Evaluation and Its Impact of SARS-CoV-2 Inactivated Vaccine Candidate in K18-hACE2 Mice

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Abstract

The COVID-19 pandemic caused by SARS-CoV-2 requires effective vaccines to be developed. This study aimed to assess the impact of a SARS-CoV-2 inactivated vaccine candidate in k18-hACE2 mice by monitoring their body weight, immune activation, and inflammatory cytokines including IL-4, IL-6, TNF-α, and IFN-γ. The study utilized k18-hACE2 mice expressing the human angiotensin-converting enzyme 2 (hACE2) receptor. The mice were administered the inactivated vaccine candidate compared with sham and vehicle. Body weight was monitored, and serum samples were collected to measure IL-4, IL-6, TNF-α, and IFN-γ levels using ELISA. Data were evaluated using SPSS statistical analysis software. The administration of the SARS-CoV-2 inactivated vaccine candidate in k18-hACE2 mice did not result in significant changes in body weight compared to the control group. Furthermore, the levels of IL-4, IL-6, TNF-α, and IFN-γ were significantly reduced in the vaccinated mice compared to the control group, suggesting a dampening effect on the inflammatory response. This study demonstrates that the SARS-CoV-2 inactivated vaccine candidate has a minimal impact on the body weight of k18-hACE2 mice. Nevertheless, it successfully regulates the levels of IL-4, IL-6, TNF-α, and IFN-γ, suggesting its safety and beneficial impact. These findings contribute to understanding the vaccine's efficacy and safety profile in vaccine development.

Keywords: COVID-19, K18-hACE2, SARS-CoV-2, transgenic, vaccination

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INTRODUCTION

The ongoing global pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has prompted an urgent need for effective vaccines to combat the spread of the virus (Arora *et al*., 2021; Dhawan *et al*., 2022). Inactivated vaccines have shown promise as a safe and viable approach for generating protective immune responses against viral infections, including SARS-CoV-2 (Law *et al*., 2023; Li *et al*., 2022). However, preclinical evaluation of these vaccine candidates in relevant animal models is crucial to assess their efficacy and safety profiles (Gimenes *et al*., 2022). We focused on evaluating the impact of a SARS-CoV-2 inactivated vaccine candidate in K18-hACE2 mice, a widely used transgenic mouse model that expresses the human angiotensin-converting enzyme 2 (hACE2) receptor as the primary receptor for SARS-CoV-2 (Wang *et al*., 2021).

Weight loss has been observed as a clinical manifestation of SARS-CoV-2 infection both in humans and animal models (Yinda *et al*., 2021). These parameters are important indicators of the overall health status and disease progression. Additionally, the dysregulation of inflammatory cytokines, such as IL-6 and TNF-α, has been

implicated in the immunopathogenesis of COVID-19, leading to severe lung injury and systemic inflammation through cytokine storm (Han *et al*., 2020). On the other hand, vaccine-induced immunity was also related to several signaling molecules such as IL-4 for its potency to induce IgE class switching related to allergies while lowering IFN-γ production which plays an important role in viral immunity.

By assessing the impact of the inactivated vaccine candidate on the mice's weight, immune activation, and inflammatory cytokine expression, we aim to gain insights into its potential effectiveness in preventing SARS-CoV-2 infection and associated inflammatory responses (Yinda *et al*., 2021). This study aimed to investigate the effects of a SARS-CoV-2 inactivated vaccine candidate on the mice's weight along with immune activation and inflammatory cytokines (IL-4, IL-6, TNF-α, and IFN-γ) for safety parameter evaluation. The findings from this study will contribute to the understanding of vaccine-induced immune responses and guide further development and optimization of SARS-CoV-2 vaccines for human use (Han *et al*., 2021). This study aimed to investigate the vaccine's effects on the mice's weight, immune activation, and inflammatory cytokines levels including IL-4, IL-6, TNF-α, and IFN-γ.

MATERIALS AND METHODS

Ethical Approval

Ethical clearance for the animal study was obtained from the Faculty of Veterinary Medicine, Airlangga University (Certificate Number: 2.KE.118.03.2020), ensuring compliance with animal welfare guideline and ethical standard.

Study Period and Location

The entire study took place in an animal biosafety level-3 (ABSL-3) facility of Biotis Pharmaceuticals Indonesia, Ltd., which ensured the containment of infectious agents and the safety of the animals and personnel involved in the study. Stringent protocols were applied to execute animal handling, housing, and waste disposal to maintain biosafety standards (Guo *et al*., 2019).

Study Design

This study involved k18-hACE2 mice obtained from InVivos, Pte. Ltd. Singapore and housed in a specific pathogen-free (SPF) Animal Biosafety Level-3 Laboratory (Yeh *et al*., 2021). The mice were allowed to acclimate for 7 days to adapt to the housing conditions. The temperature, humidity, and light-dark cycle were maintained at optimal levels throughout the study.

The mice were then randomly divided into two groups: the control group and the vaccine group. The control group received NaCl (0.9%), adjuvant (0.3 mg alhydrogel), and whole SARS-CoV-2 antigen, and the vaccine group received different vaccine doses (different antigen concentrations) based on 50% tissue culture infectious dose ($TCID_{50}$) that indicate the amount of virus needed to infect 50% of a cell culture based on the dilution at which 50% of the wells show signs of infection (plaque formation). The vaccine group received 10^6 $TCID_{50}$, 10^7 $TCID_{50}$, and 10^8 $TCID_{50}$ SARS-CoV-2 inactivated vaccines (adjuvanted with 0.3 mg alhydrogel). Each respective compound was administered through intramuscular injection in the mice's femoral muscle, with a volume of 0.1 mL. Two injections were performed on day 0 (1st dose) and day 14th (2nd dose) of the study (Turner *et al*., 2011).

Weight Measurement

To assess the mice's weight, individual measurements were taken using a calibrated analytical scale (Fujitsu, Japan) at regular intervals during the study (Oli *et al*., 2016). This scale allowed the monitoring of any changes in body weight as an indicator of general health and response to the vaccine and challenge.

Intranasal SARS-CoV-2 Challenge

On the 28th day of the study were the mice intranasally challenged with 1×10^5 $TCID_{50}$ live SARS-CoV-2 virus to simulate a real-world infection scenario and assess the vaccine's safety

and immune response (Moreau *et al*., 2020). The mice were humanely sacrificed using approved methods at the end of the study. The intracardiac puncture was performed to collect blood samples for serum cytokines analysis. The samples were processed to obtain serum and then stored at -80°C until further analysis (Ruiz *et al*., 2016).

Immune Activation and Pro-Inflammatory Cytokine Measurement

To measure the levels of IL-4, IL-6, TNF-α, and IFN-γ in the mice's serum, an Enzyme-Linked Immunosorbent Assay (ELISA) was performed according to manufacture manufacturer-supplied protocol of ELISA Kit. Blood samples were collected on days 0, 14, 28, 31, 33, and 35 through intracardiac puncture procedures. The blood samples were then centrifuged to separate the serum. ELISA kit (Biolegend, USA) was used to measure each cytokine concentration of IL-4, IL-6, TNF-α, and IFN-γ in the mice's serum. Briefly, the serum samples were diluted with PBS appropriately and added to the wells of microplate coated with specific antibodies against respective analyte and incubated.

After incubation and washing steps, a secondary antibody conjugated with an enzyme was added to the wells. Following the secondary antibody incubation and washing, a substrate solution was added, which resulted in substrate color change. The reaction was stopped using a stopper solution after substrate incubation time. The intensity of the color changes was measured using a microplate reader in 450 nm wavelength absorbance (Biobase), and the optical density-concentration standard curve was calculated before correlating with the optical density in the serum samples well (Stadlbauer *et al*., 2020).

Data Analysis

Data were analyzed using a one-way analysis of variance followed by a Duncan post-hoc test or Games-Howell post-hoc test with Prism Version 10.0. Results were expressed as mean ± standard deviation. If p was less than 0.05, it was considered significant.

RESULTS AND DISCUSSION

Mice Weight

Regarding body weight, both the Control and Vaccine groups showed a gradual increase before the challenge with live SARS-CoV-2 virus. A significant decrease in body weight post-challenge was observed in the control group compared to the vaccine group (Figure 1). This study demonstrated a protective effect against SARS-CoV-2 infection-induced weight loss in the vaccine group compared to the control group. Weight loss is the risk of infection, and disease progression is one of the clinical manifestations that could be evaluated during the vaccine development, as reported in a previous study regarding SARS-CoV-2 vaccine and animal model development (Moreau *et al*., 2020). Previous studies of vaccine efficacy also evaluated protection against weight loss (Johnson *et al*., 2023; Pérez *et al*., 2023).

The present study aimed to evaluate the effects of a SARS-CoV-2 inactivated vaccine candidate on K18-hACE2 mice, focusing on changes in body weight, as well as immune activation and inflammatory cytokines i.e., IL-4, IL-6, TNF-α, and IFN-γ (Soto *et al*., 2022). The mice were divided into two groups: the control group, which received NaCl, adjuvant, and antigen, and the vaccine group, which received different doses of vaccine antigen (1×10^6 $TCID_{50}$, 1×10^7 $TCID_{50}$, and 1×10^8 $TCID_{50}$). Measurements were performed on the 0, 14^{th}, 28^{th}, 31^{st}, 33^{rd}, and 35^{th} days of the study. The results were compared with those of similar studies conducted by Sinovac, Sinopharm, and Bharat Biotech to establish a relative baseline for the observed outcomes (Gao *et al*., 2020; Wang *et al*., 2020; Yadav *et al*., 2021).

Both the Control and Vaccine groups exhibited fluctuations in body weight over time and similar trends without a significant difference. However, it was observed that the mice in the vaccine group generally maintained a stable weight, while the control group showed a decrease in weight after challenge with intranasal 1×10^5 $TCID_{50}$ SARS-CoV-2 (Moreau *et al*., 2020). This suggests that SARS-CoV-2

inactivated vaccine exhibits a protective effect against SARS-CoV-2 infection-induced weight loss in K18-hACE2 mice. These findings were similarly reported in a previous study (Yinda *et al*., 2021).

Inflammatory Cytokines

The analysis of immune activation cytokines IL-4 in the serum samples demonstrated insignificant differences between the control and vaccine groups during the entire period of study (Figure 2). The analysis of IL-4 immune activation cytokine showed an insignificant difference between vaccine and control group (IL-4). These results might suggest that IL-4 responses were not stimulatedin both pre-challenge and post-challenge because of several mechanisms. IL-4 as an immune activation cytokine plays an important role in allergy and atopy; while maintenance of baseline level of IL-4 might support vaccine-relative safety in the scope of allergenicity and provide information regarding the potential of vaccine-induced hypersensitivity reaction in animal or clinical trials (Arora *et al*., 2021).

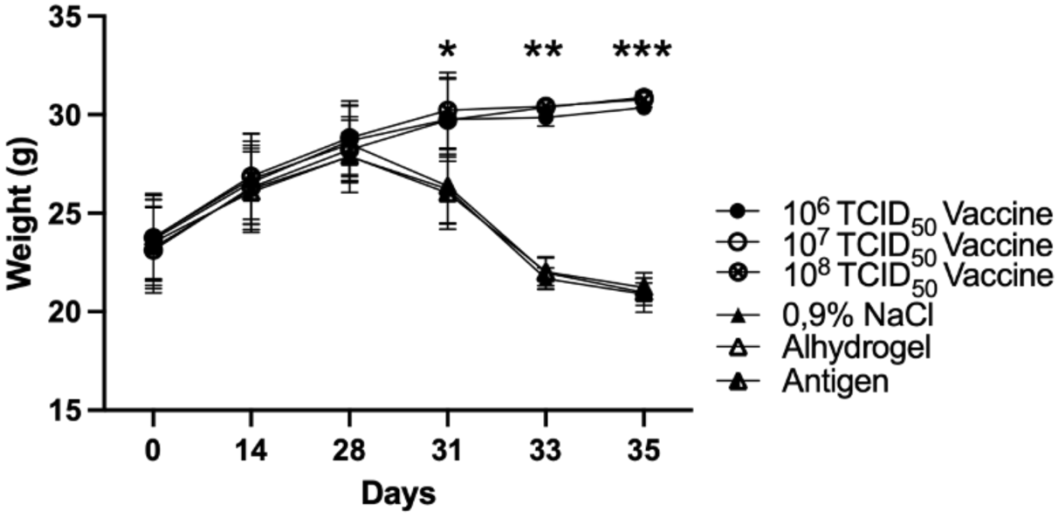

Figure 1. The body weight of mice during day of treatments.

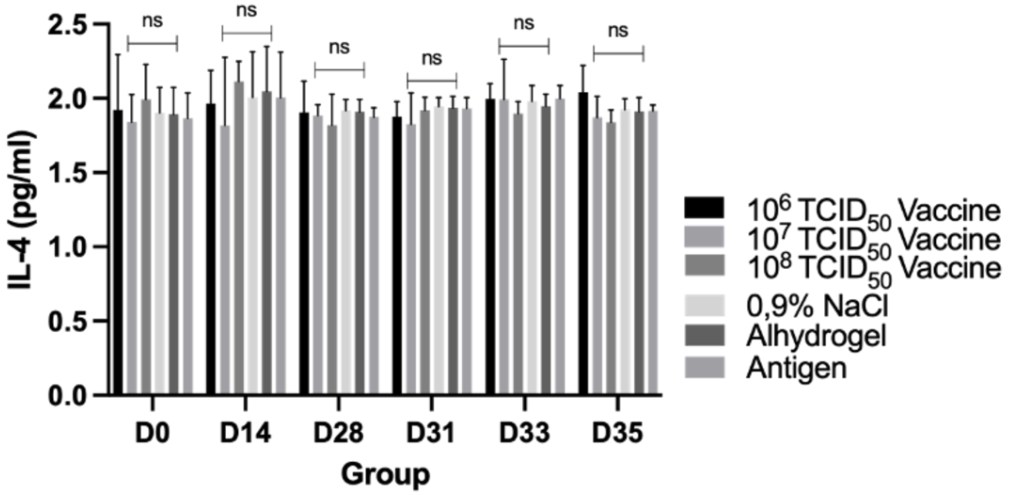

Figure 2. Level of IL-4 observed in the vaccine group compared to the control group.

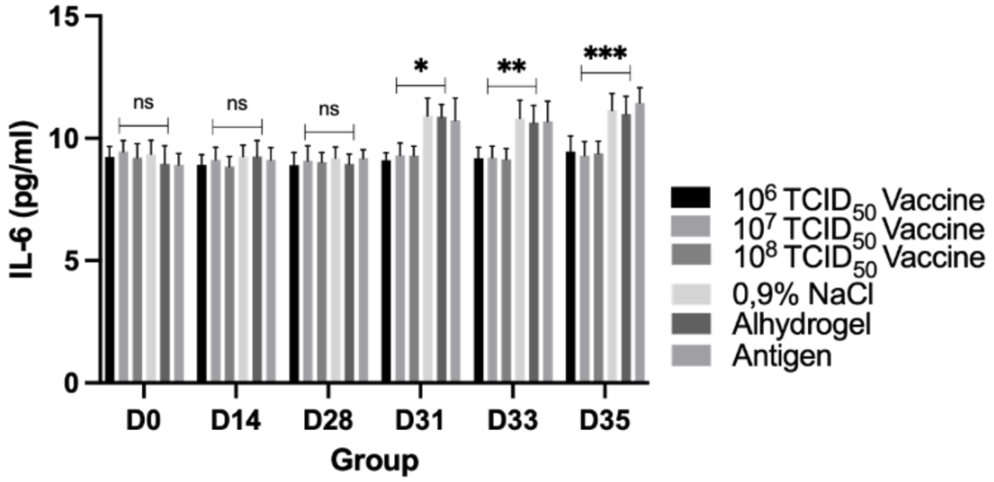

Figure 3. Level of IL-6 observed in the vaccine group compared to the control group.

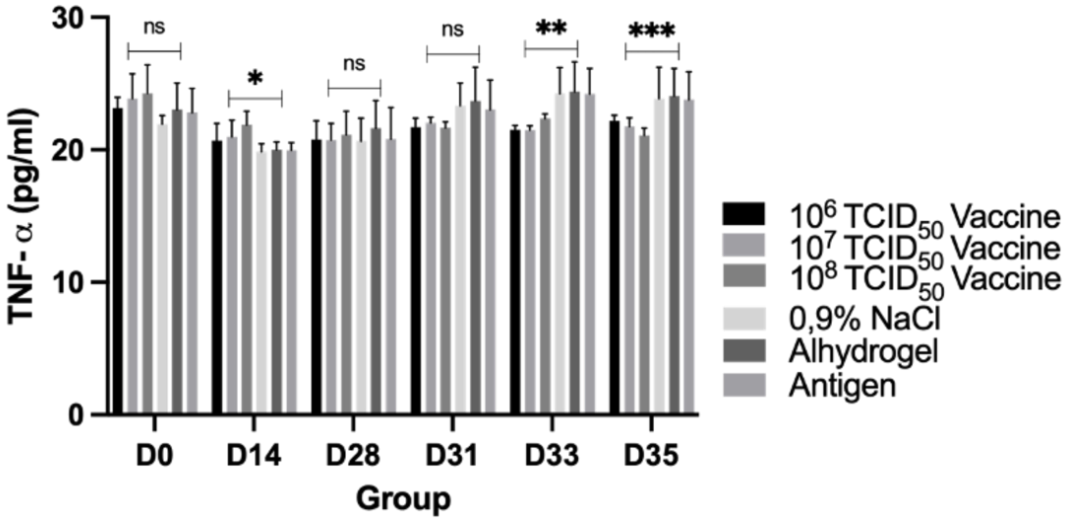

Figure 4. Level of TNF-α observed in the vaccine group compared to the control group.

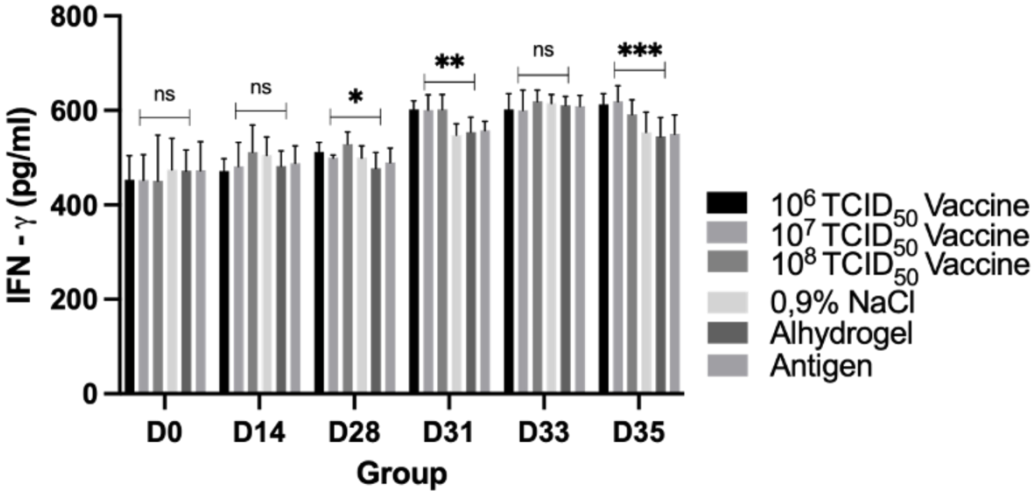

Figure 5. Level of INF-γ observed in the vaccine group compared to the control group.

The analysis of inflammatory cytokines IL-6 in the serum samples demonstrated a relatively similar trend before the challenge. A notable difference between the control and vaccine groups was observed in the post-challenge (on the 31st day, 33rd day, and 35th day). A significant

increase in IL-6 levels in the control group followed the viral challenge. Conversely, the vaccine group exhibited significantly lower levels of IL-6 (Figure 3). The levels of IL-6 were measured using ELISA and showed relatively similar IL-6 serum levels in the entire group before the challenge. The results suggest that the vaccination did not induce an inflammation response through IL-6 inflammatory signaling pathway. These findings were similar to those in the previous study regarding the SARS-CoV-2 inactivated vaccine. However, after the intranasal 1×10^5 $TCID_{50}$ SARS-CoV-2 challenge, the control group exhibited significantly elevated levels of IL-6 compared to the vaccine group. This suggests that the vaccine could potentially modulate the immune response, leading to lower levels of pro-inflammatory cytokines in the serum of vaccinated mice (Solfaine *et al*., 2024[a]). Various mechanisms might explain why the vaccine group exhibited lower levels of IL-6, including the vaccine-modulated immune response that led to a reduction in IL-6 production, vaccine-induced neutralizing antibodies that neutralized the virus before entry to host cells, and rapid clearance of viral-infected cells by the vaccine-trained immune response. However, it is important to note that further research and analysis would be needed to fully understand the specific mechanism for the observed reduction in IL-6 levels in the vaccine group (Natekar *et al*., 2022).

The analysis of inflammatory cytokines TNF-α in the serum samples showed insignificant differences between the control and vaccine groups on day 0 (before vaccination). Meanwhile, a significant increase in TNF-α post vaccination on day 14th ultimately returned to be relatively similar on day 28th. On the other hand, post-challenge TNF-α significantly increased on day 33rd and 35th in the control group without any significant difference on day 31st (Figure 4). TNF-α evaluation showed an increase in the TNF-α level during vaccination in the vaccine group compared to the control group on day 14th (pre-challenge), possibly indicating vaccine-induced inflammation with relatively common vaccine-induced fever and other mild reactions which came back to be relatively similar on day 28th. On the other hand, post-challenge TNF-α analysis demonstrated opposite characteristics of TNF-α on day 31st. An insignificant difference in TNF-α was found between groups. A relatively higher TNF-α was identified in the control group, followed by more significantly higher TNF-α on days 33rd and 35th (Gimenes *et al*., 2022).

The analysis of immune activation cytokines (IFN-γ) in the serum samples demonstrate similar trends between the control and vaccine groups before the challenge on day 0 and day 14th. However, a significant decrease in IFN-γ levels was detected on the 28th day before the challenge. Observation of post-challenge IFN-γ showed that an insignificant difference in IFN-γ between the groups was found; nevertheless, a higher IFN-γ was observed in the control group. Followed by days 33rd and 35th days, the control group had significantly higher IFN-γ than the vaccine group (Figure 5). Analysis of IFN-γ showed that IFN-γ was relatively higher in the vaccine group during vaccination on day 28th. Post-challenge analysis showed that lower IFN-γ was observed in the control group on day 31st, followed by a relatively similar IFN-γ level on day 33rd, and eventually became significantly lower in the control group on day 35th (Yinda *et al*., 2021). Changes in IFN-γ levels might suggest that a relatively sustained antiviral response and adequate resolve of IFN-γ are needed to combat SARS-CoV-2 infection and protect from weight loss (Solfaine *et al*., 2024[b]).

The SARS-CoV-2 inactivated vaccine candidate showed promising effects on K18-hACE2 mice, as supported by the protection from SARS-CoV-2, body weight reduction; relatively stable IL-4 levels; stabilization of IL-6 and TNF-α post-challenge serum levels with IL-6 level changes in the serum after vaccination and maintenance of body weight, and reduced levels of IL-6 after challenge in the vaccine group (Hamid *et al*., 2022). These findings were in line with previously reported studies conducted by Sinovac, Sinopharm, and Bharat Biotech, further supporting the potential efficacy of finding a vaccine candidate. However, future research is needed to validate these results such as assessing the long-term protective effects of the vaccine.

Further investigations are required to elucidate the underlying mechanisms and to assess the long-term efficacy and safety of the vaccine candidate. Nonetheless, these findings contribute to the understanding of the vaccine's potential in combating SARS-CoV-2 and highlight the vaccines during the ongoing pandemic.

CONCLUSION

Protective effect against SARS-CoV-2 induced weight loss, protection against increasing inflammatory cytokines (IL-6 dan TNF-α), fast increase and decreases in IFN-γ levels as rapid antiviral immune response and controlled IL-4 levels. This study suggested that the SARS-CoV-2 inactivated vaccine candidate can attenuate the hyperthermic response and modulate inflammatory cytokine production in k18-hACE2 mice. The results also mention the vaccine's ability to elicit an immune response and potentially provide protection against SARS-CoV-2 infection.

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AUTHORS' CONTRIBUTIONS

FAR and JR: Conceived and designed the study. RA, AYW, HS, DD, and AA: Collected samples. RA, AYW, HS, and SK: Performed the experimental works. FAR, RA, and AYW: Analysed and interpreted the data. FAR, RA, AYW, DD, LTS, BSL, ISY, BS: Drafted and revised the manuscripts. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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