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Original Research

Immunogenicity and Safety of SpikoGen, an Adjuvanted Recombinant SARS-CoV-2 Spike Protein, as a Heterologous Third Booster Dose in Kidney Transplant Patients: A Single-arm Clinical Trial

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Abstract

Purpose: Studies have found that immunocompromised patients have suboptimal responses to COVID-19 vaccines, leading to approval of a need for booster doses in this population. SpikoGen® is a subunit recombinant spike protein vaccine combined with Advax-CpG55.2™ adjuvant to protect against COVID-19. Previous clinical trials found this vaccine to be tolerable, immunogenic, and efficacious in reducing the risk of COVID-19, including severe disease. However, the effects of this vaccine have not been assessed in immunocompromised patients. This study sought to assess the immunogenicity and safety of the SpikoGen vaccine as a third booster dose in patients undergoing kidney transplant who were receiving immunosuppressive therapy and had received their primary vaccination based on an inactivated whole virus platform (Sinopharm).

Methods: This single-arm trial was performed with 43 patients undergoing kidney transplant. The participants received a single booster dose of the SpikoGen vaccine 1 to 3 months after primary vaccination with 2 doses of the Sinopharm vaccine. Immunogenicity assessments were performed at baseline and 30 days after the booster dose. The primary outcomes were seroconversion rates of anti-S₁ and surrogate virus neutralizing antibodies. Safety outcomes included the incidence of solicited and unsolicited adverse events in the 7 days and 1 month after the booster dose, respectively.

Findings: The SpikoGen vaccine induced positive humoral and cellular responses 30 days after the booster dose in those patients who were seropositive or seronegative after 2 primary doses of the Sinopharm vaccine. Thirty days after the SpikoGen vaccine booster, seroconversion rates were 35.29% (95% CI, 19.75%–53.51%) to anti-S₁ and 29.41% (95% CI, 13.27%–46.57%) to surrogate neutralizing antibodies. The most common local and systemic reported solicited adverse events were injection site pain and fatigue.

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which were largely mild and transient. No serious adverse events were reported.

**Implications:** A single booster dose of SpikoGen vaccine given 1 to 3 months after primary vaccination with 2 doses of Sinopharm vaccine induced positive humoral and cellular immune responses in immunosuppressed patients undergoing renal transplant, thereby achieving spike antibody levels predictive of protection. This study was performed as a single-center study, and it will be important for future large multicenter studies to extend these results to other immunocompromised patient groups. (Clin Ther. 2022;44:1566–1576.) © 2022 Elsevier Inc.

**Keywords:** SpikoGen, Kidney transplant, Booster dose, COVID-19, SARS-CoV-2.

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**INTRODUCTION**

As of September 2022, SARS-CoV-2 is still infecting many people worldwide, with vaccination playing an important role in helping to reduce the burden of disease. However, the problems of rapidly waning immunity after vaccination as well as immune escape caused by new variants, including B.1.617 or B.1.1.529, have led to calls for booster vaccinations to restore waning immunity and protection.

Low rates of seroconversion have been reported in solid organ transplant recipients receiving mRNA SARS-CoV-2 vaccines. In a study of BNT162b2 on renal transplant recipients, a third vaccine dose led to induction of neutralizing antibodies in populations with and without response to the primary vaccination. In another study, a third dose of BNT162b2 restored neutralizing titers of anti-receptor-binding domain antibodies in 40% of participants who had not responded to the previous vaccination course. On the basis of all the positive findings, a meta-analysis has also recommended a third dose of mRNA vaccines in patients with solid organ transplants, considering its enhanced effects of immunogenicity and acceptable safety profile.

SpikoGen is an adjuvanted recombinant S protein trimer vaccine that induces strong humoral and cellular responses in previous Phase II and a pivotal Phase III trials, indicating positive tolerability, immunogenicity, and efficacy, resulting in emergency use authorization from Iran’s Food and Drug Administration in October 2021.

Because patients under immunosuppressive therapy were excluded from the previous SpikoGen clinical trials, its immunogenicity and tolerability have not previously been assessed in this population. Hence, this study aimed to investigate the immunogenicity and safety of a SpikoGen booster shot in patients undergoing kidney transplant who are receiving immunosuppressive therapy and had previously received primary vaccination with 2 doses of an inactivated whole virus platform (Sinopharm) vaccine.

**METHODS**

**Setting**

This study was a single-arm, open-label, prospective clinical trial conducted in 43 patients during February and March 2022 at Shahid Labbafinezhad clinic, affiliated with Shahid Beheshti University of Medical Sciences, Tehran, Iran, which is considered a referral center for kidney transplantation in Iran.

**Patients**

Patients undergoing renal transplant who were 18 years and older were eligible to enter the study if they had received a primary course of vaccination with Sinopharm vaccine 1 to 3 months earlier. The exclusion criteria were as follows: pregnant or lactating women; patients with active infection or symptoms of COVID-19 at the screening visit; history of COVID-19 between the primary vaccination and the third booster dose; active cytomegalovirus infection; history of receiving rituximab or intravenous immunoglobulin during the past 6 months; patients with a history of severe allergic reactions (e.g., anaphylaxis) to the study vaccine or any components of the vaccine; individuals who had received any other investigational product within 30 days before the screening visit or intend to participate in other clinical studies during this trial; history of transplant rejection during the past 30 days; and individuals with special circumstances that may increase the risk of participating in the study or interfering with the evaluation of the primary end points of the study according to the researcher’s opinion.

**Informed Consent**

All participants signed the informed consent forms before enrollment. The study was approved by the Ethics Committee of the Urology and...
Nephrology Research Center; Shahid Beheshti University of Medical Sciences (ethics code number IR.SBMU.UNRC.REC.1400.017). The trial was registered at the Iranian Registry of Clinical Trials with the registration code IRCT20150303021315N28 and ClinicalTrials.gov with the registration code NCT05285384.

**Interventions**

A total of 43 eligible patients were included in this study (Figure 1). Medical histories and immunogenicity assessments were performed at baseline and 30 days after the booster dose. Eligible participants received a single booster dose of 25 μg of SpikoGen in their deltoid muscle.

**Outcomes**

The primary outcomes were the seroconversion rates of anti-S₁-binding IgG and neutralizing antibodies measured via a surrogate virus neutralizing test 1 month after the booster dose. Secondary outcomes included the geometric mean fold rise of anti-S₁ and neutralizing antibodies 1 month after the SpikoGen booster dose. T-cell responses were also assessed at baseline and 1 month after the booster dose using an interferon-γ release assay after stimulation with spike protein peptides. Other secondary outcomes included immunogenicity assessments in subgroups of participants with and without previous humoral response to their primary vaccination course. Patients with primary response were defined as positive status of anti-S₁ or neutralizing antibodies at baseline,
whereas patients without response to the primary vaccination were defined as being negative for anti-\( S_1 \) and neutralizing antibodies at baseline.

Seroconversion was defined as a change in the status of antibody levels from negative to positive based on the prespecified commercial ELISA kit threshold in the seronegative populations. In the baseline seropositive population, seroconversion was defined as at least a fourfold increase in the antibody levels on day 30. T-cell responses were performed based on the QuantiFERON SARS-CoV-2 RUO (Qiagen, Hilden, Germany) toolset. Tube 1 contained CD4\(^+\) epitopes, and tube 2 contained CD4\(^+\) and CD8\(^+\) epitopes from the spike protein. The levels of interferon-\( \gamma \) in plasma samples were reported in international units per milliliter according to the manufacturer’s instructions.

Safety assessments were performed as secondary outcomes, including the occurrence of local and systemic solicited adverse events for 7 days after the booster dose. Unsolicited adverse events were assessed until 1 month after the booster dose. These outcomes were reported based on Medical Dictionary for Regulatory Activities (MedDRA) classification. The participant’s severity score was assessed based on the US Food and Drug Administration toxicity grading scale.\(^7\)

**Statistical Analysis**

The initial recruitment target of 100 individuals was planned according to the available population of patients undergoing kidney transplant who had not received the booster dose. This estimation was not based on any power calculation, with 43 patients ultimately being enrolled. All patients who received a booster dose of the study treatment were included in the safety population. Tolerability was presented as incidences and percentages of patients with solicited (local and systemic) and unsolicited adverse events. The immunogenicity outcomes were reported based on 34 available samples of patients who received a booster dose of the vaccine.

Missing data were not imputed. No multiplicity adjustments were made in this study. Continuous data were compared using a \( t \) test, and categorical data were assessed using the \( \chi^2 \) or Fisher exact test. Participants with seroconversion due to vaccination were provided with 2-sided 95% CIs using the Clopper-Pearson method. Hypothesis testing was 2-sided, and \( P < 0.05 \) was considered significant. The 95% CIs for the geometric mean concentration and geometric mean fold rise were calculated based on the \( t \) distribution of the log-transformed values then back-transformed to the original scale in each time point for presentation. The subgroup analyses were performed based on baseline characteristics. Wilcoxon tests were used to compare the paired samples to evaluate the difference in the concentrations of interferon-\( \gamma \) and antibody levels. We used R, version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria) and STATA, version 14 (StataCorp, College Station, Texas) for all statistical analyses.

**RESULTS**

In total, 43 patients were enrolled in the study. The screening process of the participants is provided in the CONSORT diagram in Figure 1. Table 1 gives the demographic data and baseline characteristics of the participants, including drug histories, reasons for transplantation, seropositivity status at baseline, days passed from transplant, history of rejection, number of transplants, type of transplant, and history of new-onset diabetes after transplant.

**Immunogenicity Outcomes**

After booster day 30, the seroconversion rate of anti-\( S_1 \)-binding IgG was 35.29% (95% CI, 19.75%–53.51%). The seroconversion rate of neutralizing antibodies was 29.41% (95% CI, 13.27%–46.57%). The results of the geometric mean fold rise and subgroup analysis based on the baseline characteristics are provided in Table II.

Results of the T-cell response are provided in Table III. The interferon-\( \gamma \) response in blood samples taken 1 month after the booster dose was significantly higher after stimulation with antigen 2 (CD4\(^+\) and CD8\(^+\) epitopes) when compared with baseline levels, and the interferon-\( \gamma \) response to antigen 1 also trended higher at day 30 when compared with day 0. However, this finding did not reach statistical significance.

**Safety Outcomes**

The results of the solicited adverse events after the booster injection are provided in Table IV. As can be seen, injection site pain was the most common adverse event among the participants (44.19%). Among systemic complications, fatigue was the most reported adverse event, being detected in 10 patients.
Table I. Demographic and clinical characteristics of the patients at baseline.*

| Characteristic                                      | Finding (N = 43)                           |
|-----------------------------------------------------|--------------------------------------------|
| Age, mean (SD), y                                   | 41.99 (11.83)                              |
| Sex                                                 |                                            |
| Male                                                | 28 (65.12)                                 |
| Female                                              | 15 (34.88)                                 |
| Medication                                          |                                            |
| Cyclosporine                                        | 18 (41.86)                                 |
| Tacrolimus                                          | 24 (55.81)                                 |
| Sirolimus                                           | 1 (2.33)                                   |
| Everolimus                                          | 0 (0)                                      |
| Prednisolone                                        | 43 (100)                                   |
| Mycophenolate mofetil                               | 41 (95.35)                                 |
| Azathioprine                                        | 1 (2.33)                                   |
| Reason for transplantation                         |                                            |
| Autosomal dominant polycystic kidney disease        | 3 (6.98)                                   |
| Diabetes mellitus                                   | 6 (13.95)                                  |
| Glomerulonephritis                                  | 9 (20.93)                                  |
| Hypertension                                        | 15 (34.88)                                 |
| Nephrolithiasis                                     | 1 (2.33)                                   |
| Other                                               | 15 (34.88)                                 |
| Patients without primary response to Sinopharm vaccine | 12(35.29)                                 |
| Time from last transplantation, mean (SD), d        | 2775.42 (2110.78)                          |
| History of rejection                                | 0 (0)                                      |
| Transplantation type                                |                                            |
| Living                                              | 23 (53.49)                                 |
| Deceased                                            | 20 (46.51)                                 |
| Transplantation count                               |                                            |
| 0                                                   | 1 (2.33)                                   |
| 1                                                   | 36 (83.72)                                 |
| 2                                                   | 6 (13.95)                                  |
| New-onset diabetes after transplantation            | 2 (4.65)                                   |

* Data are presented as number (percentage) of patients unless otherwise indicated.

The results of the unsolicited adverse events are provided in Table V.

**Post Hoc Analysis**

The geometric mean concentration results for anti-S₁-binding IgG and neutralizing antibodies are provided in Table VI. The anti-S₁ and neutralizing antibody levels were significantly greater at day 30 compared with baseline values in the pooled population and those seropositive and seronegative after the primary vaccination course.

The use of World Health Organization standards allowed us to convert the S₁ antibody results to World Health Organization binding antibody units (BAU). The results are provided in Table VII. A total of 25 of the 34 participants (73.5%) receiving the SpikoGen booster dose achieved S₁ antibody levels of ≥154 BAU/mL 30 days after booster.
Table II. Seroconversion rate and GMFR of $S_1$-binding IgG and sVNT.*

| Population set                        | SCR, No. (%) (95% CI) | GMFR (95% CI) |
|---------------------------------------|-----------------------|--------------|
|                                       | $S_1$-Binding IgG, RU/mL | sVNT, $\mu g/mL$ | $S_1$-Binding IgG, RU/mL | sVNT, $\mu g/mL$ |
| Pooled                                | 12 (35.29) (19.75–53.51) | 10 (29.41) (13.27–46.55) | 5.01 (2.41–10.45) | 3.51 (1.97–6.25) |
| Without primary response to Sinopharm | 5 (41.67) (15.17–72.33) | 4 (33.33) (9.92–65.11) | 15.58 (2.37–102.23) | 4.77 (1.07–21.17) |
| With primary response to Sinopharm    | 7 (31.82) (13.86–54.87) | 6 (27.27) (10.73–50.22) | 2.70 (1.65–4.41) | 2.96 (1.77–4.97) |
| Sex                                   |                       |                    |                          |                  |
| Male                                  | 9 (40.91) (20.71–63.65) | 8 (36.36) (17.20–59.34) | 8.45 (2.95–24.23) | 4.45 (1.99–10.00) |
| Female                                | 3 (25) (5.49–57.19)   | 2 (16.67) (2.09–48.41) | 1.92 (1.03–3.59) | 2.26 (1.02–5.01) |
| Medication                            |                       |                    |                          |                  |
| Cyclosporine                          | 4 (26.67) (7.79–55.10) | 3 (20.00) (4.33–48.09) | 2.62 (1.38–4.98) | 2.54 (1.36–4.75) |
| Tacrolimus                            | 8 (42.11) (20.25–66.50) | 7 (36.84) (16.29–61.64) | 8.37 (2.46–28.45) | 4.52 (1.75–11.71) |
| Prednisolone                          | 12 (35.29) (19.75–53.51) | 10 (29.41) (15.10–47.48) | 5.01 (2.41–10.45) | 3.51 (1.97–6.25) |
| Mycophenolate mofetil                 |                       |                    |                          |                  |
| Medical history                       | 11 (33.33) (17.96–51.83) | 9 (27.27) (13.30–45.52) | 4.92 (2.31–10.48) | 3.34 (1.85–6.00) |
| Diabetes mellitus                     | 2 (33.33) (4.33–77.72) | 2 (33.33) (4.33–77.72) | 15.99 (0.67–380.08) | 6.95 (0.46–105.61) |
| Glomerulonephritis                    | 2 (40.00) (5.27–85.34) | 1 (20.00) (0.51–71.64) | 3.55 (0.45–27.88) | 1.92 (0.40–9.21) |
| Hypertension                          | 5 (41.67) (15.17–72.33) | 5 (41.67) (15.17–72.33) | 6.00 (1.75–20.59) | 5.12 (1.53–17.22) |
| Other                                 | 3 (23.08) (5.04–53.81) | 2 (15.38) (1.92–45.45) | 2.81 (0.94–8.41) | 2.23 (1.16–4.27) |
| Transplantation type                  |                       |                    |                          |                  |
| Living                                | 7 (36.84) (16.29–61.64) | 6 (31.58) (12.58–56.55) | 5.34 (1.75–16.38) | 4.54 (1.84–11.16) |
| Deceased                              | 5 (33.33) (11.82–61.62) | 4 (26.67) (7.79–55.10) | 4.62 (1.64–13.00) | 2.53 (1.20–5.32) |

GMFR = geometric mean fold rise; sVNT = surrogate virus neutralizing test.

* Percentages for seroconversion rate were calculated as the number of patients who reported the event divided by the total number of patients in each population set with nonmissing data multiplied by 100. The 95% CI for seroconversion rate was calculated using the exact Clopper-Pearson method. The 95% CI for GMFR was calculated based on the t distribution of the log-transformed values, then back-transformed to the original scale for presentation.
DISCUSSION
The results of this study indicate that the SpikoGen vaccine is able to induce humoral and cellular responses in patients receiving kidney transplants who are undergoing treatment with immunosuppressors. The third booster dose of the SpikoGen vaccine significantly elevated anti–S₁ and neutralizing antibody levels in patients who had previously received primary vaccination with an inactivated whole virus vaccine (Sinopharm). This boosting effect was robust in both baseline seropositive and seronegative patients.

Previous studies have found weak immune response to 2 doses of other SARS-CoV-2 vaccines in patients receiving solid organ transplants while undergoing immunosuppressive therapy. According to our baseline results, after 1 to 3 months of a second dose of an inactivated whole virus platform, approximately 35% of the patients had no detectable anti–S₁-binding or neutralizing antibodies. A booster dose of the SpikoGen vaccine successfully induced both humoral and cellular immune responses in these immunosuppressed populations.

A previous study reported a seroconversion rate of 44% at 30 days after a third dose of mRNA COVID-19 vaccine in solid organ transplant recipients without response to the primary vaccination. The seroconversion rate of approximately 35% in the pooled participants (approximately 32% and 41% in participants with and without primary response to the inactivated whole virus platform in our study, respectively), appears comparable to that study. In individuals primed with inactivated vaccine, exposure to the much larger amount of spike protein in the SpikoGen vaccine may better stimulate a recall memory B-cell response, helping explain the antibody response induced by the SpikoGen vaccine despite the lack of apparent response to the primary vaccination course. The Advax-CpG adjuvant in the SpikoGen vaccine may also have contributed to this response in nonresponders to the primary vaccine course.
Table V. Patients experiencing unsolicited adverse events.

| System Organ Class/Preferred Term | No. (%) of Patients (N = 43) |
|-----------------------------------|------------------------------|
| Any adverse event                 | 9 (20.93)                    |
| Gastrointestinal disorders        | 1 (2.33)                     |
| Diarrhea                          | 1 (2.33)                     |
| General disorders and administration site conditions |
| Fatigue                           | 1 (2.33)                     |
| Nervous system disorders          | 3 (6.98)                     |
| Dizziness                         | 2 (4.65)                     |
| Parosmia/dysgeusia                | 1 (2.33)                     |
| Respiratory, thoracic, and mediastinal disorders |
| Cough                             | 2 (4.65)                     |
| Dyspnea                           | 2 (4.65)                     |
| Oropharyngeal pain                | 2 (4.65)                     |
| Rhinorrhea                        | 2 (4.65)                     |
| Skin and subcutaneous tissue      | 1 (2.33)                     |
| disorders                         |                              |
| Pruritus                          | 1 (2.33)                     |
| Vascular disorders                | 1 (2.33)                     |
| Hypotension                       | 1 (2.33)                     |

How might the spike antibody levels achieved with the SpikoGen booster dose in solid organ transplant recipients in our study translate into actual SARS-CoV-2 protection? Goldblatt et al.9 suggested that the ratio of trial participants who achieve a level of 154 BAU/mL could be used to predict the level of protection against wild-type virus, and for studies with antibody distributions that enabled precise estimation of thresholds, this threshold was considered to be 60 BAU/mL. On the basis of this protection algorithm, 25 of 34 patients (73.5%) had spike IgG levels >154 BAU/mL 30 days after the SpikoGen booster dose. Notably, the mean concentrations of anti-S1 antibodies after the booster dose even in those without a primary response to the Sinopharm vaccine was greater than the cutoff of 60 BAU/mL. These results would predict a SpikoGen booster dose efficacy against symptomatic infection in these transplant patients of approximately 70% against the ancestral strains. This algorithm came from a ChAdOx1 viral vector vaccine study that found that an anti-S1 levels of 54 BAU/mL translated to 60% efficacy against symptomatic infection with Alpha (B.1.1.7), 113 BAU/mL with a 70% efficacy, and 264 BAU/mL with 80% vaccine efficacy.10 Notably, this algorithm was derived on antibody responses obtained in immunocompetent individuals, whereas our prediction of 70% SpikoGen efficacy against symptomatic infection was achieved in immunosuppressed patients receiving transplants. The most important outcome of vaccination, however, is protection against severe disease. Notably, in its pivotal Phase III trial, the SpikoGen vaccine provided approximately 78% protection against severe disease caused by the Delta variant.6 This finding is consistent with trial results for other COVID-19 vaccines, which have all consistently found higher levels of protection against severe disease caused by SARS-CoV-2 than against only symptomatic infection. This finding provides additional confidence that the booster dose of the SpikoGen vaccine in patients receiving renal transplants should provide even more robust protection against severe disease as predicted by this algorithm for symptomatic infection.

Overall, our results confirm that a third dose of a spike protein–based vaccine may be beneficial at an interval of 1 to 3 months after an initial primary vaccine course in immunocompromised patients. This early third booster dose could be particularly important given the rise of new vaccine-resistant variants, such as Omicron, to which vaccine immunity wanes even faster than against the ancestral strains.11

Although humoral responses are clearly highly important, T-cell responses may also play an important role in SARS-CoV-2 protection in immunocompromised patients.12 SpikoGen vaccine induced a considerable rise in interferon-γ responses after stimulation with CD4+ and CD8+ spike protein epitopes, consistent with previous trials of SpikoGen indicating its ability to induce T-cell responses.13 Notably, Advax-CpG adjuvant induces potent antiviral CD8+ T-cell responses.14 In this booster study, T-cell interferon-γ responses were increased by the SpikoGen booster, which is a valuable finding in immunocompromised patients for whom the activation of both arms of the immune response may be particularly important for protection.

On the basis of the safety outcomes, the SpikoGen booster shot was well tolerated, and the solicited adverse events were consistent with those normally
A recently published SpikoGen booster trial in immune-competent participants who had received a primary vaccination course of inactivated whole virus or other vaccines 4 to 6 months earlier showed a seroconversion rate of 68% in anti-S₁ antibodies and 80% in neutralizing antibodies 14 days after the booster dose.15 Although the seroconversion rates were lower in the current trial, this previous booster trial was in immunocompetent healthy individuals, whereas the current trial was in transplant recipients receiving immunosuppressive therapy in whom seroconversion rates would be expected to be significantly lower.

This study had some limitations, including the relatively low number of transplant patients and the lack of a suitable control group. At the time of the study design, we planned to enroll 100 patients. However, according to the national vaccination program, most of the available patients had already become vaccinated during the approval process, and we could only recruit 43 patients. Among these patients, only 34 had samples available for immunogenicity results. The values of other 9 samples could not be assessed because of the leaking and broken blood specimen containers. Another limitation of this study includes the lack

seen after vaccination. Most adverse reactions were mild and short-lived with full recovery, consistent with the clean safety profile seen in previous SpikoGe trials.⁶,¹⁵,¹⁶
of measurement for actual infection, although an attempt was made to try and predict protection by extrapolating from antibody levels. Furthermore, the results were based on just a single time point 30 days after the booster dose, and it is not currently known how the spike antibody responses induced by the booster dose might decay over time, whether additional boosters of SpikoGen may be required, and, if so, what the optimal dose window for such additional boosters may be. Similarly, it is not currently known what levels of protection this might afford patients against evolving Omicron variants. A final limitation was that this study was performed as a single-center study, and it will be important for future large, multicenter studies to extend these results to other immunocompromised patient groups.

CONCLUSIONS
In patients receiving renal transplants who had received a primary course of an inactivated whole virus vaccine 1 to 3 months previously, a single booster dose of heterologous SpikoGen, a recombinant spike protein vaccine, induced positive humoral and cellular immune responses predictive of protection against SARS-CoV-2 infection.

DECLARATION OF INTEREST
SB, NA, HK, RSH, MB, and SK are members of the Orchid Pharmed medical department which is in collaboration with CinnaGen company with respect to conducting clinical trials. The remaining authors have no other relevant affiliations.

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M. Nafar conceptualized the study. N. Mostafaloo, A. Firouzan, F. Poorrezagholi, F. Samadian, and N. Dalili were involved in performing the research. S. Barati wrote the original draft of the manuscript. N. Anjidani, H. Kafi, and S. Kianipour were involved in organization, and conducting the study. R. Shahpari and M. Bayat performed the statistical analysis. S. Samavat was involved in conceptualization, trial design and performing the study. All authors critically reviewed the manuscript and approved the final version. All authors had full access to all data in the studies and had final responsibility for the decision to submit for publication.

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