.12$ for 80 µg dose group, $p = 0.83$ for 120 µg dose group, and $p = 0.58$ for placebo). The IL-10 cytokine tend to increase in the vaccinated group and had a significant change in 80 µg dose and 120 µg doses ($p = 0.02$ for 80 µg dose group, $p = 0.007$ for 120 µg dose group, and $p = 0.48$ for placebo). The IL-12 cytokine was increased in the 80 µg group and 120 µg group ($p = 0.04$ for 80 µg dose group, $p = 0.01$ for 120 µg dose group, and $p = 0.62$ for placebo). The IFN-γ cytokine also showed an increase in vaccinated participants and no significant changes were detected in the placebo ($p = 0.04$ for 80 µg dose group, $p = 0.001$ for 120 µg dose group, and $p = 0.42$ for placebo). CTR, Control; IFN-γ, interferon gamma; PHA, phytohemagglutinin; RBD, receptor-binding domain

immunogenicity in vivo models against SARS-CoV-2. The BNT162b2, an RBD-based COVID-19 vaccine candidate, showed good immunogenicity results in healthy volunteers.[29] Other vaccines such as NovoVax and Zhifei are other RBD-based vaccines that have shown considerable immunogenicity.[22,26] Current data demonstrate promising results of the RBD-based protein subunit as a COVID-19 vaccine candidate. Humoral immune responses have been considered as an important factor that correlates with protection against SARS-CoV-2.[31] Up to now, immunogenicity results for several COVID-19 vaccine candidates have been published. Remarkably, it is challenging to compare the results of the immunogenicity of different vaccine candidates as they have used various methods in this regard and there is not a unique standardized neutralization assay. However, BNT162b1 reported 1.8 to 2.8 fold rises in neutralizing antibody[32] and ChadOx1 nCoV-19 reported that 91% and 100% participants achieved 80% plaque reduction neutralization titer.[33] The NovoVax vaccine also reported neutralizing antibody raised four times in the vaccinated group. The Zhifei vaccine, another protein subunit vaccine, reported seroconversion in more than 90% of the vaccinated participants.[26] The relative immunogenicity results showed that three doses of Noora vaccine increased anti-RBD IgG titer four times more than the placebo and 88% of the vaccinated group was seroconverted. Notably, mRNA-based vaccines and adenovirus-vectored vaccines have reported 70%–95% efficacy against COVID-19, respectively.[27,34] This efficacy is correlated with an amount of seroconversion which has been previously mentioned. They found a considerable humoral immunity response in their trial. The VNT test showed that among the vaccinated group, 78% of the individuals are

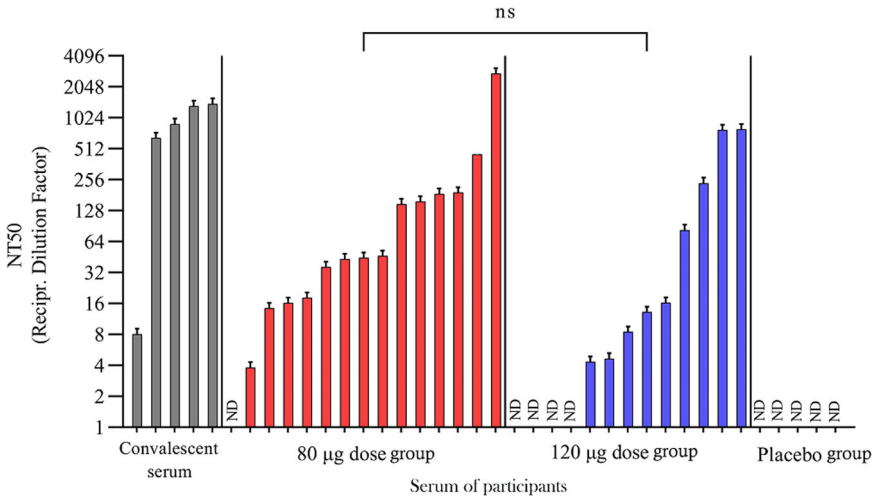

FIGURE 9 The results of the virus neutralizing test (VNT). It was performed on Day 49. Briefly, Vero cells were treated with the serial dilutions of both the virus and pseudo-virus, and the IC50 dilution was determined. The convalescent human sera as well as vaccinated and placebo groups were incubated with 100 TCID50 and exposed to Vero cell culture. About 78% of the vaccinated cases in both vaccinated groups were able to neutralize the wild and pseudo-type virus. No significant difference was seen between the two vaccinated groups ($p = 0.62$). Despite a bite increase in anti-RBD Ig in the placebo group, the sera were not able to neutralize the virus. Each bar indicates each participant's serum. ND, not determined

seroprotected. After exposure of PBMC of the vaccinated group with RBD protein, Both RBD-specific $CD4^+$ and $CD8^+$ T cells with their intracellular IFN-γ increased. Also, IFN-γ and IL-12 cytokines were released after RBD exposure. These results showed that cell-mediated immunity (Th1) response was inducted in response to RBD vaccination. However, IL-4 did not change after vaccination. BNT162b1 vaccine, induced functional $CD4^+$ and $CD8^+$ responses in all vaccinated cases, mainly Th1 helper responses.[35] The mRNA1273 vaccines also induced TH1 helper response with minimal Th2 response.[36] The rAd26-S and rAd5-S vaccine also increased the 100% formation of $CD4^+$ and $CD8^+$ cells and increased the level of IFN-γ.[27] NVX-CoV2373 induced Th1 phenotype response with the increased levels of IFN-γ, IL-2, and TNF-α with slight induction in Th2 responses which was measured by IL-5 and IL-13 cytokines.[37,38] Other vaccines also did not assess cellular immunogenicity and it is not feasible to compare their results with the current study.

BIV1-CovIran, an inactivated whole virus particle vaccine, showed acceptable safety results, and only mild to moderate transient adverse effects were reported. Similar to the current study, the seroconversion for neutralizing and anti-RBD antibodies was 82.8%.[39] The results of SpikoGen, a subunit COVID-19 vaccine, showed no serious adverse events. They reported that the seroconversion rate against S1 was 63.55%.[40]

It seems in response to inflammatory cytokines, IL-10 as an anti-inflammatory cytokine was increased to regulate the cytokine network. Additionally, the 120 µg dose showed no better immunogenicity results than the 80 µg dose. Hence, it can be concluded that the 80 µg dose is sufficient to induce the anticipated immune response. Phases 2 and 3 of the trial are also under investigation and the results of future studies can complement the current results.

The current study faced some limitations. The volunteers were young adults aged between 18 and 50 years old. Children and older adults were not included, so the results may not be extendable. The study populations were Caucasian and other ethnicities were not included. Furthermore, the Noora vaccine, induced neutralizing titer more than those in the placebo groups. The clinical protective efficacy of the vaccine cannot yet be confirmed. These limitations should be considered in the next phases of the trial.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Jafar Salimian, Ali Ahmadi, Jafar Amania, Gholamreza Oladd, Gholamhossein Alishiri, and Hassan Abolghasemi. The first draft of the manuscript was written by Ali Saffaei and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data set is available on request.

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