Vitamin D levels and mortality with SARS-COV-2 infection: a retrospective two-centre cohort study

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ABSTRACT

Background The role of vitamin D in increased mortality with SARS-COV-2 virus, namely, COVID-19, remains uncertain. We analysed all the patients who were treated as COVID-19-positive with or without a positive swab and were tested for vitamin D levels.

Methods This was a retrospective, study involving 1226 patients swabbed for SARS-CoV-2 between the 10 February 2020 and 1 May 2020 at two hospitals of East Sussex Healthcare NHS Trust. Patients who were swab-positive for COVID-19 or treated as COVID-19-positive on clinical grounds even though swab results were negative were included in this study. We analysed the association of vitamin D levels and mortality, assessing linear and non-linear associations.

Results A total of 1226 patients had SARS-CoV-2 RNA swabs in this period with age range from 1 year to 101 years. A cohort of 433 of these patients had swabs and recent vitamin D levels anytime in the previous 3 months. Mortality rates were not found to be associated with vitamin D levels (OR=1.04, 95% CI 0.96 to 1.12).

Conclusion Our findings suggest similar mortality risk from COVID-19 irrespective of the levels of vitamin D. Larger prospective studies will be needed to confirm these findings.

INTRODUCTION

Vitamin D has an important role in maintaining healthy teeth, bones and immunity. Sunlight remains the most important source of vitamin D in nature.[1 2] Although vitamin D is available in certain foods like oily fish, eggs, fortified margarines and some others, the amount of available vitamin D is not enough even with increased consumption to meet the daily vitamin D requirement. Vitamin D is biologically inert and must undergo two successive hydroxylation in the liver and kidney to become the biologically active 1,25 -dihydroxy vitamin D.[3] The two most important forms are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). In contrast to vitamin D_3, vitamin D_2 is available as fortified food or given by the supplements. Both are bound to the vitamin D binding protein (VDBP) and transported to the liver and converted to 25-hydroxyvitamin D and are commonly agreed to be the metabolite as the major storage form of vitamin D in the body. The half-life of circulating 25-hydroxyvitamin D is 2–3 weeks. 25-Hydroxyvitamin D is converted to 24,25-dihydroxyvitamin D, which is the most abundant product of 25-hydroxyvitamin D catabolism with half-life of approximately 7 days, with serum concentrations up to approximately 10 nmol/L.[4] Most of the 25-hydroxyvitamin D measured in the serum is 25-hydroxyvitamin D_3. It has been linked to diabetes, different forms of cancers, cardiovascular disease, autoimmune diseases, innate immunity and expression of over 200 different genes.[5]

The lockdowns and self-isolation implemented by the governments in the UK and other places in the world have raised concerns about further deficiency in vitamin D levels. The prognosis of patients infected with SARS-COV-2 remains poorly understood. Some studies have suggested association of vitamin D deficiency with COVID-19-related mortality and morbidity. Vitamin D has been stated to be protective in patients with SARS-COV-2 infection.[6–8]

The challenges during this study include the sensitivity of swab testing to accurately diagnose COVID-19, being only 70%. With a pretest probability of 50%, the post-test probability, with a negative test appearing to be 23%, would be far too high to assume someone is not infected.[9] Many patients therefore were treated on clinical grounds even though the swab is negative as 'treat as positive' (TAP). All patients were managed as per NHS England guidelines (publications approval reference: 001559) as of 16 March 2020.[10]

We aimed to investigate any relationship of vitamin D level with 30-day mortality with COVID-19.

MATERIALS AND METHODS

This was a retrospective study involving 1226 patients swabbed for SARS-CoV-2 between 10

February 2020 and 1 May 2020 at Conquest Hospital and Eastbourne District General Hospital.

Demographic data, medical history, blood test results and final outcomes were analysed. A total of 433 patients were included in this study. These patients presented to accident and emergency (A & E) and were swabbed for COVID-19 and had vitamin D levels available either from the time of presentation (n=161) or within 3 months prior to the visit (n=262).

Vitamin D levels were analysed via Elecsys' Vitamin D Total II using Cobas e411, e601 and e602 analysers by Roche Diagnostics. This assay is intended for the quantitative determination of total 25-hydroxyvitamin D in serum and plasma. The Elecsys Vitamin D Total II assay employs a VDBP labelled with a ruthenium complex as capture protein to bind 25-hydroxyvitamin D_3 and D_2. Cross reactivity to 24,25 dihydroxy vitamin D is blocked by a specific monoclonal antibody. Calibration is standardised using internal standards which are traceable to the ID-LC-MS/MS-25-hydroxyvitamin D reference measurement procedure.[11]

Linear and non-linear associations of vitamin D levels with mortality were assessed. In addition, patients with vitamin D levels less than 25 nmol/L were compared with those >25 nmol/L. All patients who were treated as COVID-19-positive by the attending teams due to clinical picture, for example, diarrhoea or bilateral chest X-ray infiltrates, were included in the analysis (TAP), although some of these were swab-negative. In addition, the analysis was repeated using only COVID-19-positive patients to rule out bias due to inclusion of patients without COVID-19. Furthermore, vitamin D levels were also analysed by comorbidity.

Statistical analyses

Categorical variables were expressed in terms of frequency and percentages and were compared using χ^2 test or Fisher's exact test. Continuous variables were described as mean (SD) or median (IQR) and were compared between groups using two-sample t-tests or Mann-Whitney U tests. For vitamin D levels, descriptive statistics have been shown on both the original and loge-scale and include mean (SD), median (IQR) and range. Estimates of the difference in medians between groups and confidence levels have been calculated using quantile regression. Mortality was assessed using logistic regression models. Vitamin D was log-transformed to give a normal distribution before inclusion in the models. Results are presented as OR associated with a 20% increase in vitamin D. Adjustment was made for patient characteristics and comorbidities by including them as covariates. As the prevalence for some covariates was low, we used a penalised model (Firth logistic regression) to deal with any possible bias due to sparse data. A p value of <0.05 was taken to be significant. Non-linearity was assessed using restricted cubic splines. All available data over the study period were used in the analysis. A retrospective power calculation shows that the study was powered to detect an 11 nmol/L difference (or 18% decrease) in those who died compared with survivors with 80% power at the 5% significance level based on an SD of 30 nmol/L. The data were analysed using Stata V.16.

RESULTS

A total of 433 patients were tested for SARS-CoV-2 RNA swab test and had blood tests for evaluating vitamin D levels within last 3 months. The median age was 68 years, with an age range from 1 year to 101 years. There were 52 swab-positive and 381 swab-negative patients. Swab-positive patients were significantly more likely to have malignancy (11.8% vs 4.0%) and diarrhoea (26.0% vs 8.4%) comorbidities (table 1). Vitamin D levels did not differ significantly between swab-negative and swab-positive patients (table 1) with a difference between medians of −1 (95% CI −14.5 to 12.5, p=0.96).

Table 1 Patient characteristics and comorbidities

Variable	Swab-negative N=381	Swab-positive N=52	Total
Age (years)	63.5 (23.5)	67.0 (18.9)	63.9 (23.0)
Sex, % male (N)	48.6 (185)	61.5 (32)	50.1 (217)
Ever smoker, % (N)	5.6 (21)	11.8 (6)	6.3 (27)
Comorbidities			
Diabetes, % (N)			
Type 1	0.8 (3)	2.0 (1)	0.9 (4)
Type 2	3.5 (13)	5.9 (3)	3.8 (16)
Diarrhoea, % (N)	8.4 (30)	26.0 (13)	10.6 (43)
IHD, % (N)	19.2 (72)	25.5 (13)	19.9 (85)
Asthma, % (N)	12.0 (45)	15.7 (8)	12.4 (53)
Hypertension, % (N)	11.4 (43)	19.6 (10)	12.4 (53)
Dementia, % (N)	9.6 (36)	11.8 (6)	9.8 (42)
Frailty, % (N)	13.3 (50)	17.7 (9)	13.8 (59)
ALD-CLD, % (N)	1.3 (5)	3.9 (2)	1.6 (7)
Malignancy, % (N)	4.0 (15)	11.8 (6)	4.9 (21)
PE, % (N)	1.1 (4)	2.0% (1)	1.2 (5)
Vitamin D, nmol/L	52.1 (30.3)	51.9 (27.8)	52.1 (30.0)
Mean (SD)	51 (26–72)	49.5 (31.5–67.5)	50.5 (27–72)
Median (IQR) range	9–195	8–132	8–195
Loge vitamin D	3.77 (0.64)	3.79 (0.60)	3.77 (0.64)
Mean (SD)	3.93 (3.26–4.28)	3.90 (3.44–4.22)	3.93 (3.30–4.28)
Median (IQR) range	2.20–5.27	2.08–4.88	2.08–5.27
Vitamin D <25 nmol/L	23.4 (89)	19.2 (10)	22.9 (99)

ALD, alcoholic liver disease; CLD, chronic liver disease; IHD, ischaemic heart disease; PE, pulmonary embolism.

Among the total of 433 patients, 364 (84.1%) survived, while 69 (15.9%) died within 30 days. Those who died were significantly older, more likely to be ever smokers and to have comorbidities (table 2). Those who died were was also more likely to be swab-positive than survivors (20.3% vs 10.4%, p=0.02) (table 2).

Age, ever smoking and comorbidities were associated with mortality (table 2). Vitamin D levels did not differ significantly between survivors and those who died (table 2 and figure 1), with a difference between medians of 6 (95% CI −5.2 to 17.2, p=0.35).

Vitamin D levels by comorbidity are shown in figure 2. After adjustment for age, sex, ever smoking and comorbidities, no significant association was seen for vitamin D with mortality (table 3). In addition, we found no non-linear association between vitamin D and mortality (figures 3 and 4; p=0.88 and p=0.31 for the model with restricted cubic splines vs the linear model, before and after inclusion of comorbidities). In the sensitivity analysis restricting the analysis sample to those with vitamin D levels measured at presentation (n=161 patients), we found a non-significant decrease in mortality as vitamin D levels increased (table 4).

DISCUSSION

We analysed patients who were treated as COVID-19-positive with or without positive swab at two hospitals and found no difference in the mortality in people with vitamin D deficiency

Table 2 Patient characteristics and comorbidities by mortality

Variable	All patients		Positive patients	
	Alive N=364	Deceased N=69	Alive N=38	Deceased N=14
Age (years)	61.3 (23.5)	77.5 (13.2)	63.6 (19.5)	76.2 (14.0)
Sex, % male (N)	48.4 (176)	59.4 (41)	60.5 (23)	64.3 (9)
Ever smoker, % (N)	3.1 (11)	23.5 (16)	0 (0)	46.2 (6)
Comorbidities				
Diabetes, % (N)				
Type 1	0.6 (2)	2.9 (2)	2.6 (1)	0 (0)
Type 2	0.8 (3)	19.1 (13)	0 (0)	23.1 (3)
Diarrhoea, % (N)	10.7 (36)	10.1 (7)	25.0 (9)	28.6 (4)
IHD, % (N)	13.4 (48)	54.4 (37)	10.5 (4)	69.2 (9)
Asthma, % (N)	6.1 (22)	45.6 (31)	2.6 (1)	53.9 (7)
Hypertension, % (N)	7.5 (27)	38.2 (26)	7.9 (3)	53.9 (7)
Dementia, % (N)	7.8 (28)	20.6 (14)	7.9 (3)	23.1 (3)
Frailty, % (N)	9.8 (35)	35.3 (24)	7.9 (3)	46.2 (6)
ALD-CLD, % (N)	0.3 (1)	8.8 (6)	0 (0)	15.4 (2)
Malignancy, % (N)	2.2 (8)	19.1 (13)	0 (0)	46.2 (6)
PE, % (N)	0 (0)	7.4 (5)	0 (0)	7.7 (1)
Vitamin D (nmol/L)				
Mean (SD)	51.8 (30.6)	53.9 (26.9)	51.9 (30.7)	51.9 (18.6)
Median (IQR)	49 (26–71)	55 (28–73)	43 (29–72)	54 (49–60)
Range	8–195	10–132	8–132	14–86
Loge vitamin D				
Mean (SD)	3.76 (0.64)	3.83 (0.60)	3.77 (0.65)	3.87 (0.47)
Median (IQR)	3.89 (3.26–4.26)	4.01 (3.31–4.29)	3.76 (3.36–4.29)	3.99 (3.80–4.11)
Range	2.08–5.27	2.30–4.88	2.08–4.88	2.64–4.45
Vitamin D <25 nmol/L	23.6 (86)	18.8 (13)	21.1 (8)	14.3 (2)
COVID-19 swab-positive	10.4 (38)	20.3 (14)	–	–

Frailty: Dalhousie Frailty (Rockwood) score of 4 or more classified as frail.
ALD, alcohol liver disease; CLD, chronic liver disease; COPD, chronic obstructive pulmonary disease; IHD, ischaemic heart disease; ILD, interstitial ung disease; PE, pulmonary embolism.

compared with those with normal vitamin D levels after adjusting for comorbidities.

There was increased overall mortality irrespective of vitamin D levels among patients with positive COVID-19 swab tests, older patients, smokers and those with comorbidities.

We did not find an association between vitamin D levels and mortality. We have conducted a comprehensive analysis which investigated the possibility of both linear and non-linear associations.

One study has reported increased mortality with low vitamin D levels; however, the results were not adjusted for comorbidities and frailty.[12] Another study found a protective role of vitamin D in patients with parkinsonism as compared with their healthy relatives as controls towards COVID-19 infection. These findings were in a specific group and may not be generalisable.[13]

Some previous studies have found an association between low vitamin D levels and COVID-19 infections,[14] but the other studies have failed to find an association.[15] Mortality data were not included in these studies.

A letter to the editor of the *British Medical Journal* reports increased mortality with COVID-19 in Nordic countries, Spain and Italy; however, younger people were not represented. The studies referenced looked only at the elderly population and recommended vitamin D supplements and dosing with assumption for association with other respiratory viruses in the past; therefore, findings may not be generalisable.[15] This letter to the

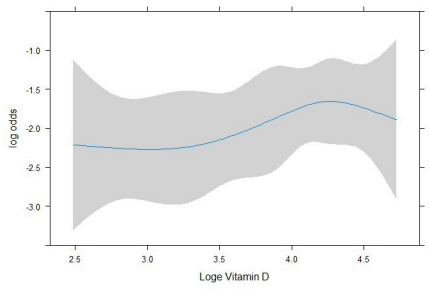

Figure 1 Box plot for distribution of vitamin D levels by mortality.

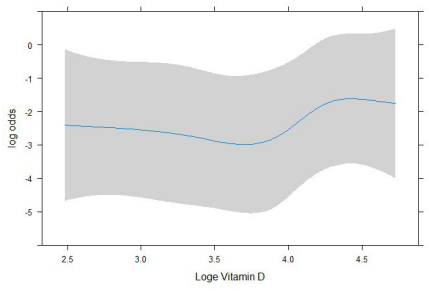

Figure 2 Median vitamin D by comorbidities.

Table 3 Firth logistic regression models for mortality by vitamin D level

	All patients		Swab-positive patients	
	OR (95% CI)	P value	OR (95% CI)	P value
Unadjusted	1.04 (0.96 to 1.12)	0.37	1.05 (0.87 to 1.28)	0.60
Model2	1.06 (0.97 to 1.16)	0.19	1.00 (0.79 to 1.26)	0.99
Model 3	1.09 (0.97 to 1.20)	0.09	0.94 (0.71 to 1.24)	0.66

Model 2: adjusted for age, sex, ever smoking and swab positivity.
Model 3: adjusted for age, sex, ever smoking, swab positivity and comorbidities.

editor was based on previous association findings with respiratory viruses.[16]

One study reported an association between low vitamin D level and COVID-19 infection and mortality with COVID-19; however, the results were not adjusted for comorbidities, and unlike our study, younger patients were not included.[17] Another study has shown 50% mortality in people with vitamin D deficiency compared with 5% mortality in people with vitamin D levels of >10 ng/mL. The study, however, does not comment on comorbidity adjustment.[17 18]

Perhaps it would be more appropriate for clinicians to take into account the overall comorbidities a patient may present with as opposed to solely looking at vitamin D levels to assess for COVID-19 risk, along with optimising management plan.

There were challenges during this study. This relates to sensitivity of the swab results and clinical dilemma towards patients presenting with symptoms and signs of COVID-19, with negative swab results, who were treated on clinical grounds as TAP. Grädel *et al* performed a systematic review and summarised the evidence from observational and randomised controlled studies on the influence of vitamin D deficiency and its treatment on patient outcomes. They concluded no available evidence of general vitamin D screening in the acute setting, and hence, vitamin D levels are not routinely done.[19]

In our sample of study, there were patients with vitamin D levels less than 25 nmol/L who were compared with those with >25 nmol/L as inclusion criteria. This was done on all patients who had these levels done any time from admission going back to 3 months' time prior to admission. This may raise the question of patients who had low vitamin D levels prior to presentation to the hospital and perhaps were on vitamin D supplements already towards correcting the levels. However, there are three debatable issues. Firstly, it is suggested that it

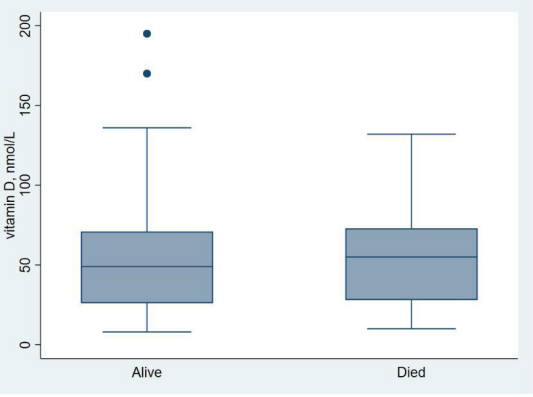

Figure 3 Restricted cubic splines for non-linear association of vitamin D levels with mortality. Adjusted for age, sex, smoking and COVID-19 positivity.

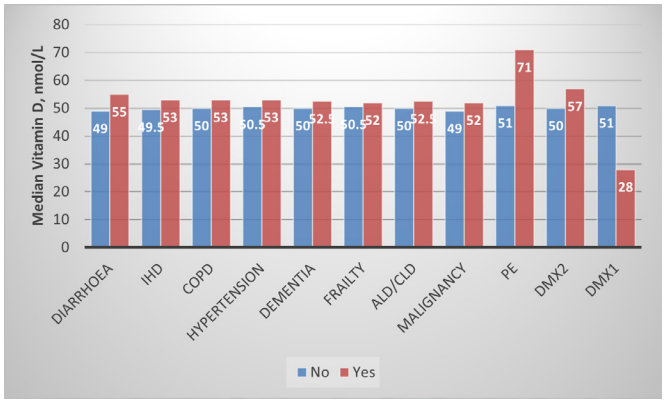

Figure 4 Restricted cubic splines for non-linear association of vitamin D levels with mortality. Adjusted for age, sex, smoking, COVID-19 positivity and comorbidities. ALD, alcoholic liver disease; CLD, chronic liver disease; COPD, chronic obstructive pulmonary disease; DMx1,diabetes mellitus type 1; DMx2, diabetes mellitus type 2; IHD, ischaemic heart disease; PE, pulmonary embolism.

takes 6–12 weeks for levels to normalise, and additionally, it may require higher doses for longer periods of time to maintain optimal blood levels of vitamin D.[20] Pinzon *et al* have done a study and found prevalence of vitamin D deficiency to be 90% (vitamin D levels <20 ng/mL) and 10% of insufficiency (vitamin D levels <30 ng/mL), while the incidence of diarrhoea reported is 10%.[21] Secondly, the association of diarrhoeal illness to deficiency of fat-soluble vitamins including vitamin D, and the role of diarrhoea as one of the major common symptoms of COVID-19 infection, towards malabsorption of vitamin D supplements even if they were previously on the vitamin D supplements.[22 23] Thirdly, the concerns for patients managed with intravenous fluid resuscitation, non-invasive ventilation and intubation make it questionable how efficient oral intake was in the face of very limited oral intake of food or medications. For this confounding variability, we decided to include all patients who had vitamin D levels screened from the time of presentation to going back to 3 months' time, and hence, the contribution of diarrhoea and associated malabsorption leading to persisting vitamin D deficiency towards COVID-19 symptoms with or without the COVID-19 swab results was included. This leaves the clinical dilemma whether the patients who previously had low vitamin D levels and were on supplement should they be assumed to have corrected or near-corrected vitamin D levels, when one of the symptoms of COVID-19 is diarrhoea with significant decreased positive predictive value of the COVID-19 swab itself.

Our study does has several limitations. The number of events in our study was small (n=69), raising the possibility of a false-negative result. Our study was powered to detect an 11 nmol/L

Table 4 Sensitivity analysis: Firth logistic regression models for mortality by vitamin D level in those with levels measured at presentation (n=31 deaths)

	All patients (n=161)	
	OR (95% CI)	P value
Unadjusted	0.98 (0.89 to 1.09)	0.73
Model2	0.96 (0.85 to 1.09)	0.52
Model 3	0.90 (0.76 to 1.07)	0.26

Model 2: adjusted for age, sex, ever smoking and swab positivity.
Model 3: adjusted for age, sex, ever smoking, swab positivity and comorbidities.

difference in vitamin D levels for those who died compared with survivors, compared with a clinically important difference of 15 nmol/L defined using the distribution method (0.5 SD).[24] Our observed difference was small (6 nmol/L, Cohen effect size d=0.2) and so unlikely to be clinically meaningful. It was an observational study with no standardisation for ethnicity, type of presentation or other factors. Patients who had swab tests done and did not require further hospital management were not tested for vitamin D levels and hence were excluded from the study. As the analysis is restricted to this group of patients, there is the possibility of selection bias (collider bias), which could bias associations with outcome. We included only patients who had vitamin D levels measured within the previous 3 months, and therefore, the results may not be generalisable for the whole population of patients. In addition, it is possible that the effect of vitamin D on mortality may be diluted if some patients took vitamin D supplementation following low results measured prior to presentation. For this reason, we repeated the analysis including only those patients with measures made at presentation and found no significant effect. In addition, the inclusion of TAP patients may have led to bias in the overall effect due to the inclusion of patients who were COVID-19-negative. While we have addressed this by also looking within swab-positive patients, this has significantly reduced the numbers, and the power is low within in this group. There are similar studies towards comorbidities that highlight the risks of COVID-19-associated mortality[25 26] and morbidity, including vaccination for prevention of COVID-19 infection.[27]

Main messages

- We did not find a significant relationship between vitamin D levels and mortality among COVID-19 tested or COVID-19-positive patients.
- The most vulnerable group was one with multiple comorbidities such as ischaemic heart disease, hypertension, chronic obstructive pulmonary disease, interstitial lung diseases, asthma, alcoholic liver disease, chronic liver diseases, dementia, frailty, current smoker, ex-smoker, diabetes mellitus types 1 and 2.
- There was increased mortality among swab-positive patients versus patients with negative treated as positive COVID-19 swab results ($p<0.05$).
- Perhaps it would more appropriate for clinicians to take into account the overall comorbidities a patient may present with as opposed to solely looking at vitamin D levels to assess for COVID-19 risk, along with optimising management plan.

Current research questions

- Would the outcome be similar or different if study is standardised for ethnicity?
- Would it not be cost effective to have vitamin D levels checked for patients who were advised isolation at home, following negative COVID-19 swab results and clinically asymptomatic?
- Any significance of extending to include vitamin D levels checked beyond 3 months?

CONCLUSION

In conclusion, we did not find a significant relationship between vitamin D levels and mortality among COVID-19 tested or COVID-19-positive patients. The most vulnerable group was one with multiple comorbidities such as ischaemic heart disease (IHD), hypertension, chronic obstructive pulmonary disease, interstitial lung diseases, asthma, alcoholic liver disease, chronic liver diseases, dementia, frailty, current smoker, ex-smoker, diabetes mellitus type 1 and type 2.

There was increased mortality among swab-positive patients versus patients with negative TAP COVID-19 swab results ($p<0.05$).

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Collaborators Information is entered in the paper as coauthors in the main paper as previously advised by the journal.

Contributors MZ designed the study and formed the steering group, which was responsible for ongoing evaluation for study design development, and led the methodological data collection from hospital electronic system towards comorbidities and access to blood test results. SM and WO assisted with electronic record for COVID-19 swab results for all patients from Conquest Hospital and Eastbourne District General Hospital. MZ, MK, MS, AK, LB, SA, KL, BP, RE, OM, DS, MF, HN, FC, KS, RSR, JH, OO, AE, BA, MP, MA, ZM, BK, AEM, GC, MJZ, NZ, MP, RG, AH and TM contributed with data acquisition and data entry. MZ, MK, MS, RSR, RE, SA and BP were responsible for the ongoing evaluation for study design development. SA, JH, DS, BP, ZM, BK, MK, JH and MP assisted with data assimilation, assisted by all other contributors. MJZ and NZ proofread the entire data for any errors. MZ and SA verified the data. MZ acted as guarantor. Statistical analysis was led by MZ, with intellectual review and support by Ms Jackie Cooper. MZ wrote the manuscript, which was reviewed by UD, MW and PM. All authors and UD approved the final version of the manuscript.

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