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A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol

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ABSTRACT

Introduction: Presently, there are no standardised strategies to address SARS-COV-2 infection except preventative measures such as vaccination. Micronutrient deficiency, particularly vitamin D and zinc deficiency, has been associated with dysregulated host responses, and may play an important role in COVID-19.

Methods and analysis: We have designed a 2×2 factorial, randomized, double-blind, multi-centre placebo-controlled trial to evaluate the effect of vitamin D and zinc on COVID-19 outcomes in Maharashtra, India. COVID-19 positive individuals are recruited from hospitals in Mumbai and Pune. Participants are provided (1) vitamin D3 bolus (180,000 IU) maintained by daily dose of 2000 IU, and/or (2) zinc gluconate (40 mg daily), versus placebo for 8 weeks. Participants undergo a detailed assessment at baseline and at 8 weeks, and are followed up daily in hospital or every three days after leaving the hospital to monitor symptoms and other clinical measures. A final follow up telephone call occurs 12 weeks post-enrolment to assess long-term outcomes. The primary outcome of the study is to time to recovery, defined as time to resolution of all of fever, cough and shortness of breath. Secondary outcomes include: duration of hospital stay, all-cause mortality, necessity of assisted ventilation, change in blood biomarker levels, and individual symptoms duration. Participant recruitment commenced on April 2021.

Ethics and dissemination: Ethical approval was obtained from institutional ethical committees of all participating institutions. The study findings will be presented in peer-reviewed medical journals.

Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060

STRENGTHS AND LIMITATIONS OF THIS STUDY

- To our knowledge, this is the first factorial trial designed primarily to assess the effect of vitamin D (high-bolus dose maintained by daily doses) and zinc gluconate in COVID-19. A few other trials have been based in South Asia – this is key given the notable recent burden of COVID-19 and high prevalence of micronutrient deficiency in this region.
- The randomized, doubled-blind, placebo-controlled design of this trial will enable a better understanding of the role of vitamin D and zinc in COVID-19, informing relevant recommendations and action. The location of the study in two large cities in India will facilitate more generalizable results.
- With frequent follow up of participants, this study collects information across a range of domains including sociodemographic and clinical measures, and biomarker data, which will allow for a detailed investigation of the effect of supplementation on disease progression.
- This study is powered to detect a modest effect (25-30%) of either treatment on the primary outcome. One limitation to the study design is that with the current sample size, the statistical power to detect modification of the effects of each supplement by other factors may be limited.

INTRODUCTION

COVID-19 continues to be a problem globally, with over 16 million incident cases and 200,000 deaths reported in November 2021.[1] Concerted global efforts have resulted in the development of vaccines, which may reduce the burden and impact of COVID-19, although suboptimal vaccine coverage and the rapid mutation of the virus continue to prolong the pandemic.[2–5] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

Observational and experimental evidence link vitamin D to an array of communicable and non-communicable diseases.[7] Vitamin D deficiency (VDD; serum vitamin D <20 ng/ml) [8] is common in urban and rural India despite the country's sunny climate, due to environmental, sociological, and biological factors,[9] including skin pigmentation and cultural practices related to clothing and sun exposure. Countrywide studies suggest VDD may affect at least 70% of the Indian population. Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[10–14] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[15] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[16]

Zinc is an essential mineral that plays critical roles in gene expression, cell division, and immunity.[17] In India, dietary predominance of micronutrient-sparse staples, limited consumption of animal foods, and high consumption of zinc absorption inhibitors render the population at extremely high risk of inadequacy, which is exacerbated due to global climate change.[18] About 25% of the Indian population is zinc inadequate, and 4.3 million child deaths (<5 years) were attributable to zinc deficiency in 2017.[19] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[16,20–23] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[24] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[25] who note that Zn^{2+} cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[26]

Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India.

OBJECTIVES

The primary objectives of this trial are**:**

- To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19
- To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19

Secondary objectives include**:**

- To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration
- To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers

METHODS AND ANALYSIS

Trial design, population, and enrolment sites

This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (**Figure 1**). Maharashtra has the highest cumulative number of COVID-19 cases and fatalities out of all states in India.[27] Within the state, both Pune and Mumbai have emerged as COVID-19 hotspots.[28,29]

The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services. The trial is targeting a sample size of 700; participant recruitment commenced in April 2021. While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[30]

Eligibility criteria

The original inclusion criteria for this study were as follows: (1) men and women aged ≥18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO2) ≥90, and (4) written informed consent.

The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.

To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use.

Study procedures

An overview of trial procedures is summarised in **Figure 2**.

Recruitment and obtaining informed consent

Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. As part of the process, potential participants are informed that their participation is completely voluntary and they can withdraw any time at any stage of the study without providing any reasons. The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.

Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.

Baseline data and sample collection

Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows:

- Screening and background: the initial screening form is extended to collect information including participants' demographic background, socio-economic status, and health and prevention behaviours (smoking and drinking)
- Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in India and has been adapted to the Maharashtra context
- Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 vaccination status, COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications, complications, and medical history

A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described above.

Randomization and blinding

Participants are assigned randomly to one of four groups: (1) vitamin D, (2) zinc, (3) vitamin D and zinc, or (4) placebo. Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic. Extra codes were generated to account for unforeseen circumstances such as lost supplements, or abrasion of labels.

Intervention

Patients are randomized to one of four groups:

1. Placebo-Placebo group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily vitamin D3 maintenance doses and *placebo* daily zinc supplements
2. Vitamin D-Placebo group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and daily *placebo* zinc supplements
3. Placebo-Zinc group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily vitamin D3 maintenance doses and *actual* daily zinc supplements (40 mg daily)

4. Vitamin D3-Zinc group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and *actual* daily zinc supplements (40 mg daily)

A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[16]

Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count.

Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India) with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited, Mumbai, India).

All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell.

Study outcomes and follow up

Following baseline assessment and provision of supplements, participants are regularly followed up as described below:

- Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants
- Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is conducted in a follow up call every three days after leaving the hospital for all participants
- 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible)
- 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms

All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.

The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These and additional symptoms are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline.

Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above.

A list of collected data and blood investigations with time points at baseline, during follow up visits or calls, and at 8 and 12 weeks is summarized in **Table 1**.

Table 1. Collection of data points in the trial.

Data category	Baseline (enrolment)	Follow up	8 weeks	12 weeks
Demographic and background information	Age, gender, education, marital status, occupation, socio-economic status, health and prevention behaviours, COVID-19 vaccination			
Dietary information	Food frequency questionnaire: consumption frequency of 25 diverse food groups in last three months			
Clinical examination	Medical history, comorbidities, preadmission medications, clinical symptoms	**Hospital and telephone follow up:** Clinical symptoms	Medical history, comorbidities, pre-assessment medications, clinical symptoms	Clinical symptoms
Clinical measurements	Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight and height	**Hospital follow up only:** Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight, requirement for non-invasive ventilation or intubation/ventilator support, need for dialysis, lab investigations	Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight and height	
Blood and other investigations and biomarkers	SARS-COV-2 RT-PCR, chest X-Ray, complete blood count, blood glucose, serum creatinine, CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1		CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1	
Other information		**Hospital and telephone follow up:** Compliance, adverse events		

SpO2: Oxygen saturation, CRP: C-reactive protein, LDH: lactate dehydrogenase, IgG: Immunoglobulin G, IgM: immunoglobulin M, Ang2: angiopoietin-2, IL-6: interleukin 6, sTREM-1: soluble triggering receptor expressed on myeloid cells-1.

Adverse events and reporting

Any undesirable circumstance or experiences reported by study participants during the study are categorised as adverse events. All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant. Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events.

Data and sample management

All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.

Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored securely at the Foundation for Medical Research for a maximum of three years.

Data analysis

Planned analyses

An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared between participants randomized to vitamin D vs. placebo and zinc vs. placebo using Cox regression. We will investigate effect modification of either treatment effect by the other, and by third variables collected at baseline (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. There are no *a priori* effect modifiers hypothesized, and unless there is strong modification of a treatment effect, our power to detect these may be low. We will assess the success of randomization by comparing baseline variables by treatment group using $\chi 2$ and t-tests and use multivariate modelling to adjust for imbalances if needed.

The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach. The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using $\chi 2$ tests,

and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects.

Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.

Statistical power calculations

With a single endpoint for both interventions, the factorial design does not provide a "two-for-one" power advantage.[33] Power will decrease if each treatment has a moderate effect; we accounted for this in calculating the sample size. Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[15,16,20–23] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times.[34] We calculated power for detecting specified hazard ratios associated with vitamin D or zinc given a specified true effect of the other treatment. Assuming average time to recovery of 22.2 days,[35] and a low (5%) rate of loss to follow-up, enrolment of 700 patients will yield the statistical power estimates in the **Table 2**.This analysis indicates that we will have at least 80% power to detect a moderate (25-30%) effect of either treatment, given a maximum 30% true effect of the other treatment.

Table 2. Statistical power estimation.

	True effect of Treatment B						
Effect of Treatment A	**0%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
30%	99%	99%	99%	99%	98%	98%	97%
25%	95%	94%	93%	92%	90%	88%	86%
20%	81%	79%	76%	74%	71%	69%	66%

Patient and public involvement

Patients and the public were not involved in the design of this study.

DATA AND SAFETY MONITORING BOARD

The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings.

The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]

ETHICS AND DISSEMINATION

This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027). The trial is registered on ClincialTrials.gov (NCT04641195). Permission for the study was also obtained from the Health Management and Screening Comments (HMSC), Government of India (HMSC (GOI)-2021-0060), and the study was registered prospectively in the Clinical Trials Registry India (CTRI/2021/04/032593). Since the study intervention is related to micronutrient supplementation, endorsement from the Drugs Controller General of India was non-obligatory. The study findings will be presented in peer-reviewed medical journals.

DISCUSSION

With continued high incidence of global cases, COVID-19 remains a global health challenge. Alongside vaccination and other preventative measures, low-cost and efficient interventions which may help minimize the occurrence of serious disease are needed. These would be particularly valuable in low- and middle-income countries, where health systems are more overburdened and resources much fewer. In this context, and given previous evidence regarding the role of vitamin D and zinc in the development of and recovery from respiratory infections,[15,16,20–23] there is a need to explore their potential value as part of therapeutic regimens for COVID-19.

We report here the protocol of a 2x2 factorial randomized controlled trial, designed to generate evidence on the effect of vitamin D and zinc on COVID-19 progression. The frequent follow up of participants and collection of a range of sociodemographic, clinical and biomarker measures alongside blood samples will enable a detailed investigation of the effect of supplementation on disease progression, including potentially important immunological and inflammatory pathways. Importantly, in comparison with other vitamin D or zinc COVID-19 intervention studies currently registered on ClinicalTrials.gov, this would be the first conducted outside of the U.S. or Europe and other similar high-income countries. The location of this study in two large cities, alongside the broad eligibility criteria, increases the generalizability of study results. Given the current unpredictability of COVID-19 waves, one challenge to the study is to maintain recruitment during periods where cases may be on the decline. We have taken steps to mitigate any anticipated effects of this, including broadening our eligibility criteria as described previously, and rigorous training of site hospital staff to help improve recruitment of eligible individuals. Regardless, the findings of this study will have direct relevance to many settings in South Asia and sub-Saharan Africa with weak health systems and prevalent malnutrition. Ultimately, the evidence generated as part of this trial will enhance our understanding of the role of vitamin D and zinc in COVID-19 disease, and contribute high quality evidence on the potential value of supplementation of these micronutrients for the same.

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AUTHOR CONTRIBUTIONSs

WWF, KCK, YD, and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP. YD, NM, PDC, GG, KKS, YM, and SS are involved in data acquisition, and in study monitoring along with KCK, WWF and UP. MW provides statistical expertise. KKS and UP drafted the manuscript, and all authors reviewed and critically revised the draft and approved the final manuscript.

COMPETING INTERESTS

All authors declare no conflicts of interest.

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FIGURE LEGENDS

Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.

Map created with mapchart.net.

Figure 2: Overview of trial procedures. RAT: Rapid Antigen Test.

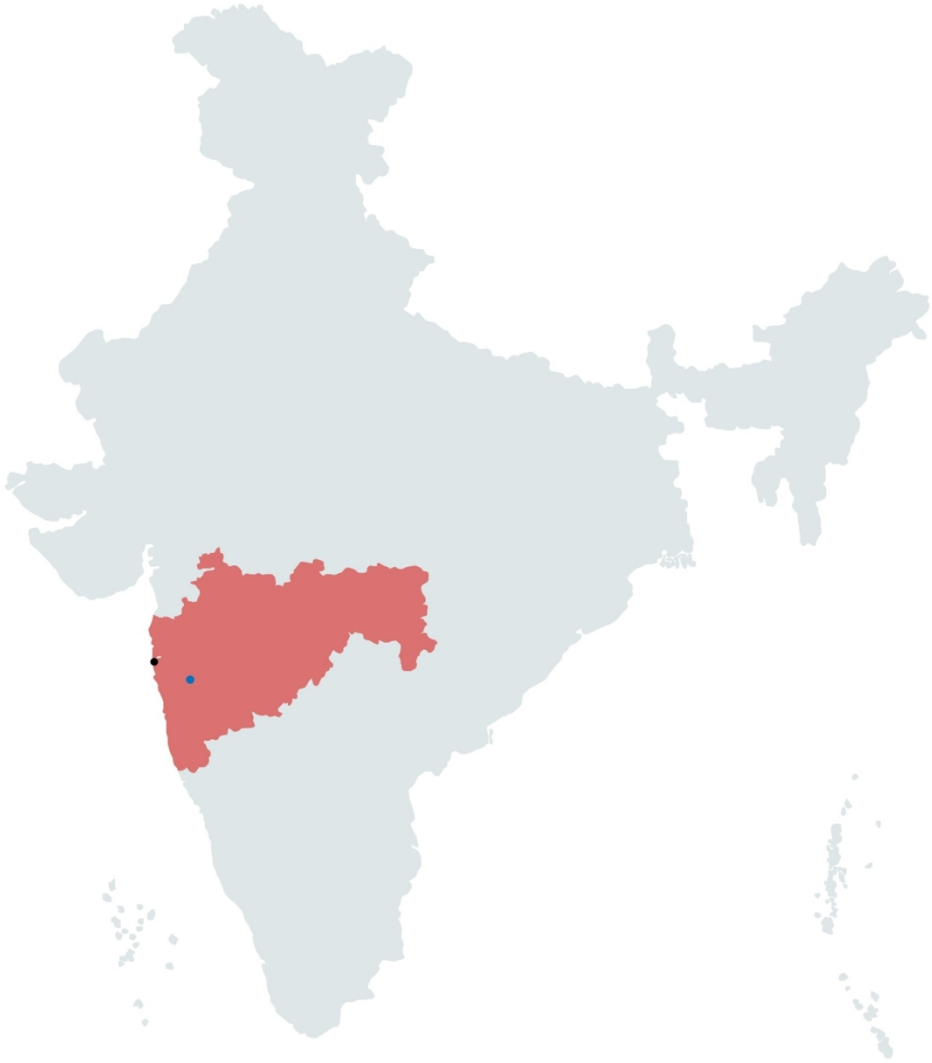

Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.
Map created with mapchart.net.

156x188mm (300 x 300 DPI)

COVID-19 patients in the hospital

Screening for eligibility

↓

Enrolment and informed consent obtained

Baseline information is obtained before allocation of supplements

↓

Randomization

(Group allocation and labelling of supplements done prior to trial initiation to maintain blinding at all levels)

(1) **Vitamin D Group** (180,000 IU bolus at enrolment, followed by 2000 IU daily)	(2) **Zinc Group** (placebo at enrolment followed by daily dose of 40 mg)	(3) **Vitamin D and zinc group** (180,000 IU vitamin D bolus at enrolment followed by daily dose of vitamin D- 2000 IU and zinc - 40 mg)	(4) **Placebo** (Placebo at enrolment and daily placebo)

Duration of intervention:

1. **Daily Supplements**: 8 weeks
2. **Bolus**: Once at enrolment

Follow up assessment:

Patient advised for home isolation (outpatients) • Direct to telephone follow up	Hospitalized patients (inpatients) • Daily hospital follow ups to discharge

Telephone follow up to 8 weeks (follow up calls every 3 days)

Final 8-week clinical assessment visit

12-week follow up for assessing any long COVID symptoms

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Title page (p1): "A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol"
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Abstract (p2): "Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060"
	2b	All items from the World Health Organization Trial Registration Data Set Manuscript: Items from the WHO Trial Registration Data Set (including elements such as trial registration, financial support, study contacts, study title, countries of recruitment and details on design and recruitment status) are noted throughout the manuscript.
Protocol version	3	Date and version identifier NA: This is a manuscript of a study protocol.
Funding	4	Sources and types of financial, material, and other support Funding (p14): "This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication."
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Author contributions (p14): "KCK, YD, WWF and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP."

5b Name and contact information for the trial sponsor
The name of the study sponsor is included in the clinical trial registration records (NCT04641195, CTRI/2021/04/032593).

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
Funding (p14):
"This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication."

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
NA

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Introduction (p4-5): "[…] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1] […] Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. […] In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[10–14] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[15] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[16] […] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[16,20–23] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[24] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[25] who note that Zn2+ cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[26] Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India."

	6b	Explanation for choice of comparators Methods and analysis // Study procedures // Intervention (p8): “A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[16]”
Objectives	7	Specific objectives or hypotheses Objectives (p5): “The primary objectives of this trial are: • To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19 • To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19 Secondary objectives include: • To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration • To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers”
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Methods and analysis // Trial design, population and enrolment sites (p5): “This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1).”

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Methods and analysis // Trial design, population and enrolment sites (p5): "This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1). […] The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services."
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) Methods and analysis // Eligibility criteria (p6): "The original inclusion criteria for this study were as follows: (1) men and women aged ≥18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO2) ≥90, and (4) written informed consent. The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month. To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use.

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Methods and analysis // Study procedures // Intervention (p7-8): Patients are randomized to one of four groups: 1. Placebo-Placebo group will receive a placebo vitamin D3 bolus at the hospital followed by placebo daily vitamin D3 maintenance doses and placebo daily zinc supplements 2. Vitamin D-Placebo group will receive an actual vitamin D3 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3 maintenance doses (2000 IU daily) and daily placebo zinc supplements 3. Placebo-Zinc group will receive a placebo vitamin D3 bolus at the hospital followed by placebo daily vitamin D3 maintenance doses and actual daily zinc supplements (40 mg daily) 4. Vitamin D3-Zinc group will receive an actual vitamin D3 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3 maintenance doses (2000 IU daily) and actual daily zinc supplements (40 mg daily) A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[16] Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count. Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India) with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited, Mumbai, India). All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell.

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)

Methods and analysis // Adverse events and reporting (p11):
"All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant."

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)

Methods and analysis // Study procedures // Intervention (p8):
"Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count."

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial

Methods and analysis // Study procedures // Intervention (p8):
"All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell."

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended Methods and analysis // Study procedures // Study outcomes and follow up (p9): "The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These and additional symptoms are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline. Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above."
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) See Methods and analysis // Study procedures section (p6) for details on enrolment, intervention, and follow up. Table 1 and Figure 2, referred to in this section, also outline the sequence and schedule of enrolment and follow up.

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations Methods and analysis // Data analysis // Statistical power calculations (p12) and Table 2: "Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[15,16,20–23] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times.[34] We calculated power for detecting specified hazard ratios associated with vitamin D or zinc given a specified true effect of the other treatment. Assuming average time to recovery of 22.2 days,[35] and a low (5%) rate of loss to follow-up, enrolment of 700 patients will yield the statistical power estimates in the Table 2.This analysis indicates that we will have at least 80% power to detect a moderate (25-30%) effect of either treatment, given a maximum 30% true effect of the other treatment."
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Methods and analysis // Trial design, population and enrolment sites (p5): "While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[30]" Methods and analysis // Eligibility criteria (p6): "To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use." Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. […]"

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Methods and analysis // Study procedures // Randomization and blinding (p7): "For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Methods and analysis // Study procedures // Randomization and blinding (p7): "Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. […] Informed consent is obtained after responding to any raised queries." Methods and analysis // Study procedures // Randomization and blinding (p7): "For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Methods and analysis // Study procedures // Randomization and blinding (p7): “Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial Data and Safety Monitoring Board (p13): “The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]”

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Methods and analysis // Study procedures // Baseline data and sample collection (p7): "Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows: • Screening and background: the initial screening form is extended to collect information including participants' demographic background, socio-economic status, and health and prevention behaviours (smoking and drinking) • Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in India and has been adapted to the Maharashtra context • Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 vaccination status, COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications, complications, and medical history A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described above." Methods and analysis // Study procedures // Study outcomes and follow up (p8 – 9): "Following baseline assessment and provision of supplements, participants are regularly followed up as described below: • Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants • Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is conducted in a follow up call every three days after leaving the hospital for all participants • 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible) • 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms […] A list of collected data and blood investigations with time points at baseline, during follow up visits or calls, and at 8 and 12 weeks is summarized in Table 1." **(Please also refer to Table 1)**

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Methods and analysis // Study procedures // Intervention (p8): “Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance.”
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): “Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.” Methods and analysis // Study procedures // Baseline data and sample collection (p7): “All information is collected securely on electronic tablets, as described above.” Methods and analysis // Study procedures // Study outcomes and follow up (p8): “All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.” Methods and analysis // Data and sample management (p11): “All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Methods and analysis // Data analysis // Planned analyses (p11): "An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared between participants randomized to vitamin D vs. placebo and zinc vs. placebo using Cox regression. […] We will assess the success of randomization by comparing baseline variables by treatment group using χ2 and t-tests and use multivariate modelling to adjust for imbalances if needed. […] The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach."
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Methods and analysis // Data analysis // Planned analyses (p11-12): "We will investigate effect modification of either treatment effect by the other, and by third variables collected at baseline (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. […] The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects. Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders."

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) Methods and analysis // Data analysis // Planned analyses (p11): "An intent-to-treat analysis will be used as the primary analytic strategy."
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Data and Safety Monitoring Board (p12-13): "The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings. The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]"
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Data and Safety Monitoring Board (p13): "The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]"

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Methods and analysis // Adverse events and reporting (p11): "Any undesirable circumstance or experiences reported by study participants during the study are categorised as adverse events. All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant. Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events."
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor NA

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Abstract // Ethics and dissemination (p2): "Ethical approval was obtained from institutional ethical committees of all participating institutions." Ethics and dissemination (p13): "This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027)."

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) NA: As this is a manuscript of a study protocol, such detail has not been included in this specific document.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Methods and analysis // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. [..] The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference."
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Methods and analysis // Recruitment and obtaining informed consent (p6): "The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries."

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent." Methods and analysis // Study procedures // Baseline data and sample collection (p7): "All information is collected securely on electronic tablets, as described above." Methods and analysis // Study procedures // Study outcomes and follow up (p8): "All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above." Methods and analysis // Data and sample management (p11): "All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only."
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Competing interests (p15): "All authors declare no conflicts of interest."
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Methods and analysis // Data and sample management (p11): "All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately."

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Methods and analysis // Adverse events and reporting (p11): "Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events."
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Ethics and dissemination (p13): "The study findings will be presented in peer-reviewed medical journals."
	31b	Authorship eligibility guidelines and any intended use of professional writers NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code NA
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates NA: As this is a manuscript of a study protocol, such detail has not been included in this specific document.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Methods and analysis // Data and sample management (p11): "Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored securely at the Foundation for Medical Research for a maximum of three years."

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol

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A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol

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ABSTRACT

Introduction: Presently, there are few population-level strategies to address SARS-COV-2 infection except preventive measures such as vaccination. Micronutrient deficiency, particularly vitamin D and zinc deficiency, has been associated with dysregulated host responses, and may play an important role in COVID-19.

Methods and analysis: We have designed a 2×2 factorial, randomized, double-blind, multi-centre placebo-controlled trial to evaluate the effect of vitamin D and zinc on COVID-19 outcomes in Maharashtra, India. COVID-19 positive individuals are recruited from hospitals in Mumbai and Pune. Participants are provided (1) vitamin D3 bolus (180,000 IU) maintained by daily dose of 2000 IU, and/or (2) zinc gluconate (40 mg daily), versus placebo for 8 weeks. Participants undergo a detailed assessment at baseline and at 8 weeks, and are monitored daily in hospital or every three days after leaving the hospital to assess symptoms and other clinical measures. A final follow up telephone call occurs 12 weeks post-enrolment to assess long-term outcomes. The primary outcome of the study is to time to recovery, defined as time to resolution of all of fever, cough and shortness of breath. Secondary outcomes include: duration of hospital stay, all-cause mortality, necessity of assisted ventilation, change in blood biomarker levels, and individual symptoms duration. Participant recruitment commenced on April 2021.

Ethics and dissemination: Ethical approval was obtained from institutional ethical committees of all participating institutions. The study findings will be presented in peer-reviewed medical journals.

Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The setting of this study in India enables applicability of findings to the wider South Asia region, where evidence on this topic remains scarce despite a notable recent burden of COVID-19 and high prevalence of micronutrient deficiency.
- As a double-blind factorial randomized controlled trial, this study enables an efficient assessment of the effect of vitamin D and zinc on COVID-19 symptoms that is less prone to confounding and bias than other observational studies on this topic.
- With frequent follow up of participants, this study collects information across a range of domains including sociodemographic and clinical measures, and biomarker data, which will allow for a detailed investigation of the effect of supplementation on disease progression.
- One limitation to the study design is that with the current sample size, the statistical power to detect modification of the effects of each supplement by other factors may be limited.

INTRODUCTION

COVID-19 continues to be a problem globally, with over 16 million incident cases and 200,000 deaths reported in November 2021.[1] Concerted global efforts have resulted in the development of vaccines, which may reduce the burden and impact of COVID-19, although suboptimal vaccine coverage and the rapid mutation of the virus continue to prolong the pandemic.[2–5] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

Observational and experimental evidence link vitamin D to an array of communicable and non-communicable diseases.[7] Vitamin D deficiency (VDD; serum vitamin D <20 ng/ml) [8] is common in urban and rural India despite the country's sunny climate, due to environmental, sociological, and biological factors,[9,10] including skin pigmentation and cultural practices related to clothing and sun exposure. Countrywide studies suggest VDD may affect at least 70% of the Indian population. Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[11–15] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[16] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[17]

Zinc is an essential mineral that plays critical roles in gene expression, cell division, and immunity.[18] In India, dietary predominance of micronutrient-sparse staples, limited consumption of animal foods, and high consumption of zinc absorption inhibitors render the population at extremely high risk of inadequacy, which is exacerbated due to global climate change.[19] About 25% of the Indian population is zinc inadequate, and 4.3 million child deaths (<5 years) were attributable to zinc deficiency in 2017.[20] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[17,21–24] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[25] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[26] who note that Zn^{2+} cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[27]

Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India.

OBJECTIVES

The primary objectives of this trial are**:**

- To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19
- To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19

Secondary objectives include**:**

- To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration
- To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers

METHODS AND ANALYSIS

Trial design, population, enrolment sites, and time frame

This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (**Figure 1**). Maharashtra has the highest cumulative number of COVID-19 cases and fatalities out of all states in India.[28] Within the state, both Pune and Mumbai have emerged as COVID-19 hotspots.[29,30]

The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services. The trial is targeting a sample size of 700. The study commenced in April 2021 and study activities are expected to continue until July 2022. While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[31]

Eligibility criteria

The original inclusion criteria for this study were as follows: (1) men and women aged ≥18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO2) ≥90, and (4) written informed consent.

The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.

To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use. Since this change was made early, when few (<6% of target population) participants were enrolled in the trial, we anticipate that the majority of the final study population will have been enrolled under the updated, broader criteria.

Study procedures

An overview of trial procedures is summarised in **Figure 2**.

Recruitment and obtaining informed consent

Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. As part of the process, potential participants are informed that their participation is completely voluntary and they can withdraw any time at any stage of the study without providing any reasons. The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.

Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[32] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.

Baseline data and sample collection

Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows:

- Screening and background: the initial screening form is extended to collect information including participants' demographic background, socio-economic status, and health and prevention behaviours (smoking and drinking), and COVID-19 vaccination status
- Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in India and has been adapted to the Maharashtra context
- Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications including those prescribed for COVID-19, nutritional supplement use, complications, and medical history

A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described above.

Randomization and blinding

Participants are assigned randomly to one of four groups: (1) vitamin D, (2) zinc, (3) vitamin D and zinc, or (4) placebo. For randomization, a computer-generated list was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic. The randomization list assigns each participant randomization identifier (ID) to a regimen code, with the actual regimen known only to the manufacturer and accessible to the statistician in a currently unopened, sealed envelope. Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and all research staff including investigators remain blinded. At each site, each participant entering the trial is given the next available randomization ID, and is provided their corresponding regimen based on the assigned regimen code.

Intervention

Patients are randomized to one of four groups:

1. Placebo-Placebo group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily vitamin D3 maintenance doses and *placebo* daily zinc supplements
2. Vitamin D-Placebo group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and daily *placebo* zinc supplements
3. Placebo-Zinc group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily vitamin D3 maintenance doses and *actual* daily zinc supplements (40 mg daily)
4. Vitamin D3-Zinc group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and *actual* daily zinc supplements (40 mg daily)

We selected vitamin D3 as it has been shown to be more effective in raising and maintaining high levels of circulating 25(OH)D than vitamin D2 [33,34]. A bolus dose followed by daily doses was chosen to boost vitamin D levels quickly and safely within the first few days and maintain levels thereafter. Previous studies have indicated the efficacy of large oral doses (>200 000 IU bolus, and 1,700-2,000 IU per day) in increasing and sustaining blood 25(OH)D concentrations, with very low risk of side effects [35–41]. The 40 mg dosage of zinc is understood to be sufficiently high to assess efficacy, while remaining within the Institute of Medicine's tolerable upper intake level for adults [42]. A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[17]

Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are observed taking supplements daily while in hospital or contacted regularly via telephone after leaving the hospital to ensure compliance. Research staff identify barriers to compliance and aim to address these via appropriate counselling, and assess compliance at 8 weeks via direct questioning and pill count.

Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India) with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited, Mumbai, India).

All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell. Indian national guidelines have evolved during the pandemic, and currently consist of appropriate treatment (which may include oxygen support, respiratory support, anti-inflammatory or immunomodulatory therapy, and anticoagulation therapy) according to disease severity; discharge of admitted patients from the hospital upon resolution of symptoms and sufficient oxygen saturation (SpO2 > 93%) for three days; and self-monitoring during home isolation [43–45].

Study outcomes and follow up

Following baseline assessment and provision of supplements, participants are regularly followed up as described below and in **Table 1**:

- Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants. Any new prescribed medications and supplements are also recorded alongside other interventions such as need for non-invasive ventilation or dialysis. Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records.
- Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is conducted in a follow up call every three days after leaving the hospital for all participants. All information is self-reported by participants.
- 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, use of any other nutritional supplements, updates to COVID-19 vaccination status, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible). Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records.
- 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms, and any updates to COVID-19 vaccination status. All information is self-reported by participants.

All data are collected using standardized questionnaire forms on electronic tablets,[32] as described above.

The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These symptoms are most commonly reported among COVID-19 patients, including in Indian populations,[46,47] and have also been assessed as part of studies examining vitamin D and zinc in respiratory illnesses [17]. These and additional symptoms (including fatigue, headache, loss of smell and taste and sore throat) are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Data on symptoms are collected using the same structured questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how many days in total including today the participant has experienced X symptom. Staff conducting in-person and telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this

information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline.

Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, and blood biomarker levels, including 25-hydroxy vitamin D, zinc, and other immunological and inflammatory biomarkers (including interleukin 6, angiopoietin-2, soluble triggering receptor expressed on myeloid cells-1, immunoglobulin G and immunoglobulin M). Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above.A list of collected data and blood investigations with time points at baseline, during follow up visits or calls, and at 8 and 12 weeks is summarized in **Table 1**.

Table 1. Collection of data points in the trial.

Data category	Baseline (enrolment)	Follow up	8 weeks	12 weeks
Demographic and background information	Age, gender, education, marital status, occupation, socio-economic status, health and prevention behaviours, COVID-19 vaccination *(Self-reported by participant, assessed by staff or abstracted from participant record)*		COVID-19 vaccination *(Self-reported by participant)*	COVID-19 vaccination *(Self-reported by participant)*
Dietary information	Food frequency questionnaire: consumption frequency of 25 diverse food groups in last three months *(Self-reported by participant)*			
Clinical examination	Medical history, comorbidities, preadmission medications, non-intervention nutritional supplement use (*Self-reported by participant, assessed by staff or abstracted from participant record*) Clinical symptoms[1] *(Self-reported by participant)*	**Hospital and telephone follow up:** Clinical symptoms[1] *(Self-reported by participant)* **Hospital follow up only:** Changes in medications, changes in non-intervention nutritional supplement use *(Assessed by staff or abstracted from participant record)*	Medical history, comorbidities, pre-assessment medications, non-intervention nutritional supplement use *(Self-reported by participant, assessed by staff or abstracted from participant record)* Clinical symptoms[1] *(Self-reported by participant)*	Clinical symptoms[1] (*Self-reported by participant*)
Clinical measurements	Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight and height *(Assessed by staff or abstracted from participant record)*	**Hospital follow up only:** Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight, requirement for non-invasive ventilation or intubation/ventilator support, need for dialysis, lab investigations *(Assessed by staff or abstracted from participant record)*	Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight and height *(Assessed by staff or abstracted from participant record)*	
Blood and other investigations and biomarkers	SARS-COV-2 RT-PCR, chest X-Ray, complete blood count, blood glucose, serum creatinine, CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1		CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1 *(Assessed by laboratory or abstracted from participant record)*	

	(Assessed by laboratory or abstracted from participant record)		
Other information		**Hospital and telephone follow up:** Compliance, adverse events *(Self-reported by participant, assessed by staff or abstracted from participant record)*	Compliance (count of remaining pills) (*Assessed by staff*)

SpO2: Oxygen saturation, CRP: C-reactive protein, LDH: lactate dehydrogenase, IgG: Immunoglobulin G, IgM: immunoglobulin M, Ang2: angiopoietin-2, IL-6: interleukin 6, sTREM-1: soluble triggering receptor expressed on myeloid cells-1.

[1]Clinical symptoms include: fever, cough, shortness of breath, fatigue, headache, loss of smell, loss of taste, diarrhea, anorexia, sore throat, nasal congestion, nausea and vomiting, and any other reported by the participant.

1

Adverse events and reporting

Any undesirable circumstance or experiences reported by study participants during the study are categorised as adverse events. All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant. Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events.

Data and sample management

All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[32] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.

Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored securely at the Foundation for Medical Research for a maximum of three years.

Data analysis

Planned analyses will initially be undertaken in blinded fashion (comparing coded treatment groups); unblinding of investigators and research staff with respect to treatment allocation will only occur once analyses are completed.

Planned analyses

An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared between participants randomized to vitamin D vs. no vitamin D and zinc vs. no zinc using Cox regression. We will investigate effect modification of either treatment effect by the other, and by third variables collected at baseline (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. There are no *a priori* effect modifiers hypothesized, and unless there is strong modification of a treatment effect, our power to detect these may be low. We will assess the success of randomization by comparing baseline variables by treatment group using $\chi 2$ and t-tests and use multivariate modelling to adjust for imbalances if needed. Additional

collected information, including data on prescribed medications and other treatments, will enable an assessment of whether important factors including non-protocol interventions are balanced across intervention groups.

The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach. The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using $\chi 2$ tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[48] we will test and modify it if needed in the whole study population, adjusting for treatment effects.

Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.

Statistical power calculations

With a single endpoint for both interventions, the factorial design does not provide a “two-for-one” power advantage.[49] Power will decrease if each treatment has a moderate effect; we accounted for this in calculating the sample size. Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[17,21–24,50] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times, which assumes exponential distribution of the time to recovery.[51] We calculated power for detecting specified hazard ratios associated with vitamin D or zinc given a specified true effect of the other treatment. Assuming average time to recovery of 22.2 days,[52] and a low (5%) rate of loss to follow-up, enrolment of 700 patients will yield the statistical power estimates in the **Table 2**. This analysis indicates that we will have at least 80% power to detect a moderate (25-30%) effect of either treatment, given a maximum 30% true effect of the other treatment. We did not further adjust our power calculations and desired sample size following changes to our eligibility criteria, which may result in the inclusion of participants with symptoms that are both more severe (SpO2 <90) and less severe (outpatients) at baseline.

Table 2. Statistical power estimation.

	True effect of Treatment B						
Effect of Treatment A	**0%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
30%	99%	99%	99%	99%	98%	98%	97%
25%	95%	94%	93%	92%	90%	88%	86%
20%	81%	79%	76%	74%	71%	69%	66%

Patient and public involvement

Patients and the public were not involved in the design of this study.

DATA AND SAFETY MONITORING BOARD

The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings.

The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[53]

ETHICS AND DISSEMINATION

This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027). The trial is registered on ClincialTrials.gov (NCT04641195). Permission for the study was also obtained from the Health Management and Screening Comments (HMSC), Government of India (HMSC (GOI)-2021-0060), and the study was registered prospectively in the Clinical Trials Registry India (CTRI/2021/04/032593). Since the study intervention is related to micronutrient supplementation, endorsement from the Drugs Controller General of India was non-obligatory. The study findings will be presented in peer-reviewed medical journals.

DISCUSSION

With continued high incidence of global cases, COVID-19 remains a global health challenge. Alongside vaccination and other preventative measures, low-cost and efficient interventions which may help minimize the occurrence of serious disease are needed. These would be particularly valuable in low- and middle-income countries, where health systems are more overburdened and resources much fewer. In this context, and given previous evidence regarding the role of vitamin D and zinc in the development of and recovery from respiratory infections,[17,21–24,50] there is a need to explore their potential value as part of therapeutic regimens for COVID-19.

We report here the protocol of a 2x2 factorial randomized controlled trial, designed to generate evidence on the effect of vitamin D and zinc on COVID-19 progression. The frequent follow up of participants and collection of a range of sociodemographic, clinical and biomarker measures alongside blood samples will enable a detailed investigation of the effect of supplementation on disease progression, including potentially important immunological and inflammatory pathways. Importantly, in comparison with other vitamin D or zinc COVID-19 intervention studies currently registered on ClinicalTrials.gov, this would be the first conducted outside of the U.S. or Europe and other similar high-income countries. The location of this study in two large cities, alongside the broad eligibility criteria, increases the generalizability of study results. Given the current unpredictability of COVID-19 waves, one challenge to the study is to maintain recruitment during periods where cases may be on the decline. We have taken steps to mitigate any anticipated effects of this, including broadening our eligibility criteria as described previously, and rigorous training of site hospital staff to help improve recruitment of eligible individuals. Regardless, the findings of this study will have direct relevance to many settings in South Asia and sub-Saharan Africa with weak health systems and prevalent malnutrition. Ultimately, the evidence generated as part of this trial will enhance our understanding of the role of vitamin D and zinc in COVID-19 disease, and contribute high quality evidence on the potential value of supplementation of these micronutrients for the same.

FUNDING

This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication.

AUTHOR CONTRIBUTIONSs

WWF, KCK, YD, and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP. YD, NM, PDC, GG, KKS, YM, and SS are involved in data acquisition, and in study monitoring along with KCK, WWF and UP. MW provides statistical expertise. KKS and UP drafted the manuscript, and all authors reviewed and critically revised the draft and approved the final manuscript.

COMPETING INTERESTS

All authors declare no conflicts of interest.

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FIGURE LEGENDS

Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.

Map created with mapchart.net.

Figure 2: Overview of trial procedures. RAT: Rapid Antigen Test.

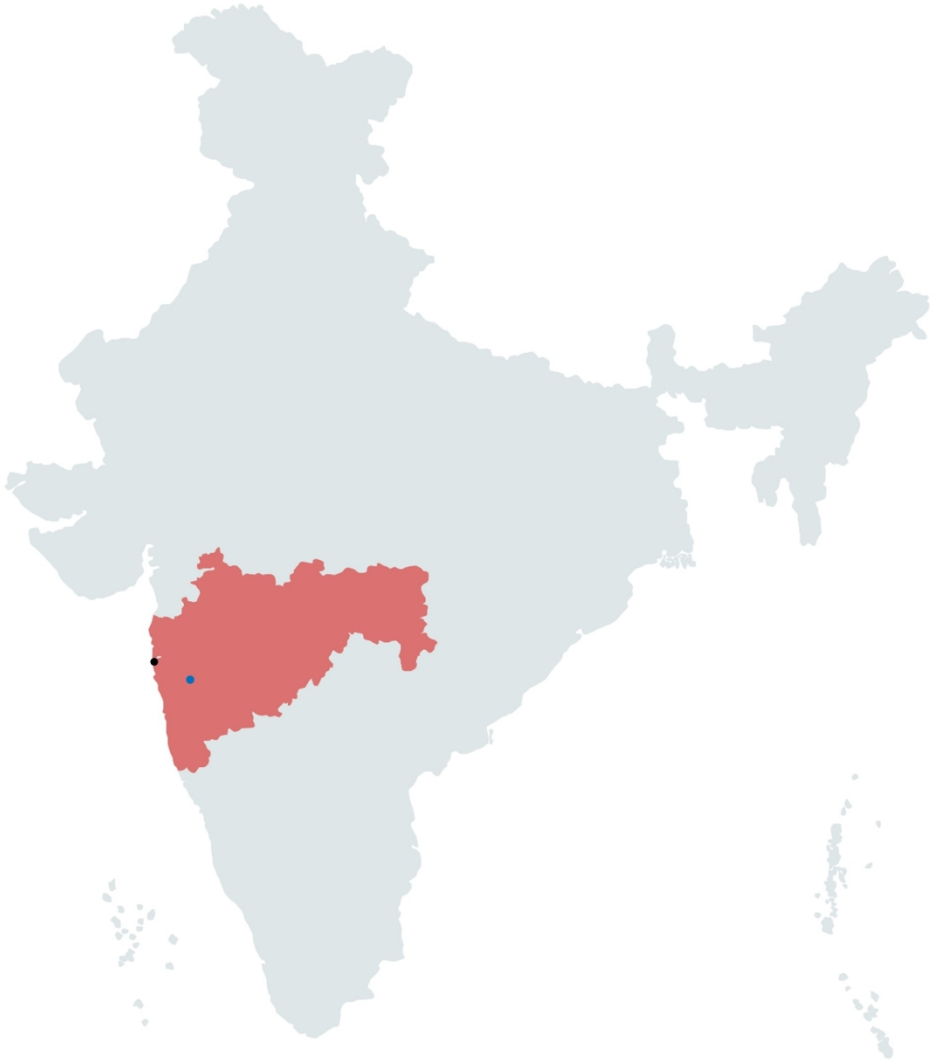

Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.
Map created with mapchart.net.

156x188mm (330 x 330 DPI)

COVID-19 patients in the hospital

Screening for eligibility

↓

Enrolment and informed consent obtained

Baseline information is obtained before allocation of supplements

↓

Randomization

(Group allocation and labelling of supplements done prior to trial initiation to maintain blinding at all levels)

(1) **Vitamin D Group** (180,000 IU bolus at enrolment, followed by 2000 IU daily)	(2) **Zinc Group** (placebo at enrolment followed by daily dose of 40 mg)	(3) **Vitamin D and zinc group** (180,000 IU vitamin D bolus at enrolment followed by daily dose of vitamin D- 2000 IU and zinc - 40 mg)	(4) **Placebo** (Placebo at enrolment and daily placebo)

Duration of intervention:

1. **Daily Supplements**: 8 weeks
2. **Bolus**: Once at enrolment

Follow up assessment:

Patient advised for home isolation (outpatients) • Direct to telephone follow up	Hospitalized patients (inpatients) • Daily hospital follow ups to discharge

Telephone follow up to 8 weeks (follow up calls every 3 days)

Final 8-week clinical assessment visit

12-week follow up for assessing any long COVID symptoms

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Title page (p1): "A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol"
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Abstract (p2): "Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060"
	2b	All items from the World Health Organization Trial Registration Data Set Manuscript: Items from the WHO Trial Registration Data Set (including elements such as trial registration, financial support, study contacts, study title, countries of recruitment and details on design and recruitment status) are noted throughout the manuscript.
Protocol version	3	Date and version identifier NA: This is a manuscript of a study protocol.
Funding	4	Sources and types of financial, material, and other support Funding (p14): "This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication."
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Author contributions (p14): "KCK, YD, WWF and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP."

5b Name and contact information for the trial sponsor
The name of the study sponsor is included in the clinical trial registration records (NCT04641195, CTRI/2021/04/032593).

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
Funding (p14):
"This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication."

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
NA

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Introduction (p4-5): "[…] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1] […] Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. […] In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[10–14] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[15] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[16] […] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[16,20–23] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[24] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[25] who note that Zn2+ cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[26] Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India."

	6b	Explanation for choice of comparators Methods and analysis // Study procedures // Intervention (p8): “A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[16]”
Objectives	7	Specific objectives or hypotheses Objectives (p5): “The primary objectives of this trial are: To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19 To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19 Secondary objectives include: To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers”
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Methods and analysis // Trial design, population and enrolment sites (p5): “This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1).”

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Methods and analysis // Trial design, population and enrolment sites (p5): "This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1). […] The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services."
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) Methods and analysis // Eligibility criteria (p6): "The original inclusion criteria for this study were as follows: (1) men and women aged ≥18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO2) ≥90, and (4) written informed consent. The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month. To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use.

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Methods and analysis // Study procedures // Intervention (p7-8): Patients are randomized to one of four groups: 1. Placebo-Placebo group will receive a placebo vitamin D3 bolus at the hospital followed by placebo daily vitamin D3 maintenance doses and placebo daily zinc supplements 2. Vitamin D-Placebo group will receive an actual vitamin D3 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3 maintenance doses (2000 IU daily) and daily placebo zinc supplements 3. Placebo-Zinc group will receive a placebo vitamin D3 bolus at the hospital followed by placebo daily vitamin D3 maintenance doses and actual daily zinc supplements (40 mg daily) 4. Vitamin D3-Zinc group will receive an actual vitamin D3 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3 maintenance doses (2000 IU daily) and actual daily zinc supplements (40 mg daily) A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[16] Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count. Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India) with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited, Mumbai, India). All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell.

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
Methods and analysis // Adverse events and reporting (p11):
"All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant."

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
Methods and analysis // Study procedures // Intervention (p8):
"Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count."

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
Methods and analysis // Study procedures // Intervention (p8):
"All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell."

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended Methods and analysis // Study procedures // Study outcomes and follow up (p9): "The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These and additional symptoms are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline. Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above."
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) See Methods and analysis // Study procedures section (p6) for details on enrolment, intervention, and follow up. Table 1 and Figure 2, referred to in this section, also outline the sequence and schedule of enrolment and follow up.

	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations Methods and analysis // Data analysis // Statistical power calculations (p12) and Table 2: "Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[15,16,20–23] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times.[34] We calculated power for detecting specified hazard ratios associated with vitamin D or zinc given a specified true effect of the other treatment. Assuming average time to recovery of 22.2 days,[35] and a low (5%) rate of loss to follow-up, enrolment of 700 patients will yield the statistical power estimates in the Table 2.This analysis indicates that we will have at least 80% power to detect a moderate (25-30%) effect of either treatment, given a maximum 30% true effect of the other treatment."
	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Methods and analysis // Trial design, population and enrolment sites (p5): "While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[30]" Methods and analysis // Eligibility criteria (p6): "To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use." Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. […]"

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Methods and analysis // Study procedures // Randomization and blinding (p7): "For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Methods and analysis // Study procedures // Randomization and blinding (p7): "Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. […] Informed consent is obtained after responding to any raised queries." Methods and analysis // Study procedures // Randomization and blinding (p7): "For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Methods and analysis // Study procedures // Randomization and blinding (p7): “Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial Data and Safety Monitoring Board (p13): “The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]”

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Methods and analysis // Study procedures // Baseline data and sample collection (p7): "Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows: • Screening and background: the initial screening form is extended to collect information including participants' demographic background, socio-economic status, and health and prevention behaviours (smoking and drinking) • Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in India and has been adapted to the Maharashtra context • Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 vaccination status, COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications, complications, and medical history A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described above." Methods and analysis // Study procedures // Study outcomes and follow up (p8 – 9): "Following baseline assessment and provision of supplements, participants are regularly followed up as described below: • Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants • Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is conducted in a follow up call every three days after leaving the hospital for all participants • 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible) • 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms […] A list of collected data and blood investigations with time points at baseline, during follow up visits or calls, and at 8 and 12 weeks is summarized in Table 1." **(Please also refer to Table 1)**

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Methods and analysis // Study procedures // Intervention (p8): “Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance.”
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): “Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.” Methods and analysis // Study procedures // Baseline data and sample collection (p7): “All information is collected securely on electronic tablets, as described above.” Methods and analysis // Study procedures // Study outcomes and follow up (p8): “All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.” Methods and analysis // Data and sample management (p11): “All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Methods and analysis // Data analysis // Planned analyses (p11): "An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared between participants randomized to vitamin D vs. placebo and zinc vs. placebo using Cox regression. […] We will assess the success of randomization by comparing baseline variables by treatment group using χ2 and t-tests and use multivariate modelling to adjust for imbalances if needed. […] The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach."
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Methods and analysis // Data analysis // Planned analyses (p11-12): "We will investigate effect modification of either treatment effect by the other, and by third variables collected at baseline (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. […] The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects. Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders."

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) Methods and analysis // Data analysis // Planned analyses (p11): "An intent-to-treat analysis will be used as the primary analytic strategy."
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Data and Safety Monitoring Board (p12-13): "The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings. The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]"
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Data and Safety Monitoring Board (p13): "The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]"

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Methods and analysis // Adverse events and reporting (p11): "Any undesirable circumstance or experiences reported by study participants during the study are categorised as adverse events. All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant. Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events."
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor NA

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Abstract // Ethics and dissemination (p2): "Ethical approval was obtained from institutional ethical committees of all participating institutions." Ethics and dissemination (p13): "This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027)."

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) NA: As this is a manuscript of a study protocol, such detail has not been included in this specific document.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Methods and analysis // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. [..] The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference."
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Methods and analysis // Recruitment and obtaining informed consent (p6): "The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries."

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent." Methods and analysis // Study procedures // Baseline data and sample collection (p7): "All information is collected securely on electronic tablets, as described above." Methods and analysis // Study procedures // Study outcomes and follow up (p8): "All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above." Methods and analysis // Data and sample management (p11): "All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only."
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Competing interests (p15): "All authors declare no conflicts of interest."
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Methods and analysis // Data and sample management (p11): "All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately."

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Methods and analysis // Adverse events and reporting (p11): "Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events."
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Ethics and dissemination (p13): "The study findings will be presented in peer-reviewed medical journals."
	31b	Authorship eligibility guidelines and any intended use of professional writers NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code NA
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates NA: As this is a manuscript of a study protocol, such detail has not been included in this specific document.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Methods and analysis // Data and sample management (p11): "Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored securely at the Foundation for Medical Research for a maximum of three years."

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol

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A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol

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ABSTRACT

Introduction: Presently, there are few population-level strategies to address SARS-COV-2 infection except preventive measures such as vaccination. Micronutrient deficiency, particularly vitamin D and zinc deficiency, has been associated with dysregulated host responses, and may play an important role in COVID-19.

Methods and analysis: We have designed a 2×2 factorial, randomized, double-blind, multi-centre placebo-controlled trial to evaluate the effect of vitamin D and zinc on COVID-19 outcomes in Maharashtra, India. COVID-19 positive individuals are recruited from hospitals in Mumbai and Pune. Participants are provided (1) vitamin D3 bolus (180,000 IU) maintained by daily dose of 2000 IU, and/or (2) zinc gluconate (40 mg daily), versus placebo for 8 weeks. Participants undergo a detailed assessment at baseline and at 8 weeks, and are monitored daily in hospital or every three days after leaving the hospital to assess symptoms and other clinical measures. A final follow up telephone call occurs 12 weeks post-enrolment to assess long-term outcomes. The primary outcome of the study is to time to recovery, defined as time to resolution of all of fever, cough and shortness of breath. Secondary outcomes include: duration of hospital stay, all-cause mortality, necessity of assisted ventilation, change in blood biomarker levels, and individual symptoms duration. Participant recruitment commenced on April 2021.

Ethics and dissemination: Ethical approval was obtained from institutional ethical committees of all participating institutions. The study findings will be presented in peer-reviewed medical journals.

Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The setting of this study in India enables applicability of findings to the wider South Asia region, where evidence on this topic remains scarce despite a notable recent burden of COVID-19 and high prevalence of micronutrient deficiency.
- As a double-blind factorial randomized controlled trial, this study enables an efficient assessment of the effect of vitamin D and zinc on COVID-19 symptoms that is less prone to confounding and bias than other observational studies on this topic.
- With frequent follow up of participants, this study collects information across a range of domains including sociodemographic and clinical measures, and biomarker data, which will allow for a detailed investigation of the effect of supplementation on disease progression.
- One limitation to the study design is that with the current sample size, the statistical power to detect modification of the effects of each supplement by other factors may be limited.

INTRODUCTION

COVID-19 continues to be a problem globally, with over 16 million incident cases and 200,000 deaths reported in November 2021.[1] Concerted global efforts have resulted in the development of vaccines, which may reduce the burden and impact of COVID-19, although suboptimal vaccine coverage and the rapid mutation of the virus continue to prolong the pandemic.[2–5] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1]

Observational and experimental evidence link vitamin D to an array of communicable and non-communicable diseases.[7] Vitamin D deficiency (VDD; serum vitamin D <20 ng/ml) [8] is common in urban and rural India despite the country's sunny climate, due to environmental, sociological, and biological factors,[9,10] including skin pigmentation and cultural practices related to clothing and sun exposure. Countrywide studies suggest VDD may affect at least 70% of the Indian population. Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[11–15] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[16] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[17]

Zinc is an essential mineral that plays critical roles in gene expression, cell division, and immunity.[18] In India, dietary predominance of micronutrient-sparse staples, limited consumption of animal foods, and high consumption of zinc absorption inhibitors render the population at extremely high risk of inadequacy, which is exacerbated due to global climate change.[19] About 25% of the Indian population is zinc inadequate, and 4.3 million child deaths (<5 years) were attributable to zinc deficiency in 2017.[20] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[17,21–24] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[25] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[26] who note that Zn^{2+} cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[27]

Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India.

OBJECTIVES

The primary objectives of this trial are**:**

- To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19
- To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19

Secondary objectives include**:**

- To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration
- To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers

METHODS AND ANALYSIS

Trial design, population, enrolment sites, and time frame

This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (**Figure 1**). Maharashtra has the highest cumulative number of COVID-19 cases and fatalities out of all states in India.[28] Within the state, both Pune and Mumbai have emerged as COVID-19 hotspots.[29,30]

The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services. The trial is targeting a sample size of 700. The study commenced in April 2021 and study activities are expected to continue until July 2022. While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[31]

Eligibility criteria

The original inclusion criteria for this study were as follows: (1) men and women aged ≥18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO2) ≥90, and (4) written informed consent.

The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month.

To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use. Since this change was made early, when few (<6% of target population) participants were enrolled in the trial, we anticipate that the majority of the final study population will have been enrolled under the updated, broader criteria.

Study procedures

An overview of trial procedures is summarised in **Figure 2**.

Recruitment and obtaining informed consent

Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. As part of the process, potential participants are informed that their participation is completely voluntary and they can withdraw any time at any stage of the study without providing any reasons. The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference.

Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[32] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.

Baseline data and sample collection

Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows:

- Screening and background: the initial screening form is extended to collect information including participants' demographic background, socio-economic status, and health and prevention behaviours (smoking and drinking), and COVID-19 vaccination status
- Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in India and has been adapted to the Maharashtra context
- Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications including those prescribed for COVID-19, nutritional supplement use, complications, and medical history

A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described above.

Randomization and blinding

Participants are assigned randomly to one of four groups: (1) vitamin D, (2) zinc, (3) vitamin D and zinc, or (4) placebo. For randomization, a computer-generated list was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic. The randomization list assigns each participant randomization identifier (ID) to a regimen code, with the actual regimen known only to the manufacturer and accessible to the statistician in a currently unopened, sealed envelope. Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and all research staff including investigators remain blinded. At each site, each participant entering the trial is given the next available randomization ID, and is provided their corresponding regimen based on the assigned regimen code.

Intervention

Patients are randomized to one of four groups:

1. Placebo-Placebo group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily vitamin D3 maintenance doses and *placebo* daily zinc supplements
2. Vitamin D-Placebo group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and daily *placebo* zinc supplements
3. Placebo-Zinc group will receive a *placebo* vitamin D3 bolus at the hospital followed by *placebo* daily vitamin D3 maintenance doses and *actual* daily zinc supplements (40 mg daily)
4. Vitamin D3-Zinc group will receive an *actual* vitamin D3 bolus (180,000 IU) at the hospital followed by *actual* daily vitamin D3 maintenance doses (2000 IU daily) and *actual* daily zinc supplements (40 mg daily)

We selected vitamin D3 as it has been shown to be more effective in raising and maintaining high levels of circulating 25(OH)D than vitamin D2 [33,34]. A bolus dose followed by daily doses was chosen to boost vitamin D levels quickly and safely within the first few days and maintain levels thereafter. Previous studies have indicated the efficacy of large oral doses (>200 000 IU bolus, and 1,700-2,000 IU per day) in increasing and sustaining blood 25(OH)D concentrations, with very low risk of side effects [35–41]. The 40 mg dosage of zinc is understood to be sufficiently high to assess efficacy, while remaining within the Institute of Medicine's tolerable upper intake level for adults [42]. A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[17]

Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are observed taking supplements daily while in hospital or contacted regularly via telephone after leaving the hospital to ensure compliance. Research staff identify barriers to compliance and aim to address these via appropriate counselling, and assess compliance at 8 weeks via direct questioning and pill count.

Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India) with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited, Mumbai, India).

All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell. Indian national guidelines have evolved during the pandemic, and currently consist of appropriate treatment (which may include oxygen support, respiratory support, anti-inflammatory or immunomodulatory therapy, and anticoagulation therapy) according to disease severity; discharge of admitted patients from the hospital upon resolution of symptoms and sufficient oxygen saturation (SpO2 > 93%) for three days; and self-monitoring during home isolation [43–45].

Study outcomes and follow up

Following baseline assessment and provision of supplements, participants are regularly followed up as described below and in **Table 1**:

- Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants. Any new prescribed medications and supplements are also recorded alongside other interventions such as need for non-invasive ventilation or dialysis. Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records. Clinical measurements are recorded in study-specific visits that are conducted independently after ward rounds, to minimize interference in care and ensure all relevant information for the day is noted.
- Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is conducted in a follow up call every three days after leaving the hospital for all participants. All information is self-reported by participants.
- 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, use of any other nutritional supplements, updates to COVID-19 vaccination status, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible). Symptoms are specifically asked to participants; other measures are asked, observed, assessed, or abstracted from the participants' records.
- 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms, and any updates to COVID-19 vaccination status. All information is self-reported by participants.

All data are collected using standardized questionnaire forms on electronic tablets,[32] as described above.

The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These symptoms are most commonly reported among COVID-19 patients, including in Indian populations,[46,47] and have also been assessed as part of studies examining vitamin D and zinc in respiratory illnesses [17]. These and additional symptoms (including fatigue, headache, loss of smell and taste and sore throat) are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Data on symptoms are collected using the same structured questions at each time point: (1) whether the participant has experienced X symptom today, and if so, (2) how

many days in total including today the participant has experienced X symptom. Staff conducting in-person and telephone follow ups are trained uniformly using a standardised telephone script with regards to collecting this information. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline.

Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, and blood biomarker levels, including 25-hydroxy vitamin D, zinc, and other immunological and inflammatory biomarkers (including interleukin 6, angiopoietin-2, soluble triggering receptor expressed on myeloid cells-1, immunoglobulin G and immunoglobulin M). Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above. A list of collected data and blood investigations with time points at baseline, during follow up visits or calls, and at 8 and 12 weeks is summarized in **Table 1**.

Table 1. Collection of data points in the trial.

Data category	Baseline (enrolment)	Follow up	8 weeks	12 weeks
Demographic and background information	Age, gender, education, marital status, occupation, socio-economic status, health and prevention behaviours, COVID-19 vaccination *(Self-reported by participant, assessed by staff or abstracted from participant record)*		COVID-19 vaccination *(Self-reported by participant)*	COVID-19 vaccination *(Self-reported by participant)*
Dietary information	Food frequency questionnaire: consumption frequency of 25 diverse food groups in last three months *(Self-reported by participant)*			
Clinical examination	Medical history, comorbidities, preadmission medications, non-intervention nutritional supplement use (*Self-reported by participant, assessed by staff or abstracted from participant record*) Clinical symptoms[1] *(Self-reported by participant)*	**Hospital and telephone follow up:** Clinical symptoms[1] *(Self-reported by participant)* **Hospital follow up only:** Changes in medications, changes in non-intervention nutritional supplement use *(Assessed by staff or abstracted from participant record)*	Medical history, comorbidities, pre-assessment medications, non-intervention nutritional supplement use *(Self-reported by participant, assessed by staff or abstracted from participant record)* Clinical symptoms[1] *(Self-reported by participant)*	Clinical symptoms[1] (*Self-reported by participant*)
Clinical measurements	Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight and height *(Assessed by staff or abstracted from participant record)*	**Hospital follow up only:** Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight, requirement for non-invasive ventilation or intubation/ventilator support, need for dialysis, lab investigations *(Assessed by staff or abstracted from participant record)*	Respiratory rate, pulse, auxiliary temperature, SpO2, systolic and diastolic blood pressure, weight and height *(Assessed by staff or abstracted from participant record)*	
Blood and other investigations and biomarkers	SARS-COV-2 RT-PCR, chest X-Ray, complete blood count, blood glucose, serum creatinine, CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1		CRP, LDH, serum ferritin, D-dimer, vitamin D, zinc, calcium, IgG, IgM, Ang2, IL-6 and sTREM-1 *(Assessed by laboratory or abstracted from participant record)*	

	(Assessed by laboratory or abstracted from participant record)		
Other information		**Hospital and telephone follow up:** Compliance, adverse events *(Self-reported by participant, assessed by staff or abstracted from participant record)*	Compliance (count of remaining pills) (*Assessed by staff*)

SpO2: Oxygen saturation, CRP: C-reactive protein, LDH: lactate dehydrogenase, IgG: Immunoglobulin G, IgM: immunoglobulin M, Ang2: angiopoietin-2, IL-6: interleukin 6, sTREM-1: soluble triggering receptor expressed on myeloid cells-1.

[1]Clinical symptoms include: fever, cough, shortness of breath, fatigue, headache, loss of smell, loss of taste, diarrhea, anorexia, sore throat, nasal congestion, nausea and vomiting, and any other reported by the participant.

1

Adverse events and reporting

Any undesirable circumstance or experiences reported by study participants during the study are categorised as adverse events. All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant. Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events.

Data and sample management

All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[32] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.

Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored securely at the Foundation for Medical Research for a maximum of three years.

Data analysis

Planned analyses will initially be undertaken in blinded fashion (comparing coded treatment groups); unblinding of investigators and research staff with respect to treatment allocation will only occur once analyses are completed.

Planned analyses

An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared between participants randomized to vitamin D vs. no vitamin D and zinc vs. no zinc using Cox regression. We will investigate effect modification of either treatment effect by the other, and by third variables collected at baseline (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. There are no *a priori* effect modifiers hypothesized, and unless there is strong modification of a treatment effect, our power to detect these may be low. We will assess the success of randomization by comparing baseline variables by treatment group using $\chi 2$ and t-tests and use multivariate modelling to adjust for imbalances if needed. Additional

collected information, including data on prescribed medications and other treatments, will enable an assessment of whether important factors including non-protocol interventions are balanced across intervention groups.

The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach. The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using $\chi 2$ tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[48] we will test and modify it if needed in the whole study population, adjusting for treatment effects.

Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders.

Statistical power calculations

With a single endpoint for both interventions, the factorial design does not provide a “two-for-one” power advantage, where the total number of participants required to test two treatments is lower using a single factorial trial compared with two parallel group trials.[49] Power will decrease if each treatment has a moderate effect; we accounted for this in calculating the sample size. Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[17,21–24,50] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times, which assumes exponential distribution of the time to recovery.[51] We calculated power for detecting specified hazard ratios associated with vitamin D or zinc given a specified true effect of the other treatment. Assuming average time to recovery of 22.2 days,[52] and a low (5%) rate of loss to follow-up, enrolment of 700 patients will yield the statistical power estimates in the **Table 2**. This analysis indicates that we will have at least 80% power to detect a moderate (25-30%) effect of either treatment, given a maximum 30% true effect of the other treatment. We did not further adjust our power calculations and desired sample size following changes to our eligibility criteria, which may result in the inclusion of participants with symptoms that are both more severe (SpO2 <90) and less severe (outpatients) at baseline.

Table 2. Statistical power estimation.

	True effect of Treatment B						
Effect of Treatment A	**0%**	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
30%	99%	99%	99%	99%	98%	98%	97%
25%	95%	94%	93%	92%	90%	88%	86%
20%	81%	79%	76%	74%	71%	69%	66%

Patient and public involvement

Patients and the public were not involved in the design of this study.

DATA AND SAFETY MONITORING BOARD

The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings.

The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[53]

ETHICS AND DISSEMINATION

This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027). The trial is registered on ClincialTrials.gov (NCT04641195). Permission for the study was also obtained from the Health Management and Screening Comments (HMSC), Government of India (HMSC (GOI)-2021-0060), and the study was registered prospectively in the Clinical Trials Registry India (CTRI/2021/04/032593). Since the study intervention is related to micronutrient supplementation, endorsement from the Drugs Controller General of India was non-obligatory. The study findings will be presented in peer-reviewed medical journals.

DISCUSSION

With continued high incidence of global cases, COVID-19 remains a global health challenge. Alongside vaccination and other preventative measures, low-cost and efficient interventions which may help minimize the occurrence of serious disease are needed. These would be particularly valuable in low- and middle-income countries, where health systems are more overburdened and resources much fewer. In this context, and given previous evidence regarding the role of vitamin D and zinc in the development of and recovery from respiratory infections,[17,21–24,50] there is a need to explore their potential value as part of therapeutic regimens for COVID-19.

We report here the protocol of a 2x2 factorial randomized controlled trial, designed to generate evidence on the effect of vitamin D and zinc on COVID-19 progression. The frequent follow up of participants and collection of a range of sociodemographic, clinical and biomarker measures alongside blood samples will enable a detailed investigation of the effect of supplementation on disease progression, including potentially important immunological and inflammatory pathways. Importantly, in comparison with other vitamin D or zinc COVID-19 intervention studies currently registered on ClinicalTrials.gov, this would be the first conducted outside of the U.S. or Europe and other similar high-income countries. The location of this study in two large cities, alongside the broad eligibility criteria, increases the generalizability of study results. Given the current unpredictability of COVID-19 waves, one challenge to the study is to maintain recruitment during periods where cases may be on the decline. We have taken steps to mitigate any anticipated effects of this, including broadening our eligibility criteria as described previously, and rigorous training of site hospital staff to help improve recruitment of eligible individuals. Another limitation is that we may not be able to ascertain differences in distribution of sun exposure (as a source of vitamin D) across treatment groups, although we would expect this to be similar due to randomization. Regardless, the findings of this study will have direct relevance to many settings in South Asia and sub-Saharan Africa with weak health systems and prevalent malnutrition. Ultimately, the evidence generated as part of this trial will enhance our understanding of the role of vitamin D and zinc in COVID-19 disease, and contribute high quality evidence on the potential value of supplementation of these micronutrients for the same.

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AUTHOR CONTRIBUTIONS

WWF, KCK, YD, and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP. YD, NM, PDC, GG, KKS, YM, and SS are involved in data acquisition, and in study monitoring along with KCK, WWF and UP. MW provides statistical expertise. KKS and UP drafted the manuscript, and all authors reviewed and critically revised the draft and approved the final manuscript.

COMPETING INTERESTS

All authors declare no conflicts of interest.

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FIGURE LEGENDS

Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.

Map created with mapchart.net.

Figure 2: Overview of trial procedures. RAT: Rapid Antigen Test.

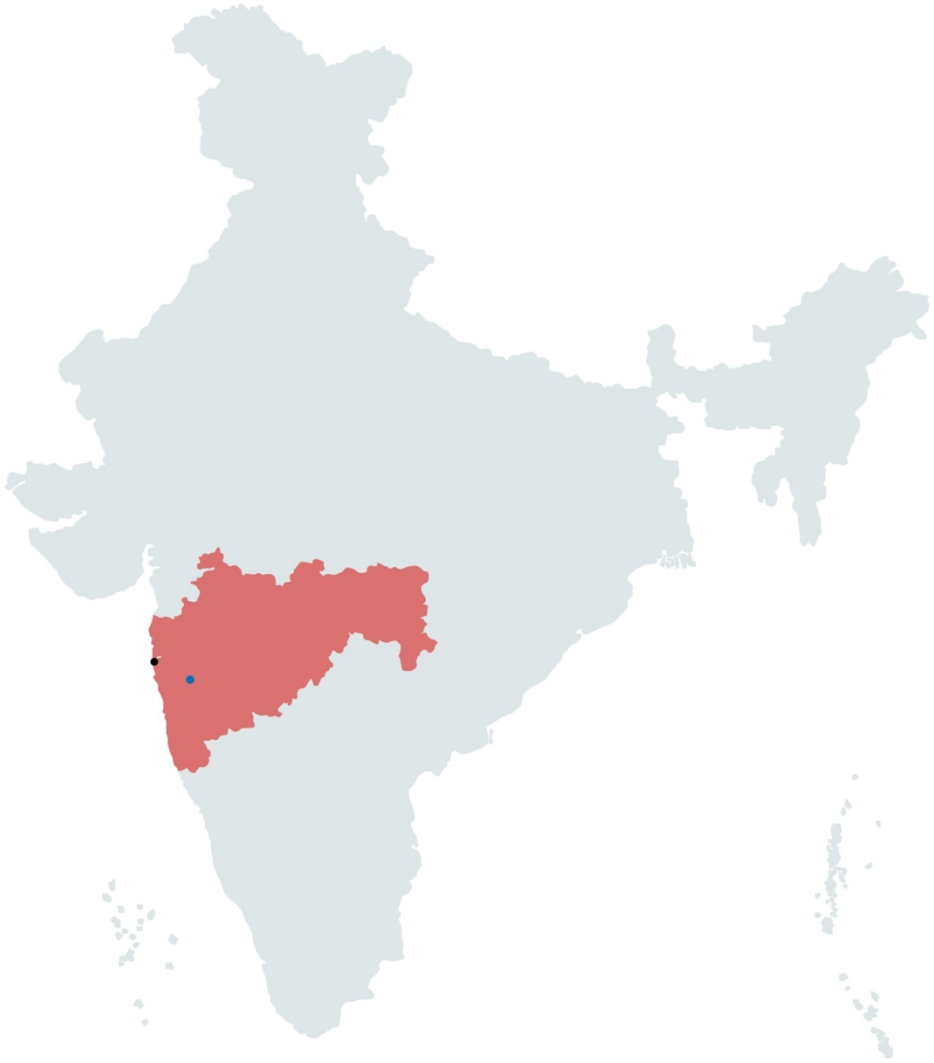

Figure 1: Map of India (grey) with Maharashtra highlighted in red, and Mumbai (black dot) and Pune (blue dot) identified.
Map created with mapchart.net.

156x188mm (330 x 330 DPI)

COVID-19 patients in the hospital

Screening for eligibility

↓

Enrolment and informed consent obtained

Baseline information is obtained before allocation of supplements

↓

Randomization

(Group allocation and labelling of supplements done prior to trial initiation to maintain blinding at all levels)

(1) **Vitamin D Group** (180,000 IU bolus at enrolment, followed by 2000 IU daily)	(2) **Zinc Group** (placebo at enrolment followed by daily dose of 40 mg)	(3) **Vitamin D and zinc group** (180,000 IU vitamin D bolus at enrolment followed by daily dose of vitamin D- 2000 IU and zinc - 40 mg)	(4) **Placebo** (Placebo at enrolment and daily placebo)

Duration of intervention:

1. **Daily Supplements**: 8 weeks
2. **Bolus**: Once at enrolment

Follow up assessment:

Patient advised for home isolation (outpatients) • Direct to telephone follow up	Hospitalized patients (inpatients) • Daily hospital follow ups to discharge

Telephone follow up to 8 weeks (follow up calls every 3 days)

Final 8-week clinical assessment visit

12-week follow up for assessing any long COVID symptoms

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Title page (p1): "A randomized trial to determine the effect of vitamin D and zinc supplementation for improving treatment outcomes among COVID-19 patients in India: trial protocol"
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Abstract (p2): "Trial Registration number: NCT04641195, CTRI/2021/04/032593, HMSC (GOI)-2021-0060"
	2b	All items from the World Health Organization Trial Registration Data Set Manuscript: Items from the WHO Trial Registration Data Set (including elements such as trial registration, financial support, study contacts, study title, countries of recruitment and details on design and recruitment status) are noted throughout the manuscript.
Protocol version	3	Date and version identifier NA: This is a manuscript of a study protocol.
Funding	4	Sources and types of financial, material, and other support Funding (p14): "This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication."
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Author contributions (p14): "KCK, YD, WWF and NM conceptualised the project, and designed the study along with SB, KKS, ECH, YM and UP."

5b Name and contact information for the trial sponsor
The name of the study sponsor is included in the clinical trial registration records (NCT04641195, CTRI/2021/04/032593).

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
Funding (p14):
"This trial is supported by the Canadian Institutes of Health Research, Operating Grant: COVID-19 Rapid Research Funding Opportunity – Therapeutics, application number: 447092 and the Canada Research Chair program (to KCK). The funding body has no role in study design and procedures, or the decision to submit manuscripts for publication."

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
NA

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Introduction (p4-5): "[…] Additionally, with limited proven treatment regimens for COVID-19 to date, it is essential to continue exploring low cost and commonly available effective interventions which can be implemented as standardized therapeutic treatment regimens at large.[6] This is especially important in the context of low and middle-income countries in South Asia and Africa, which are particularly vulnerable given weak health systems and the co-existence of malnutrition and other co-morbidities. This includes India, which continues to report a substantial number of COVID-19 cases.[1] […] Vitamin D shows promise as a novel, cost-effective prevention and adjunctive treatment for respiratory infections. […] In laboratory studies, vitamin D metabolites support innate immune responses to rhinoviruses and respiratory syncytial virus.[10–14] In participants with influenza, high-dose vitamin D supplementation shortened durations of fever, cough and wheezing, particularly among those with low vitamin D levels.[15] In a recent systematic review and meta-analysis of randomised controlled trials, vitamin D supplementation was associated with decreased risk of acute respiratory infections and shortened duration of symptoms.[16] […] Multiple meta-analyses and pooled analyses of randomized controlled trials conducted in the US and low- and middle-income countries have shown that oral zinc supplementation reduces incidence of acute respiratory infections by 35%, shortens duration of symptoms, and improves recovery rate.[16,20–23] Zinc is a potential treatment in COVID-19, due to its immune modulatory effect, as well as direct antiviral effect.[24] The mechanisms by which zinc may serve as adjunct therapy in COVID-19 has been recently reviewed by Skalny et al. 2020,[25] who note that Zn2+ cations, especially in combination with zinc ionophore pyrithione inhibit SARS-coronavirus RNA polymerase activity by decreasing replication.[26] Vitamin D and zinc are safe, inexpensive, and widely available therapies; therefore, experimental evidence that these nutrient supplements are effective against COVID-19 would readily support their inclusion in standard of care. Therefore, we are undertaking a randomized controlled trial to determine the effect of vitamin D and zinc supplementation on treatment outcomes among individuals with COVID-19 in India."

	6b	Explanation for choice of comparators Methods and analysis // Study procedures // Intervention (p8): “A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[16]”
Objectives	7	Specific objectives or hypotheses Objectives (p5): “The primary objectives of this trial are: To determine the effect of vitamin D supplementation versus placebo on time to recovery among patients with COVID-19 To determine the effect of zinc supplementation versus placebo on time to recovery among patients with COVID-19 Secondary objectives include: To determine the effect of vitamin D or zinc supplementation on duration of hospital stay, all-cause mortality, necessity for assisted ventilation, and individual symptoms duration To examine the effect of vitamin D or zinc supplementation on key blood biomarkers, including serum vitamin D and zinc, and immunological and inflammatory markers”
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) Methods and analysis // Trial design, population and enrolment sites (p5): “This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1).”

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Methods and analysis // Trial design, population and enrolment sites (p5): "This is a double-blind, placebo-controlled, randomized superiority trial with 2x2 factorial design and 1:1:1:1 allocation ratio, being conducted at two site hospitals in Mumbai and Pune, Maharashtra, India (Figure 1). […] The two study sites (King Edward Memorial Hospital and Research Centre, Pune, and Saifee Hospital, Mumbai) are established medical institutions located within the cities of Pune and Mumbai. These hospitals have been designated as COVID-19 dedicated hospitals by local municipal corporations, where people can avail COVID-19-related treatment and services."
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) Methods and analysis // Eligibility criteria (p6): "The original inclusion criteria for this study were as follows: (1) men and women aged ≥18 years, (2) RT-PCR-confirmed infection with SARS-COV-2, (3) oxygen saturation level (SpO2) ≥90, and (4) written informed consent. The exclusion criteria were as following: (1) pregnant women, (2) individuals enrolled in other clinical trials, (3) daily use of multivitamins for the past 1 month. To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use.

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered Methods and analysis // Study procedures // Intervention (p7-8): Patients are randomized to one of four groups: 1. Placebo-Placebo group will receive a placebo vitamin D3 bolus at the hospital followed by placebo daily vitamin D3 maintenance doses and placebo daily zinc supplements 2. Vitamin D-Placebo group will receive an actual vitamin D3 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3 maintenance doses (2000 IU daily) and daily placebo zinc supplements 3. Placebo-Zinc group will receive a placebo vitamin D3 bolus at the hospital followed by placebo daily vitamin D3 maintenance doses and actual daily zinc supplements (40 mg daily) 4. Vitamin D3-Zinc group will receive an actual vitamin D3 bolus (180,000 IU) at the hospital followed by actual daily vitamin D3 maintenance doses (2000 IU daily) and actual daily zinc supplements (40 mg daily) A placebo was chosen as the comparator group given that there is currently no widespread consensus on the use of any nutritional supplement as part of standard or routine treatment for COVID-19.[16] Participants receive a pre-labelled daily supplement bottle with 60 tablets, and an envelope which contains three vitamin D3/placebo bolus tablets to be consumed at baseline under supervision of site hospital staff. Following the bolus dose, participants are instructed to take supplements daily for 8 weeks. Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count. Supplement and placebo tablets were manufactured by Excellamed Laboratories Private Limited (Mumbai, India) with an external quality check done by an independent service provider (Bee Pharmo Labs Private Limited, Mumbai, India). All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell.

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
Methods and analysis // Adverse events and reporting (p11):
"All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant."

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
Methods and analysis // Study procedures // Intervention (p8):
"Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance. Research nurses identify barriers to compliance, and assess compliance at 8 weeks via direct questioning and pill count."

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
Methods and analysis // Study procedures // Intervention (p8):
"All participants are provided with care and treatment consistent with Indian national guidelines, and are encouraged to visit the study clinics seven days a week for medical attention if they feel unwell."

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended Methods and analysis // Study procedures // Study outcomes and follow up (p9): "The primary outcome of the study is time to resolution of all of the following symptoms: (1) fever, (2) cough and (3) shortness of breath. These and additional symptoms are captured on multiple time points, including baseline, daily hospital follow ups for admitted patients, telephone follow ups every three days after leaving the hospital until 8 weeks post-enrolment, the 8-week clinical assessment, and finally at a 12-week assessment call. Metrics of individual symptoms and combination of symptoms are used to identify the time point of resolution symptoms from baseline. Secondary outcomes include duration of hospital stay, need for assisted ventilation, individual symptoms duration, all-cause mortality, occurrence of other severe adverse events and change in blood biomarker levels, including 25-hydroxy vitamin D, zinc and calcium, and other immunological and inflammatory biomarkers. Biomarker levels are assessed using blood samples collected at baseline and at the 8-week clinical assessment. Occurrence of any other secondary endpoints between baseline to 8-week clinical assessment is recorded during follow up calls or visits as described above."
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) See Methods and analysis // Study procedures section (p6) for details on enrolment, intervention, and follow up. Table 1 and Figure 2, referred to in this section, also outline the sequence and schedule of enrolment and follow up.

	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations Methods and analysis // Data analysis // Statistical power calculations (p12) and Table 2: "Assumptions related to treatment effects may be reasonably inferred from meta-analyses of well-designed randomized controlled trials studying these supplements in other acute respiratory illnesses.[15,16,20–23] We based power analysis on the primary outcome of time from onset of disease to clinical recovery, using methodology for survival times.[34] We calculated power for detecting specified hazard ratios associated with vitamin D or zinc given a specified true effect of the other treatment. Assuming average time to recovery of 22.2 days,[35] and a low (5%) rate of loss to follow-up, enrolment of 700 patients will yield the statistical power estimates in the Table 2.This analysis indicates that we will have at least 80% power to detect a moderate (25-30%) effect of either treatment, given a maximum 30% true effect of the other treatment."
	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Methods and analysis // Trial design, population and enrolment sites (p5): "While we initially targeted only hospitalized inpatients at each site for the study, we broadened our target population in June 2021 to include all hospital outpatients. This was done in order to increase generalizability of results and maintain enrolment in light of decreasing COVID-19 cases.[30]" Methods and analysis // Eligibility criteria (p6): "To capture the greatest possible number and range of symptomatic COVID-19 cases and increase generalizability, we made the following alterations to our eligibility criteria from June 2021 (within 2 months of recruitment commencement): (1) added inclusion criterion of individuals with Rapid Antigen Test-confirmed SARS-COV-2 infection (with confirmatory PCR tests performed subsequently on all such enrolled individuals), (2) removed inclusion criterion of SpO2 ≥90, and (3) removed exclusion criterion of recent daily multivitamin use." Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. […]"

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Methods and analysis // Study procedures // Randomization and blinding (p7): "For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Methods and analysis // Study procedures // Randomization and blinding (p7): "Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. […] Informed consent is obtained after responding to any raised queries." Methods and analysis // Study procedures // Randomization and blinding (p7): "For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic."

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Methods and analysis // Study procedures // Randomization and blinding (p7): “Supplement bottles and envelopes are pre-labelled with codes, and active tablets and placebo are indistinguishable, so that participants and investigators are blinded. For randomization, a computer-generated list from 1 to 1000 was prepared by the study statistician, according to a randomization sequence in blocks of 20 and stratified by follow up clinic.”
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant’s allocated intervention during the trial Data and Safety Monitoring Board (p13): “The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]”

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Methods and analysis // Study procedures // Baseline data and sample collection (p7): "Following informed consent, participants undergo baseline data and sample collection, including recording of key background and clinical information as follows: • Screening and background: the initial screening form is extended to collect information including participants' demographic background, socio-economic status, and health and prevention behaviours (smoking and drinking) • Baseline dietary information: a food frequency questionnaire (FFQ) is administered, collecting information on dietary practices and habits in relation to 25 food groups. The FFQ is validated for use in India and has been adapted to the Maharashtra context • Clinical baseline: clinical and physical measures are collected alongside information on COVID-19 vaccination status, COVID-19 symptoms, vital signs, blood investigations, medical conditions, treatment and medications, complications, and medical history A blood sample is also collected at baseline. All information is collected securely on electronic tablets, as described above." Methods and analysis // Study procedures // Study outcomes and follow up (p8 – 9): "Following baseline assessment and provision of supplements, participants are regularly followed up as described below: • Daily hospital follow up: Daily assessment of COVID-19 symptoms, vital signs, complications, medical conditions and study supplement compliance is recorded for hospitalised participants • Telephone follow up: Assessment of COVID-19 symptoms, supplement compliance and adverse events is conducted in a follow up call every three days after leaving the hospital for all participants • 8-week clinical assessment: After completion of study supplements at 8 weeks, information is gathered on results of a clinical and physical examination, COVID-19 symptoms, compliance with regimen (including direct questioning and pill count), vital signs, blood investigations (from a collected blood sample), medical conditions, treatment and medications, complications, and history. This assessment is conducted in person at the hospital, or at a location convenient to the participant where privacy can be ensured (including an option to collect some information via telephone if an in-person visit is not possible) • 12-week telephone follow up: A final assessment is conducted of long-term COVID-19 symptoms […] A list of collected data and blood investigations with time points at baseline, during follow up visits or calls, and at 8 and 12 weeks is summarized in Table 1." **(Please also refer to Table 1)**

	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Methods and analysis // Study procedures // Intervention (p8): “Participants are contacted daily while in hospital or regularly via telephone after leaving the hospital to ensure compliance.”
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): “Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent.” Methods and analysis // Study procedures // Baseline data and sample collection (p7): “All information is collected securely on electronic tablets, as described above.” Methods and analysis // Study procedures // Study outcomes and follow up (p8): “All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above.” Methods and analysis // Data and sample management (p11): “All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only.”

Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol Methods and analysis // Data analysis // Planned analyses (p11): "An intent-to-treat analysis will be used as the primary analytic strategy. Time to primary outcome will be compared between participants randomized to vitamin D vs. placebo and zinc vs. placebo using Cox regression. […] We will assess the success of randomization by comparing baseline variables by treatment group using χ2 and t-tests and use multivariate modelling to adjust for imbalances if needed. […] The effect of vitamin D or zinc on dichotomous secondary outcomes will be analyzed in a similar approach."
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) Methods and analysis // Data analysis // Planned analyses (p11-12): "We will investigate effect modification of either treatment effect by the other, and by third variables collected at baseline (including, anthropometric status, and vitamin D status). Effect modification will be assessed by including interaction terms in Cox regression models, and statistical significance assessed via likelihood ratio tests. […] The proportion of individuals experiencing hypercalcemia will be compared between treatment groups using χ2 tests, and effects of the supplements on blood biomarkers will be compared via Wilcoxon and t-tests. This study will measure numerous risk factors for COVID-19 progression and severe treatment outcomes including hemoglobin; co-morbidities; medications including chloroquine, hydroxychloroquine, and ACE inhibitors; and socio-demographic, clinical, nutritional, and lifestyle-related risk factors. We will examine relationships of these factors in the placebo group first, to avoid complex questions concerning interactions between risk factors and treatments. Once we find a satisfactory parsimonious model using principles of model selection as detailed by Greenland,[32] we will test and modify it if needed in the whole study population, adjusting for treatment effects. Analyses will consider sex and gender throughout, by disaggregating findings, and attempting to elucidate the roles of sex and gender in the clinical course and immune response by controlling for potential sociodemographic, nutritional, and immunological confounders."

	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) Methods and analysis // Data analysis // Planned analyses (p11): "An intent-to-treat analysis will be used as the primary analytic strategy."
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed Data and Safety Monitoring Board (p12-13): "The Data and Safety Monitoring Board (DSMB) was established prior to commencement of the trial. It consists of independent experts in respiratory infection and communicable diseases, public health and nutrition, clinical research, and biostatistics. The role of the board is to provide their inputs, recommendations, review the trial protocols and progress by ensuring the rights and safety of involving participants in the study through periodic trial review meetings. The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]"
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial Data and Safety Monitoring Board (p13): "The trial DSMB will examine efficacy endpoints by study arms when half of individuals are enrolled. In accordance with the Haybittle-Peto rule, if the difference in the primary outcome between study arms is <0.001, unblinding of the DSMB and stopping will be considered.[36]"

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Methods and analysis // Adverse events and reporting (p11): "Any undesirable circumstance or experiences reported by study participants during the study are categorised as adverse events. All adverse events which are possibly, probably or very likely related to administration of any supplement are monitored and reported to site institutional review boards (IRBs) within 72 hours (serious adverse events) or 1 month (all other adverse events), using a standardized reporting format. The trial data and safety monitoring board (DSMB) is also notified. Site principal investigators and independent physicians are responsible for assessing the causal relationship and making the conclusive decision about continuation of the trial for a particular participant. Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events."
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor NA

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval Abstract // Ethics and dissemination (p2): "Ethical approval was obtained from institutional ethical committees of all participating institutions." Ethics and dissemination (p13): "This study is being conducted in the accordance with the Declaration of Helsinki 2013. The study was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health (Protocol No. IRB20-1425), the University Health Network Research Ethics Board (20-5775), the Institutional Research Ethics Committee of the Foundation for Medical Research (IREC No. FMR/IREC/C19/02/2020), the Institutional Review Board of Saifee Hospital (Project No. EC/008/2020) and the KEM Hospital Research Centre Ethics Committee (KEMHRC ID No. 2027)."

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) NA: As this is a manuscript of a study protocol, such detail has not been included in this specific document.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) Methods and analysis // Recruitment and obtaining informed consent (p6): "Potential participants are approached by trained site hospital staff members when they present to site hospitals. Site hospital staff members undergo intensive training and refresher training in order to ensure that potential participants are able to make an informed decision regarding participation. These dedicated site hospital staff members determine their interest and eligibility, and provide a brief introduction including key details about the study and what participation involves. The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries. [..] The informed consent process is completed once participants provide their signature on two copies of the consent document; one copy for the trial record and another provided to participants for their reference."
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Methods and analysis // Recruitment and obtaining informed consent (p6): "The staff members read out the participant information sheet in the appropriate conversational language (English, Hindi or Marathi), and discuss the trial components and the role of the participant in the study. Information provided includes a clear outline of potential benefits and harms, the length of the follow up period, remuneration that can be expected, future use of information and samples, and resources available to the participant such as access to study clinics. Informed consent is obtained after responding to any raised queries."

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial Methods and analysis // Study procedures // Recruitment and obtaining informed consent (p6): "Information regarding eligibility of potential participants is collected on a secure electronic tablet using Open Data Kit (ODK),[31] with questionnaires including built-in checks and data uploaded to a secure server. No identifiable data are collected until the participant has provided informed consent." Methods and analysis // Study procedures // Baseline data and sample collection (p7): "All information is collected securely on electronic tablets, as described above." Methods and analysis // Study procedures // Study outcomes and follow up (p8): "All data are collected using standardized questionnaire forms on electronic tablets,[31] as described above." Methods and analysis // Data and sample management (p11): "All data collected as part of this trial are entered into password-protected android electronic tablets, with pre-programmed questionnaires using ODK.[31] All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately. All analyses and data checks are conducted on anonymised data only."
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site Competing interests (p15): "All authors declare no conflicts of interest."
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Methods and analysis // Data and sample management (p11): "All data are automatically and directly uploaded from the tablets onto a secure electronic server, and entered into a password-protected database accessible only to authorised study team members. Data are stored in linked-anonymised form, with identifiable information and the linking key stored separately."

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Methods and analysis // Adverse events and reporting (p11): "Additionally, medical insurance is provided to all study participants to take care of any progression of severe adverse events."
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Ethics and dissemination (p13): "The study findings will be presented in peer-reviewed medical journals."
	31b	Authorship eligibility guidelines and any intended use of professional writers NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code NA
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates NA: As this is a manuscript of a study protocol, such detail has not been included in this specific document.
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Methods and analysis // Data and sample management (p11): "Blood samples collected as part of this trial are processed at the Foundation for Medical Research, Mumbai, and accredited laboratories in India including at the site hospitals. Specimens are linked-anonymised and are stored securely at the Foundation for Medical Research for a maximum of three years."

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.