Prognostic and therapeutic role of vitamin D in COVID-19: systematic review and meta-analysis

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Abstract

Purpose

Vitamin D deficiency/insufficiency may increase the susceptibility to COVID-19. We aimed to determine the association between vitamin D deficiency/insufficiency and susceptibility to COVID-19, its severity, mortality and role of vitamin D in its treatment.

Methods

We searched CINHAL, Cochrane library, EMBASE, PubMED, Scopus, and Web of Science up to 30.05.2021 for observational studies on association between vitamin D deficiency/insufficiency and susceptibility to COVID-19, severe disease and death among adults, and, randomized controlled trials (RCTs) comparing vitamin D treatment against standard care or placebo, in improving severity or mortality among adults with COVID-19. Risk of bias was assessed using Newcastle-Ottawa scale for observational studies and AUB-KQ1 Cochrane tool for RCTs. Study-level data were analyzed using RevMan 5.3 and R (v4·1·0). Heterogeneity was determined by I^2 and sources were explored through pre-specified sensitivity analyses, subgroup analyses and meta-regressions.

Results

Of 1877 search results, 76 studies satisfying eligibility criteria were included. Seventy-two observational studies were included in the meta-analysis (n=1976099). Vitamin D deficiency/insufficiency increased the odds of developing COVID-19 (OR 1·46, 95% CI 1·28–1·65, p<0·0001, I^2=92%), severe disease (OR 1·90, 95% CI 1·52–2·38, p<0.0001, I^2=81%) and death (OR 2·07, 95% CI 1·28–3·35, p=0.003, I^2=73%). 25-hydroxy vitamin D (25(OH)D) concentration were lower in individuals with COVID-19 compared to controls (mean difference [MD] -3·85 ng/mL, 95% CI -5·44,-2·26, p=<0.0001), in patients with severe COVID-19 compared to controls with non-severe COVID19 (MD -4·84 ng/mL, 95% CI -7·32,-2·35, p=0·0001) and in non-survivors compared to survivors (MD -4·80 ng/mL, 95%-CI -7·89,-1·71, p=0·002). The association between vitamin D deficiency/insufficiency and death was insignificant when studies with high risk of bias or studies

reporting unadjusted effect estimates were excluded. Risk of bias and heterogeneity were high across all analyses. Discrepancies in timing of vitamin D testing, definitions of severe COVID-19 and vitamin D deficiency/insufficiency partly explained the heterogeneity. Four RCTs were widely heterogeneous precluding meta-analysis.

Conclusion

Multiple observational studies involving nearly two million adults suggest vitamin D deficiency/insufficiency increases susceptibility to COVID-19 and severe COVID-19, although with a high risk of bias and heterogeneity. Association with mortality was less robust. Heterogeneity in RCTs precluded their meta-analysis.

Key words vitamin D, COVID-19, SARS-CoV2

Introduction

COVID-19 pandemic remains a global health challenge, claiming over 4 million lives worldwide.[1] Despite vaccine roll-out at scale, it is expected to remain a problem due to inequities in resource allocation and chance of new mutants evading vaccine-mediated protection. Therefore, other treatment and prevention strategies for COVID-19 have been an area of extensive research.

Vitamin D is implicated in optimum function of the immune system. Its deficiency has been linked to susceptibility to respiratory infections.[2,3] It is postulated that vitamin D deficiency/insufficiency is also associated with COVID-19. Low cost, wider availability and ease of administration would make it an attractive and practice-changing intervention if proven effective. These hypotheses have been tested in several observational and interventional studies. Despite strong scientific suspicion, those have yielded variable results. Conclusions of meta-analyses summarizing these have also been mixed.[4–12] Significant but unexplained heterogeneity is common to all analyses. Since the publication of those reports, several more studies have been published.

Therefore, we aimed to systematically review the literature and determine:

1. Does vitamin D deficiency/insufficiency increase the susceptibility to COVID-19 infection, risk of developing severe COVID-19 and risk of death from COVID-19 among adults?
2. In adults with COVID-19, does treatment with vitamin D compared to standard care/placebo improve clinical outcome?

Materials and methods

Search strategy and selection criteria

We conducted a systematic review and independent meta-analyses for three different outcomes of interest: susceptibility to COVID-19, risk of developing severe COVID-19 and death from COVID-19.

We searched for observational studies (prospective or retrospective cohort or case-control) in adults (above 18 years) comparing the rates of above outcomes in groups with and without vitamin D deficiency/insufficiency, and, for observational studies comparing 25-hydroxy vitamin D (25(OH)D) concentration in people with or without above outcomes. We also searched for randomized controlled trials comparing vitamin D therapy against placebo/standard care in improving clinical end-points (length of hospital stay, severe COVID-19, death or any combination) when used to treat adults with COVID-19.

We searched CINHAL, Cochrane library, EMBASE, PubMED, Scopus, and Web of Science databases from their inception to 30·05·2021, using keywords "SARS-CoV-2" OR "COVID-19" OR "Coronavirus" OR "Coronavirus disease 2019" OR "new coronavirus infection" OR "novel coronavirus infection" OR "Coronavirus infection" OR "SARS" OR "severe acute respiratory syndrome" / "vitamin D deficiency" OR "vitamin D insufficiency" OR "hypovitaminosis D" / "treatment" / "vitamin D" OR "cholecalciferol" OR "calcidol" OR "alfa-calcidol" OR "calcitriol" OR "calcifediol" in all fields. The search strategy in full is available in supplementary data file 1, section 1.[13] Articles published in English language, analyzing individual patient-level data were selected. Additional references were identified by manually screening references of the published articles. If abstracts alone were published, we contacted authors to request full-texts. If reported data were inadequate to synthesize effect estimates for the meta-analysis, we contacted authors for additional information. If required data could not be obtained, those studies were excluded from the

meta-analysis. The other exclusion criteria were reporting of population level data, not reporting the outcomes of interest or analyses in done in duplicate (2 reports based on the same population)

Titles/abstracts, and full-texts were screened by two authors independently (SDNdS and CD). Conflicts were resolved by a third author (HAD). When abstract / research letter alone was available, the data were included in the meta-analysis. Their impact on the pooled effect estimate were assessed through sensitivity analysis.

Definition of variables

We used the following definitions to categorize studies in to subgroups for subgroup analysis and/or meta-regression.

Timing of vitamin D testing: We defined 4 categories of timing of vitamin D testing: "long ago" (more than 1 year before the outcome), "before COVID-19" (within a year preceding COVID-19), "after COVID-19" and "variable timing" (before, during or after COVID-19).

Cut off for vitamin D testing: We categorized the studies according to 25(OH)D cut-off used in analysis into the following categories: category 1 (studies using a cut-off 10±3 ng/mL), category 2 (studies using a cut-off 20±3 ng/mL) and category 3 (studies using a cut-off 30±3 ng/mL). One study that used a cut-off of 15 ng/mL was included in category 1. These approximations were required because different studies used different cut-points to dichotomize the data: either based on local or regional protocols or based on the distribution of the study cohorts' 25(OH)D distribution. We use the term 'vitamin D deficiency/insufficiency' to denote vitamin D insufficiency or deficiency of any severity.

Criteria for severe COVID-19: For subgroup analyses, we classified the severity criteria as follows: "hospitalization" (when hospitalization defines severe disease), "hypoxia" (when need for oxygen, non-invasive or invasive ventilation, acute respiratory distress syndrome, or a combination of these

define severe COVID-19), "death" (when death defines severe disease) or "composite" (when a composite of hospitalization, hypoxia or death defines severe COVID-19).

Data analysis

Two authors independently extracted data from selected articles (NLdS and KKKG) under the domains: publication details, setting, design, participant selection criteria, characteristic of participants, exposure and outcome assessment, statistical analysis, raw data relevant for meta-analysis and adjusted and/or unadjusted effect estimates (format in Supplementary data file 1 section 2).[14] When two studies reported data from the same dataset, authors of both studies were inquired for clarification and the most updated dataset were included in the analysis.

Statistical analysis was conducted in R (v·4·1·0) and RevMan 5·3. Inverse variance method and random-effects model was used to pool effect estimates because we anticipated significant between-study heterogeneity. We used DerSimonian-Laird method to calculate the heterogeneity variance τ^2 and Knapp-Hartung adjustments[14] to calculate the confidence interval around the pooled effect. Forest plots were used for graphical representation. Statistical assessments were two-tailed and a p-value less than 0·05 was considered significant.

Susceptibility to infection / severe disease / death was reported as odds ratio in most selected studies. When it was not available, raw data were extracted from the articles to calculate the odds ratios. When adjusted odds ratios were reported (with or without unadjusted odds ratios), those were used for the meta-analysis. All 25(OH)D concentration were converted to ng/mL (1 ng/mL = 2.5 nmol/L) units and mean differences were determined. When median and range or interquartile range of vitamin D were reported, approximate mean and standard deviations were calculated and adopted for the meta-analysis.[15–17]

Robustness of findings were assessed by sensitivity analysis. Heterogeneity across studies was estimated with I^2 statistic. Source of heterogeneity was explored using subgroup analyses and meta-regression.

Elements for sensitivity analyses defined *a priori* were extreme effect estimates (odds ratios < 0·2 or > 5·0; mean differences greater than the 95% confidence interval limits), extreme sample sizes (<100 or >10000), type of publication (with and without abstract-only publications), risk of bias (with and without publications with high risk of bias), and type of effect estimate (adjusted vs unadjusted).

Sub-group analyses were conducted to determine the impact of risk of bias, type of effect estimate (adjusted vs unadjusted), geographical territory (Africa, Asia, Europe, Middle East, North America, South America), definition of COVID-19 severity, definition of vitamin D deficiency/insufficiency and timing of vitamin D testing. We used inverse variance random effects model for subgroup analysis. Tau^2 and its confidence intervals were estimated by DerSimonian-Laird and Jackson methods respectively.

Cut off for definition of vitamin D deficiency/insufficiency, criteria to define severe COVID-19 and geographical territory of the study were the predictor variables defined *a priori* for meta-regression. The model fit was assessed by weighted least squares method. Multiple meta-regression models were assessed by forward selection step-wise approach. The predictor sequence was determined by single variable meta-regression analyses. The models were compared using ANOVA likelihood-ratio test and corrected Akaike's Information Criterion (AICc). Robustness of the models was ascertained by permutation testing.

Risk of bias analysis

The risk of bias was assessed using Newcastle and Ottawa scales for cohort and case-control studies and AUB KQ1 Cochrane tool for RCTs. Two authors independently assessed each publication (NLdS, KKKG). Conflicts were resolved by a third author (HAD). Abstracts and research letters were not

subjected to risk of bias analysis due to limited availability of data. Impact of publications with high risk of bias was assessed by conducting sensitivity analyses. Publication bias was assessed by Funnel plots and by Egger's test.

Results

The literature search yielded 1877 records. After excluding duplicates, 1166 titles/abstracts were screened and 100 were selected for full-text review. Twenty-nine articles were excluded at full-text review (Supplementary data 1, section 3).[13] Five additional publications were identified through manual screening of references. Seventy-six publications that matched the selection criteria were included in this review. This included 62 full papers on observational studies[18-79], 10 publications of abstracts/research letters on observational studies[80–89] and 4 full papers on randomized controlled trials[90–93] (Figure 1). The 72 observational studies selected for the meta-analysis included 1976099 participants (sample sizes range: 20 to 987849, range of mean age 32·0-81·0 years), from 6 geographic territories (Africa 2, Asia 10, Europe 24, Middle East 18, North America 12, South America 2, not reported 4). Characteristics of included studies are summarized in table 1. Summary of risk of bias of included studies is shown in figure 2.

Susceptibility to infection

Nineteen studies (1967068 participants) reported odds ratios for the association between vitamin D deficiency/insufficiency and risk of developing COVID-19. This included one abstract. Six were retrospective cohort studies and 13 were case-control. Eight studies reported adjusted odds ratios. Risk of bias was high in 15/18 and unclear in the remaining. Egger's test indicated significant asymmetry of the Funnel plot (intercept 2·842, 95%-CI 1·70-3·98, t=4·88, p=0·0001).

Vitamin D deficiency/insufficiency was associated with increased odds of developing COVID-19 (OR 1·46, 95%-CI 1·28–1·65, p<0·0001) (Figure 3). However, there was significant statistical heterogeneity (I^2=92%, p<0·0001). The association remained significant in all sensitivity analyses (supplementary data file 1, section 5).[13]

Subgroup analyses by geographic territory (Q=14·02, df 4, p = 0·0072), timing of vitamin D testing (Q=9·39, d.f.=3, p=0·025) and risk of bias (Q=5·75, d.f.=1, p=0·0165) revealed significant between-group heterogeneity. Higher odds ratios were reported in studies from Asia (4 studies, OR 2·60, 95% CI 1·52 – 4·44, tau^2=0·11, Q=4·84, I^2=38·0%), studies reporting 25(OH)D concentration tested after the diagnosis of COVID-19 (5 studies, OR 2·83, 95%-CI 1·35-5·96, tau^2=0·62, Q=45·74, I^2=91·3%) and in studies with high risk of bias (OR 1·55, 95%-CI 1·33-1·82, p<0·0001, tau^2=0·06 Q=202·79, I^2=93·1%). Other subgroup analyses did not contribute to heterogeneity (supplementary data file 1, section 5).[13]

Meta-regressions with single predictor variables indicated that timing of vitamin D testing had a significant impact on effect estimate (F(df1=3, df2=14)=3·68, p = 0·038), accounting for 49·82% of the observed heterogeneity. The model remained robust in permutation testing (F(df1=3, df2=14) = 3·6818, p=0·050). Yet, the residual heterogeneity remained significant (94·69%, p<0·0001). The other two pre-specified predictors did not have a significant impact in single variable meta-regression. On stepwise forward selection multi-model meta-regression, the model combining timing of vitamin D testing and cut-offs used to define vitamin D deficiency/insufficiency had a significant impact on effect estimate (F(df1=4, df2=10)=3·68, p=0·03), and was superior to the above single variable model in ANOVA test for model comparison (df=6, AICc=29·56 for full model vs df=3, AICc 31·00 for single variable, p=0·0013).

Eighteen studies (616261 participants) compared the difference in 25(OH)D concentration between people with COVID-19 infection and those without. Two were abstract only publications. Fourteen of the 18 studies had high risk of bias; rest had unclear risk. Funnel plot inspection and Egger's test

(intercept -1·675, 95% -CI -5·12, -1·77, t = -0·952, p=0·355) indicated a low risk of publication bias. The mean 25(OH)D concentration in people with COVID-19 infection was lower compared to those without (mean difference -3·85 ng/mL, 95% CI -5·44, -2·26, p = < 0·0001) (Figure 4). Heterogeneity across studies was high (I^2=97·7%, p<0·0001). Difference remained significant in all sensitivity analyses (supplementary data file 1, section 6). [13]

In summary, vitamin D deficiency/insufficiency increased the odds of developing COVID-19. Patients with COVID-19 had lower 25(OH)D concentration that those without. Wide heterogeneity across studies is partly explained by differences in timing of vitamin D testing, geographical territory of the study, cut-off used to define vitamin D deficiency/insufficiency and risk of bias. Most case-control studies assessing the association between vitamin D status and risk of developing COVID-19 had a high risk of bias since exposure status was determined after the onset of outcome.

Risk of developing severe COVID-19

Thirty-six studies (367852 participants, 32 full-texts) reported on the association between vitamin D deficiency/insufficiency and severe COVID-19; 18/32 had high risk of bias. Only 18/36 papers reported adjusted odds ratios. Funnel plot was asymmetric, confirmed in Egger's test (intercept 2·84, 95-CI 1·70 – 3·98, t = 4·88, p = 0·0001).

Vitamin D deficiency/insufficiency increased the odds of developing severe COVID-19 (OR 1·90, 95%-CI 1·52 – 2·38, p < 0·0001). However, there was a significant statistical heterogeneity (I^2 = 81%, p < 0·00001) (Figure 5). Association remained significant in all sensitivity analyses (supplementary data file 1, section 7).[13]

A significant between-group heterogeneity was observed when studies were grouped according to the criteria used to define disease severity (Q =9·09, d.f. = 3, p = 0·03). Studies reporting a composite of

mortality and respiratory failure reported a higher odds ratio than the others (9 studies, OR 2·63, 95% CI 1·60 – 4·36, *tau*^{2} 0·34, Q=33·40, I^2 = 76%). No significant heterogeneity was observed in other subgroup analyses (supplementary data file 1, section 7).[13]

In single variable meta-regression models, none of the tested variables (criteria for vitamin D deficiency/insufficiency, criteria for disease severity, geographical region of the study) effectively predicted the effect size. Therefore, we conducted a post-hoc multi-model analysis including the above pre-specified variables and 2 additional variables: adjusted vs non-adjusted effect estimates and risk of bias. Yet no models effectively predicted the effect size (supplementary data file 1, section 7).[13]

Eighteen studies (2566 participants) compared the levels of vitamin D in people with complicated versus uncomplicated COVID-19. Three were abstract-only publications. Fourteen (of 15) studies had a high risk of bias. Publication bias was minimal (Egger's test: Intercept 0·346, 95%-CI -2·47, -3·16, t = 0·241, p = 0·8125) (supplementary data file 1, section 8).[13] Patients with severe COVID-19 had a lower 25(OH)D concentration (mean difference -4·84 ng/mL, 95% CI -7·32, -2·35, p=0·0001). Heterogeneity across studies was high (I^2 89%, p<0.00001) (Figure 6). The significance in difference remained in sensitivity analyses conducted excluding abstract only publications, studies with high risk of bias and extreme effect size (mean difference greater than the upper limit of 95% confidence interval, ie 8·04 ng/mL).

In summary, vitamin D deficiency/insufficiency increased the odds of developing severe COVID-19. Patients with severe COVID-19 had lower 25(OH)D concentration . Heterogeneity was significant and may partly be explained by differences in definition of severe disease. Most studies, did not report the timing of vitamin D testing in relation to the stage of illness, leading to high or unclear risk of bias in the exposure and outcome assessment domain.

Mortality

We identified 20 publications (3686 participants) reporting association between vitamin D deficiency/insufficiency and risk of death from COVID-19. All were full paper publications. Ten were prospective studies while the others were retrospective. Only eight studies reported adjusted effect estimates. Twelve studies were judged to have high risk of bias. Asymmetry in funnel plot was minimal (Egger's test intercept 1·78, 95%-CI 0·09-3·48, t = 2·06, *p*=0·054).

Vitamin D deficiency/insufficiency increased the odds of death from COVID-19 (OR 2·07, 95%-CI 1·28–3·35, p=0·003, I^2=73%) (Figure 7). The significance of association was lost in sensitivity analyses excluding publications with high risk of bias (8 publications, 1368 participants, OR 1·93, 95%-CI 0·75–4·96, p=0·17, I^2 = 80%), studies reporting unadjusted odds ratios (8 publications, 1773 participants, OR 2·22, 95%-CI 0·88-5·59, p=0·09, I^2 = 83%) and studies with extreme effect estimates (13 publications, 3071 participants, OR 1·18, 95%-CI 0·78-1·78, *p*=0·44, I^2 = 56%). The significance remained in the other sensitivity analysis for sample size (Supplementary data file 1, section 9).[13]

In subgroup analysis, grouping by cut-off to define vitamin D deficiency/insufficiency showed significant between-group heterogeneity (Q=12·33, d.f.=2, p=0·0021). Higher odds ratios were observed in studies using lower cut offs (10±2 ng/mL) (OR 5·03, 95% CI 2·72-9·30, p < 0·0001, I^2=26·3%). (supplementary data file 1, section 9).[13] Other subgroup analyses for geographic territory, risk of bias, adjusted vs unadjusted effect estimates did not show significant between-group heterogeneity.

In stepwise multi-variable meta-regression analysis, only the model comprising of vitamin D cut-off as the predictor variable was significant, accounting for 59·73% of the heterogeneity (F(df1=2, df2=16) = 5·45, p=0·0157) but significant residual heterogeneity remained (I^2=53·03%, p=0·0033) (supplementary data file 1, section 9).[13]

Nine studies (n=1421) comparing 25(OH)D concentration in survivors and non-survivors of COVID-19. Eight were full-paper publications. Six (of eight) had high risk of bias. Funnel plot was asymmetric. Egger's test was not applied due to small number of studies.

Non-survivors had lower mean 25(OH)D concentration compared to the survivors (mean difference -4·80 ng/mL, 95%-CI -7·89, -1·71, p=0·002) (figure 8). Studies were significantly heterogeneous (I^2=85·1%, p<0·0001). The association lost significance when studies with extreme effect estimates (>7·89 ng/mL) were excluded (6 studies, 1147 participants, OR -2·11, 95%-CI -4·34, 0·13, p=0·06). Difference remained significant in other sensitivity analyses (supplementary data file 1, section 10).[13]

In summary, vitamin D deficiency/insufficiency increased the odds of death from COVID-19. Non-survivors had lower 25(OH)D concentration compared to survivors. However, this finding is likely influenced by studies with high risk of bias, studies reporting unadjusted effect estimates and studies with extreme effect estimates.

Vitamin D in the treatment of COVID-19

Four randomized controlled trials assessed vitamin D therapy in treatment of COVID-19 (Table 2). Of the three studies reporting hard clinical endpoints, two showed no benefit of vitamin D therapy. All studies had small number of participants. There were significant variations in participant selection criteria, vitamin D regimen and outcomes assessed. Considering this heterogeneity, their methodological limitations and risks of bias, a meta-analysis was not performed (supplementary data file 1, section 4).[13]

Discussion

Our findings indicate increased odds of developing COVID-19, progression to severe COVID-19 and death in people with vitamin D deficiency/insufficiency. People who developed COVID-19, severe COVID-19 and fatal disease had lower 25(OH)D concentration compared to people without COVID-19 or non-severe COVID-19 or non-fatal COVID-19 respectively. Association with fatal COVID-19 was less robust. Overall, the studies are largely heterogeneous with significant risk of bias. Discrepancies in timing of vitamin D testing in relation to the illness, definition of severe COVID-19 and cut-off used to define vitamin D deficiency/insufficiency were the key contributors to heterogeneity in association between vitamin D deficiency/insufficiency and susceptibility to COVID-19, severe COVID-19 and death, respectively. Our findings add evidence to the hypothesized association between vitamin D deficiency/insufficiency and COVID-19. However, observational nature and heterogeneity of the studies precludes deriving definite conclusions.

Previous meta-analyses explored the association between vitamin D deficiency/insufficiency and risk of developing COVID-19 [11,12], or developing complications of the disease,[5] or both [8,10] while another reported prevalence of vitamin D deficiency/insufficiency among patients with COVID-19 without a comparison group.[94] All included less than 40 studies in meta-analysis. A significant association was shown in some[10,11] but not others.[8] Significant heterogeneity was a common feature, but the sources remained inadequately explained. Three meta-analysis reported therapeutic benefit of vitamin D in patients with COVID-19.[6,9,95]

This meta-analysis is the most updated and largest in terms of number of studies and participants, in the topic to the best of our knowledge. We explored clinically relevant endpoints: susceptibility to COVID-19, severe disease and death. Association with each outcome was analyzed in two dimensions: risk estimate as odds ratio and the mean difference of 25(OH)D concentration. We tested

the robustness of association through multiple sensitivity analyses and recognized contributors to heterogeneity through subgroup analyses and meta-regressions.

The main source of bias in the studies stemmed from exposure and outcome assessment: ie the timing of vitamin D testing in relation to the illness. Evaluating the risk of developing COVID-19 requires a large cohort of individuals with a pre-morbid 25(OH)D concentration determined and followed-up over a period of time for development of COVID-19: a less pragmatic strategy. Evaluating the role of vitamin D in severity of the illness is methodologically less challenging. However, 25(OH)D concentration is known to decrease with acute illness or inflammation.[95] The change may have a bidirectional effect: it may be causal, driving the worsening of illness, or it may be an effect of the severe illness (i.e. reverse-causality). Most reported studies indicate the timing of vitamin D testing in relation to the day of admission rather than the stage of illness, thus obscuring the interpretation of findings.

The other source of bias arose from the challenge in having comparable groups and/or in adjusting for appropriate confounding variables. Vitamin D deficiency/insufficiency has been linked to myriad of diseases, some of which are recognized risk factors for severe COVID-19. For example, a recent study reported vitamin D deficiency to be associated with hyperglycaemia, high body mass index and worse severe COVID-19, implying complex interplay between risk factors.[97] Therefore, a comprehensive adjustment for such confounding variables is likely to be overly exhaustive and meaningless. But, it is important to consider the comparability of clinical profiles of studied subjects and adapt methods to adjust for variations in the common and strong risk factors for severe COVID-19 like atherosclerotic cardiovascular disease, hypertension and metabolic syndrome.

The four interventional studies reported some benefit in vitamin D in the treatment of COVID-19. Improvement in inflammatory markers was consistent but only one study showed clinical benefit while the others were neutral. However, there is marked heterogeneity in study population characteristics and type of intervention (dose, duration and timing). Furthermore, it is questionable

whether administration of vitamin D after the onset of illness raises the body's active 25(OH)D concentration fast enough to have a significant impact. Therefore, more randomized controlled trials with early administration of adequately high doses of vitamin D are needed.

There are several limitations in our analysis. First, most studies had a high risk of bias, hence the need for cautious interpretation of the findings. Second, we could not establish a model to fully explain the wide heterogeneity in observed results across studies, with the pre-specified predictors as well as with other post-hoc analyses. This is probably due to wide clinical and methodological heterogeneity and bias. Third, we could not analyze several important predictors of heterogeneity like sex, ethnicity, body mass index and co-morbidities due to lack of disaggregated data. Fourth, we could not determine outcomes like length of hospital stay, thromboembolic complications, cost-effectiveness of treatment and impact on patient-perceived outcomes (wellbeing and quality of life during and after COVID-19). Another problem in pooling data from different 25(OH)D studies is the differences in 25(OH)D testing methods. While some assays measure cholecalciferol and ergocalciferol in combination, others measure cholecalciferol only. The specific method is not reported in most studies. In the absence of a standardized method for vitamin D testing, the measured 25(OH)D concentrations may not reflect the true circulating 25(OH)D concentration. Finally, although we identified four randomized controlled trials evaluating the therapeutic role of vitamin D, meta-analysis of those findings was precluded by significant heterogeneity.

Nevertheless, the finding of possible increased susceptibility to COVID-19 and severe COVID-19 with vitamin D deficiency/insufficiency calls for future research. Therapeutic role of vitamin D needs urgent evaluation in well-designed randomized trials. Interventional studies should examine clinically relevant endpoints and adopt standardized definitions of vitamin D status and outcomes, thus ensuring relevance and comparability across studies. Vitamin D is a relatively inexpensive treatment. If proven effective, it has the potential to change the course of COVID-19 pandemic.

Conclusions

Vitamin D deficiency/insufficiency may increase the risk of developing COVID-19 infection and susceptibility to more severe disease. Its association with mortality is less robust. The data arise from a heterogeneous group of studies with substantial risk of bias, hence the less certainty of evidence and need need for cautious interpretation. Randomized controlled trials investigating the therapeutic role of vitamin D were largely heterogeneous in design, precluding a meta-analysis.

Declarations

Author contributions

PK and MS conceived the research question and provided leadership. MS, HAD, PK, SP and NLdS defined the research questions. DCK planned and conducted the literature search. SDNdS, and CD screened abstracts and full-texts. NLdS, KKKG and HAD conducted data extraction and risk of bias analysis. HAD planned and conducted the statistical analysis. PR critically reviewed the statistical methods and results. HAD drafted the manuscript and compiled supplementary data files. HAD and SDNdS developed figures. PK, SP, MS critically reviewed the manuscript. HAD, NLdS and KKKG vouch for fidelity of the data. All authors read and approved the final manuscript for submission.

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Data availability Additional data (protocol, data extractions of individual studies, summary of extracted data of all studies and for studies in different meta-analyses, analytical codes and detailed results) are available with HAD and can be provided upon request.

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Figure legends

Figure 1. PRISMA diagram

Figure 2. Summary of risk of bias

Figure 3. Forest plot of studies reporting association between vitamin D deficiency / insufficiency and susceptibility to COVID-19

Figure 4. Forest plot of studies reporting comparison of 25(OH)D concentration in patients with and without COVID-19

Figure 5. Forest plot of studies reporting association between vitamin D deficiency / insufficiency and severe COVID-19

Figure 6. Forest plot of studies reporting comparison of 25(OH)D concentration in patients with severe and non-severe COVID-19

Figure 7. Forest plot of studies reporting association between vitamin D deficiency / insufficiency and death due to COVID-19

Figure 8. Forest plot of studies reporting comparison of 25(OH)D concentration in non-survivors and survivors of COVID-19

Table 1. Characteristics of studies included in the meta-analysis

Study	Design	Country	Population characteristics			Exposure		Outcome
			Sample size: total (vit D not sufficient and sufficient groups / cases and controls)	Age in years mean (SD) / median (IQR)	Males (%)	Vitamin D cut off for analysis (ng/mL)	Timing of vitamin D testing	
Abdollahi 2020 [20]	Case control	Iran	402 (201, 201)	48.0	46.3	30	after diagnosis	COVID-19 infection (by RT PCR on NPA)
Abrishami 2020 [21]	Retrospective cohort	Iran	73 (12, 61)	55.18 (14.98)	64	25	on admission	Mortality
Adami 2021 [22]	Retrospective cohort	Italy	61 (44, 17)	69.4 (15.3)	69.4	20	on admission	hypoxia (< 60 mmHg), mortality
Alsafar 2021 [25]	Prospective cohort	UAE	464 (309, 155)	46.6 (14.9)	80.2	20	on recruitment	severity of COVID, according to WHO 2020 criteria
Alsegai 2021 [26]	case control	Egypt	58 (31, 27)	60.7 (14.3)	46.6	32	on admission	Mortality
Al-azzawy 2021 [23]	case control	Iraq	150 (120, 30)	NR	among cases 62.5	NA	NR	COVID-19 based on RT PCR on a nasopharyngeal aspirate
Al-Daghri 2021 [24]	case control	Saudi Arabia	220 (138, 82)	43(15) cases 50 (13) controls 32 (13)	54.5 (cases 57.2, control 50)	NA	on admission	mild COVID-19 (no hypoxia / pneumonia)
Angelidi 2020 [27]	retrospective cohort	USA	144 (79, 65)	VDD 60 (48-72) VDS 68 (63.5-76.0)	VDD: 51.9, VDS: 35.4	30 (and 20)	within preceding 6 months	death and need for mechanical ventilation
Anjum 2020 [28]	Prospective cohort	Pakistan	140 (82, 58)	42.46 (14.73)	59.0	10	NR	Mortality
Ansari 2020 [29]	Prospective cohort	India	125 (14, 111)	45.58 (15.66)	60.0	10	NR	Mortality
Backtash 2020 [30]	Case control	UK	105 (70, 35)	81 (range: 65-102)	54.3	12	on admission	COVID-19 infection (by RT PCR on NPA)
Backtash 2020 [30]	Prospective cohort	UK	70 (39, 21)	Vit D deficient: 79.46 (9.52) Vit D sufficient: 81.16 (7.23)	VDD: 61.5, VDS: 58.1	12	on admission	Mortality

Bennouar 2020 [31]	prospective cohort	Algeria	120 (37 deaths)	62.3 (17.6)	69.2	<10	on admission	severe COVID-19 based on WHO criteria
Brandao 2021 [32]	Retrospective cohort	Brazil	13930 (2345, 11585)	NR	NR	20	30 days before or after COVID diagnosis	COVID-19 (RT PCR on respiratory secretions)
Bychinin 2021 [33]	Retrospective cohort	Russia	50	NR	NR	20	several months before pandemic	COVIF 19
Bychinin 2021 [33]	case control	Russia	65 (40, 25)	NR	NR	20	during illness	severe COVID 19 (criteria not reported)
Bychinin 2021 [33]	Prospective cohort	Russia	40 (18, 22)	61 (52.5-80)	50	9.9 for mortality risk	On admission to ICU	Mortality
Carpagnano 2020 [34]	Retrospective cohort	Italy	42 (10, 32)	Vit D deficient: 74 (11) non-deficient: NR	VDD: 80 VDS: NR	10	NR	Mortality
Cereda 2020 [35]	Prospective cohort	Italy	129 (99, 30)	73.56 (13.9) Vit D deficient: 77 (64-85). Non-deficient: 77.5 (65-86)	54.3 (VDD: 57.6, VDS: 43.3)	20	within 48h of hospital admission	Mortality
Chang 2020 [74]	Case control	USA	10992 (992, 10000)	Cases: 49 (20)	Cases: 48	NR	1 year prior to COVID-19 diagnosis	COVID-19 infection (by RT PCR on NPA)
Charoenngam 2021 [36]	Retrospective cohort	USA	287 (sufficient 100, insufficient 91, deficienct 96)	55.9 ± 15.8 63.7 ± 14.3 66.2 ± 15.7 in deficient, insufficient and sufficient groups respectively	55.2, 53.8, 49 in deficient, insufficient and sufficient groups respectively	NA (continuous variable)	within 48h of admission	Primamry: in-hopsital mortality
D Avolio 2020 [37]	Case control	Switzerland	107 (27, 80)	73 (63-81) cases: 74 (65-81) controls: 73 (61-82)	54.2% (Cases: 70.4, controls: 48.8)	Not applicable	3 days after RT PCR test	COVID-19 infection (by RT PCR on NPA)

Davoudi 2021 [38]	Retrospective cohort	Iran	153 (96, 57)	NR	53.9	30	at the time of hospitalization	Severe COVID-19 (WHO definition)
De Smet 2020 [39]	Retrospective cohort	Belgium	186 (27, 159)	Cases: 81 (72-87) Controls: 73 (53-81)	case: 66.7, Controls: 46.8	20	On admission with COVID-19 pneumonia, within 2 hours from chest CT staging	Mortality
Demir 2020 [40]	Retrospective cohort	Turkey	487 (227, 260)	NR	NR	10	within the preceding 6 months	RT PCR positive COVID-19
Elham 2021 [41]	Case control	Iran	283 (93, 186)	51 (40-61)	44.1	NA	after symptoms onset / testing for COVID-19	25(OH)D concentration is lower in patients with COVID-19 than those without
Ersoz 2021 [42]	Retrospective cohort	Turkey	310	57.02 (18.28)	51.9	NA	within preceding 6 months	ICU admission, intubation, death
Ferrari 2020 [43]	Case control	Italy	347 (128, 219)	cases 65.0 (15.0) controls 58.7 (20.2)	cases: 64.8, controls: 48.9	30	before during or after illness (between the 1st of January and the 31st of May, 2020)	COVID 19 diagnosed based on RT PCR on a swab test
Gaudio 2021 [44]	case control	Italy	150 (50, 100)	cases 65 (24-98) controls 61 (22-89) [median and range]	cases 52, controls 44	NA	first 5 days of admission	COVID 19 (by RT PCR) severe COVID 19 (death / need for ventilatory support - invsive / non-invasive)
Gavioli 2020 [45]	Retrospective cohort	USA	437 (177, 260)	total : 67 (56-79) Vitamin D deficient: 63 (54-76) Vitamin D sufficient: 69 (58-80)	total sample: 48, VDD: 55 VDS: 43	20	within 3 months preceding the admission	Hospital admission, need for oxygen support, and 90 day mortality

Hernandez 2020 [47]	Case control	Spain	394 (197, 197)	Cases: 61.0 (47.5-70.0) Controls: 61.0 (56.0-66.0)	62.4	20	After admission for cases. Among controls: data from Vitamin D tests done in the previous year January to March	Severe COVID-19: composite of admission to the intensive care unit (ICU), requirement for mechanical ventilation, or in-hospital mortality.
Hernandez 2020 [47]	Prospective cohort	Spain	197 (162, 35)	tota sample: 61.0 (47.5-70.0)	62.4	20	not reported	COVID-19 infection (by RT PCR on NPA)
Im 2020 [48]	case control	South Korea	200 (50, 150)	cases 52.2 (20.7) controls: 52.4 (20.2)	cases 58.0, controls - NR	20	within 7 days of admission	COVID-19
Im 2020 [48]		South Korea	50 (38, 12)	52.2 (20.7)	5800%	20	within 7 days of admission	need for oxygen
Infante 2021 [49]	Case control	Italy	137 (59, 78)	cases 70 (61-80) controls 65 (55-65)	cases 78.0, controls 55.0	NA	after admission	Mortality
Israel 2020 [18]	Case control	Israel	576455 (52405, 524050)	32 (18-50)*	47.1	< 12 vs > 30	within preceding 10 years	PCR positive COVID-19
Jain 2020 [50]	retrospective cohort	India	154 (63, 91)	cases 51.41 ± 9.12 control 42.34 ± 6.41	Cases: 53, controls : 42	20	on admission	Clinical signs of pneumonia (fever, cough, breathlessness) plus one of the following: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO2 < 90% on room air. b. Signs of multi-organ involvement: altered sensorium, decreased urine output, heart Rate > 120/min, with cold extremities or low blood pressure (Systolic BP < 90 mm of Hg and/or Diastolic BP < 60 mm of Hg). c. Laboratory evidence of coagulation abnormalities, thrombocytopenia, acidosis (pH < 7.25), lactate level > 2 mmol/L, or hyperbilirubinemia.

Jevalikar 2021 [51]	Retrospective cohort	India	410 (197, 213)	54 (6-92) [median and range] VDD: .46.7 (17.1) VDS: 57.8 (14.7)	cases: 68, controls: 69.8	20	NR	Severe COVID-19 (based on outcome severity score)
Karahan 2020 [52]	Case control (Fatal Vs surviving COVID-19)	Turkey	149 (102, 47)	overall 63.5 (15.3) cases 67.0 (14.1), controls 56.1 (15.2)	overall 54.4 (cases 48.9, controls 56.9)	20	after admission	Primary outcome: all cause mortality secondary outcomes: severe - critical illness Vs moderate illness. Severe disease: The presence of any of the following criteria: i) respiratory distress (≥ 30 breaths/min); ii) oxygen saturation ≤ 93% at rest; iii) PaO2/FiO2 ≤ 300 mmHg or chest imaging shows obvious lesion progression > 50% within 24-48 hours) • Critical disease: The presence of any of the following criteria: i) respiratory failure and need for mechanical ventilation; ii) shock; iii) other organ failures that requires ICU care.
Katz 2020 [53]	Retrospective cohort	USA	987849 (31950, 955899)	NR	VDI: 69.7	NR	Most likely prior to the onset of illness (2015.10.01 to 2020.6.30)	COVID-19 infection (based on database records)
Kerget 2020 [54]	Case control (COVID-9 patients Vs asymptomatic HCW)	Turkey	108 (88, 20)	NR	cases 46.6, controls 40.0	NR	after admission	COVID 19 infection (by RT PCR or commerical kit on NPA or bronchial washings) COVID-19 with Macrophage Acivating Syndrome or ARDS data for ARDS used for meta-analysis. Data for risk of COVID-19 infection in case control design not adequate for meta analysis
Lau 2020 [55]	Case control	USA	20 (13, 7)	cases 61.5 (15.7) controls 72.0 (14.8)	cases 61.5, controls 14.3	30	after hospitalization	ICU admission
Li 2021 [56]	Retrospective cohort	UK	353299	67.7 (8.1)	45.6	10 and 20	long before (2006-2010)	COVID-19 infection (by RT PCR on NPA) and severe COVID19 and severe COVIID19 (need for hospitalization)

Livingston 2020 [57]	Case control	UK	104 (47, 57)	cases: 68.6 (18.7) controls: 68.5 (18.1)	cases: 42.6, controls: 33.3	13.76	within the 6 months preceding admissions	COVID 19 infection (by RT PCR or commerical kit on NPA or bronchial washings)
Lohia 2020 [58]	Retrospective cohort	USA	270	63.81 (14.69)	43.3	20	within 12 months preceding the infection	Mortality (ICU admission, Venous thrombosis, need for venntilation analyzed independently)
Luo 2021 [59]	Prospective cohort	China	74	62.5 (51.0–75.3)	cases 58.1	30	on admission	severe covid 19: respiratory distress, respiratory rate ≥30 breaths/min, hypoxemia, oxygen saturation (SpO2) ≤93% (at rest), or lung infiltrates of >50% within 24–48 h] critical covid 19: meeting any of the following criteria: respiratory failure requiring mechanical ventilation, shock, or multiple organ dysfunction requiring intensive care unit monitoring and treatment. Non-severe patients whose symptoms became progressively severe during hospitalization were defined as severe cases
Luo 2021 [59]	case control	China				12	before	RT PCR positive COVID-19
Ma 2021 [60]	Retrospective cohot	UK	8297 (1378, 6919)	cases 56.2 (9.2) controls: 57.8 (8.4)	cases 53.4, controls 48.7	10 (vs >20)	10-14 years ago	COVID-19 (RT PCR on respiratory secretions)
Macaya 2020 [61]	Retrospective cohort	Spain	80 (45, 35)	NR	NR	20	on admission or within 3 preceding months	severe COVID defined by: death, admission to the intensive care unit, and/or need for higher oxygen flow than that provided by a nasal cannula
Maghbooli 2020 [62]	Retrospective cohort	Iran	235 (158, 77)	NR	NR	30	on admission	Severe disease (dyspnea, respiratory frequency >30/minute, blood oxygen saturation < 93%, and/or lung infiltrates >50% of the lung field within 24–48 hours) and critical (respiratory failure, septic shock, and/or multiple organ dysfunction/failure). Patients with at least two complications, including acute respiratory distress syndrome (ARDS), acute cardiac injury (ACI), acute kidney injury (AKI) or acute liver injury consider as multiple organ damage.

Mazziotti 2021 [63]	Retrospective cohort	Italy	348	68	64.0	12	after admission (28 patients had 25(OH)D concentration before the admission, within the preceding 6 months)	Mortality
Meltzer 2020 [64]	case control	USA	489 (172, 317)	cases 45.9, controls 51.0	cases 23.0, controls 27.0	20	before the onset of illness	COVID-19 Vs no infection
Mendy 2020 [19]	case control	USA	689 (91, 598)	cases 60.5, controls 47.2	cases 50.5, controls 50.5	NR	not reported	admission to ICU and/or death during hospitalization.
Merzon 2020 [65]	Case control	Israel	7807 (782, 7025)	cases 35.58 (34.4.9 - 36.67)* controls 47.35 (46.87 - 47.85)	cases 49.2, controls 40.6	20	before illness; timing not specified	RT PCR (specimen not clearly reported)
Nasiri 2021 [66]	Prospective cohort	Iran	329 (32 deceased)	64.7 (18.5)	50.8	20	on admission	death (other outcomes - leangth of hospital stay, not included in the analysis)
Orchard 2021 [67]	retrospective cohort	UK	165 (116, 49)	NR	NR	20	on admission	Mortality, need for ICU care
Osman 2021 [68]	retrospective cohort	Oman	445 (133, 312)	50.8	62.0	20	NR	intubation, mortality,
Radujkovic 2020 [68]	Prospective cohort	Germany	185	60 (49-70)	51.0	12	on admission	death and/or need for invasive mechanimal ventilation
Raisi-Estabragh 2020 [70]	Case control	UK	4510 (1326, 2184)	cases: 68.11 (± 9.23) controls: 68.91 (± 8.72)	cases 52.5, controls 47.3	NA	between 2006-2010	COVID-19 diagnosed based on RT PCR
Susianti 2020 [71]	prospective cohort	Indonesia	50 (8, 42)	NR	54.0	20	on the first day of admission	severe COVID (clinical DVT /ISTH DIC score 5 or more)
Szeto 2021 [72]	retrospective cohort	USA	93	NR	NR	20	within 1 year preceding admission	death
Tehrani 2021 [73]	Retrospective cohort	Iran	205 (43, 162)	59.71 (14.92)	33.7	10	NR	death

Tuncay 2021 [75]	Retrospective case control	Turkey	655 (596 cases: 450 with non-severe, 146 with severe COVID-19 - 120 survived)	non-severe: 48.1 (9.4) severe-survived: 66.6 (7.2) severe-nin-survival: 68.2 (9.2)	non-severe: 75.5, severe-survived: 60.0, severe-non-survived: 69.2	NA	NR	COVID-19 (Clinical features / RT PCR / radiology based WHO critieria), severe COVID-19 (not defined), death
Unsal 2021 [76]	retrospectiive cohot	Turkey	56	median age 56 (range 26-76)	32.1	20	within preceding 6 months before COVID-19	need for respiratory support (criteria not reported)
Vanges Cedillo 2021 [77]	Prospective cohort	Mexico	551	51.92 (13.74)	64.4	12	at the time of presentation	Mortality
Vasiliou 2020 [78]	Prospective cohort	Greece	30	65 (11)	80.0	15.2	on admission to ICU	Mortality
Walk 2020 [46]	Prospective cohort	Netherlands	133 (58, 75)	68 (12)	69.0	NA	after admission	severe COVID-19 (Need for intubation/ ventilation or death)
Ye 2020 [79]	case control	China	142 (62, 80)	cases 43 (39-52) and controls 42 (31-52)*	37.0, 40.0	20	after admission	SARS CoV 2 PCR in throat swab
Ye 2020 [79]	Prospective cohort	China	60 (10, 50)	43*	37.0	20	after admission	According to guidelines of national health commission of China severe COVID and critical COVID were defined

Table 2. Summary of characteristics and findings from randomized controlled trials evaluating clinical outcomes of COVID-19 after treatment with vitamin D

Study, (Country), method	Participants	Intervention	Control	Outcome	Results
Rastogi, 2020 [93] (India) Randomised, placebo controlled (placebo not identical)	Asymptomatic or mildly symptomatic individuals with SARS-CoV-2 infection, vitamin D <20 ng/mL and without co-morbidities or ventilation. (Intervention =16, placebo= 24)	Oral Cholecalciferol 60000 IU daily for 7 days (If target 25(OH)D concentration > 50 ng/mL not achieved on day 7, same dose continued, if target achieved weekly 60000 IU supplemented)	Placebo	Proportion of patients with negative SARS-CoV-2 virus RNA by day 21. Change in the inflammatory markers.	Significant difference in SARS-CoV-2 RNA negativity in day 21 between intervention and control groups (62.5% Vs. 20.8%, p=0.018). Fibrinogen levels (ng/mL) reduced significantly in the intervention group (-0.9 Vs. -0.04, p=0.001). No difference in the other inflammatory markers. No hypercalcaemia in the intervention group.
Entrenas Castillo, 2020 [90] (Spain) Randomised open label, double-masked study	Patients older than 18 years with positive SARS-CoV-2 PCR, clinical and radiographic pattern of viral pneumonia and CURB>1 (Intervention= 50, placebo=26)	Oral calcifediol 0.532 mg on day of admission and 0.266 on day 3, 7 and weekly until discharge	Standard care	Rate of ICU admission and death	Need for ICU admission was lower in the group receiving intervention (2% Vs. 50%, p<0.001). Two patients in control group died, none in the intervention group died.
Lakkireddy, 2021 [91] (India) Randomised open label trial (intervention group had higher inflammatory markers on enrollment)	Confirmed COVID-19 with 25(OH)D concentration <30 ng/mL having mild-moderate illness, >18 years (Intervention: recruited= 65, completed=44, Control: recruited=65, completed=43)	Cholecalciferol aqueol nano solution 60000 IU daily for 8 days in participants with BMI 18-25 kg/m^2 and 10 days for participants with BMI >25 kg/m^2	Standard care	Change in level of Inflammatory markers before and after intervention, between two groups and subgroup analysis on patients who have not received any specific additional treatment.	Significant reduction of inflammatory markers (CRP, LDH, Ferritin, IL-6, N/L ratio) in intervention group compared to control group. No difference in hospital stay or mortality.
Murai 2021 [92] (Brazil) Multi-centre double blind randomised placebo-controlled study	COVID-19 confirmed by SARS-CoV-2 PCR or ELISA for IgG, Moderate- severe disease (Respiratory rate >24/min or SpO_2 <94% or presence of co-morbidities), age >18 years (Intervention: recruited =120, analysed 119, control: recruited=120, analysed=118)	Single dose of oral cholecalciferol 200000 IU	Identical placebo	Length of hospital stay, mortality, ICU admissions, need for ventilation	No significant difference between groups in median length of hospital stay (7 Vs. 7, p=0.94), mortality (7.6% Vs. 5.1%, p=0.43). No significant difference in need for ventilation or length of ventilation. No significant difference in post-hoc analysis on patients with vitamin D deficiency.

Figure 1

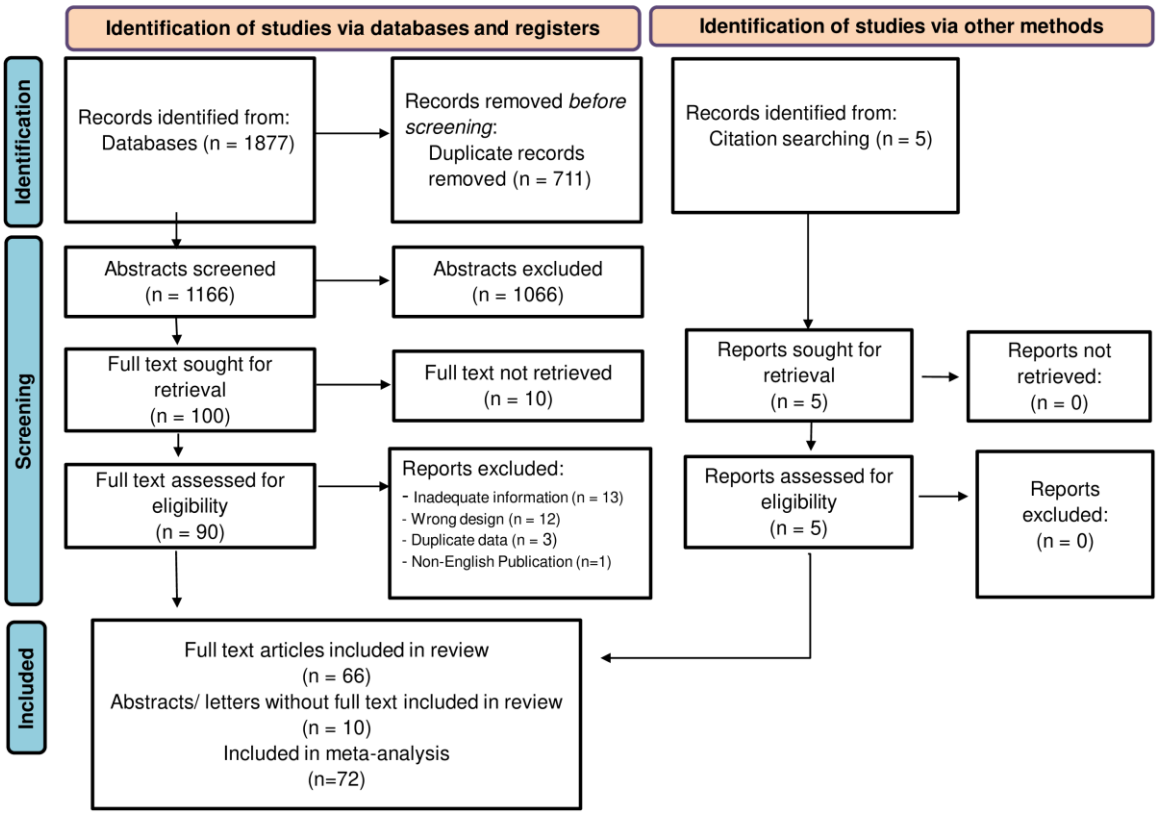

Figure 2

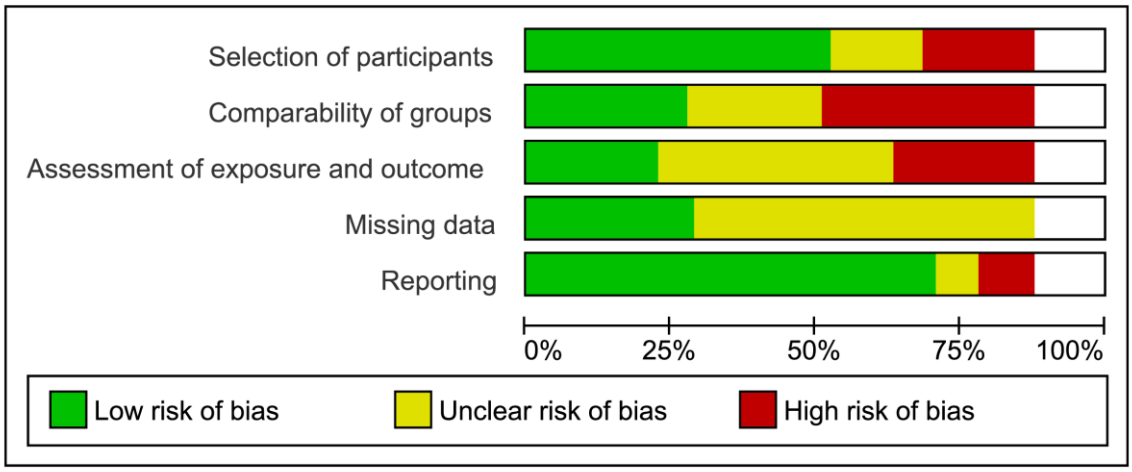

Figure 3

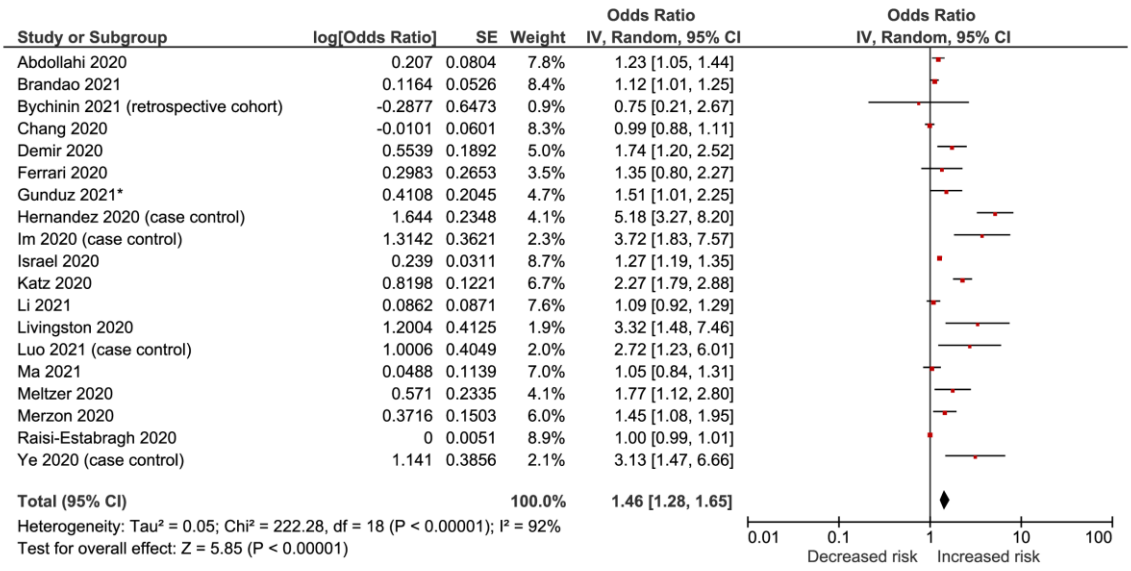

Figure 4

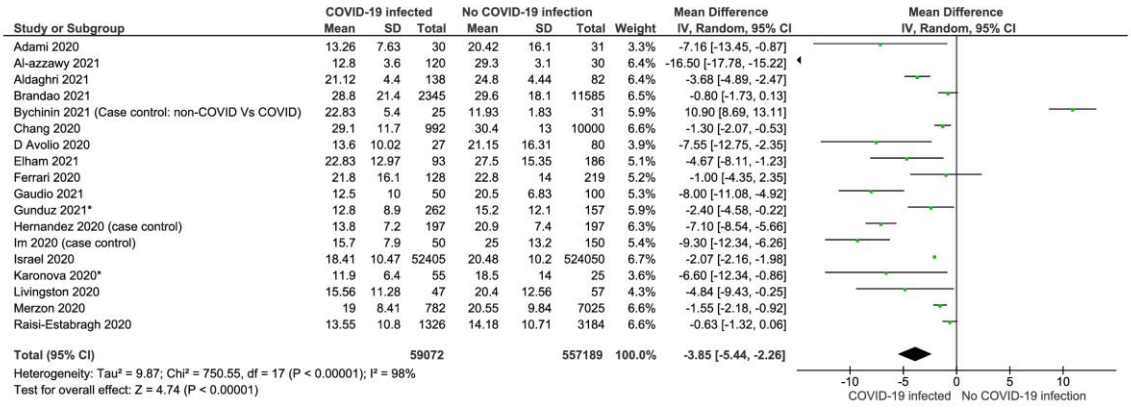

Figure 5

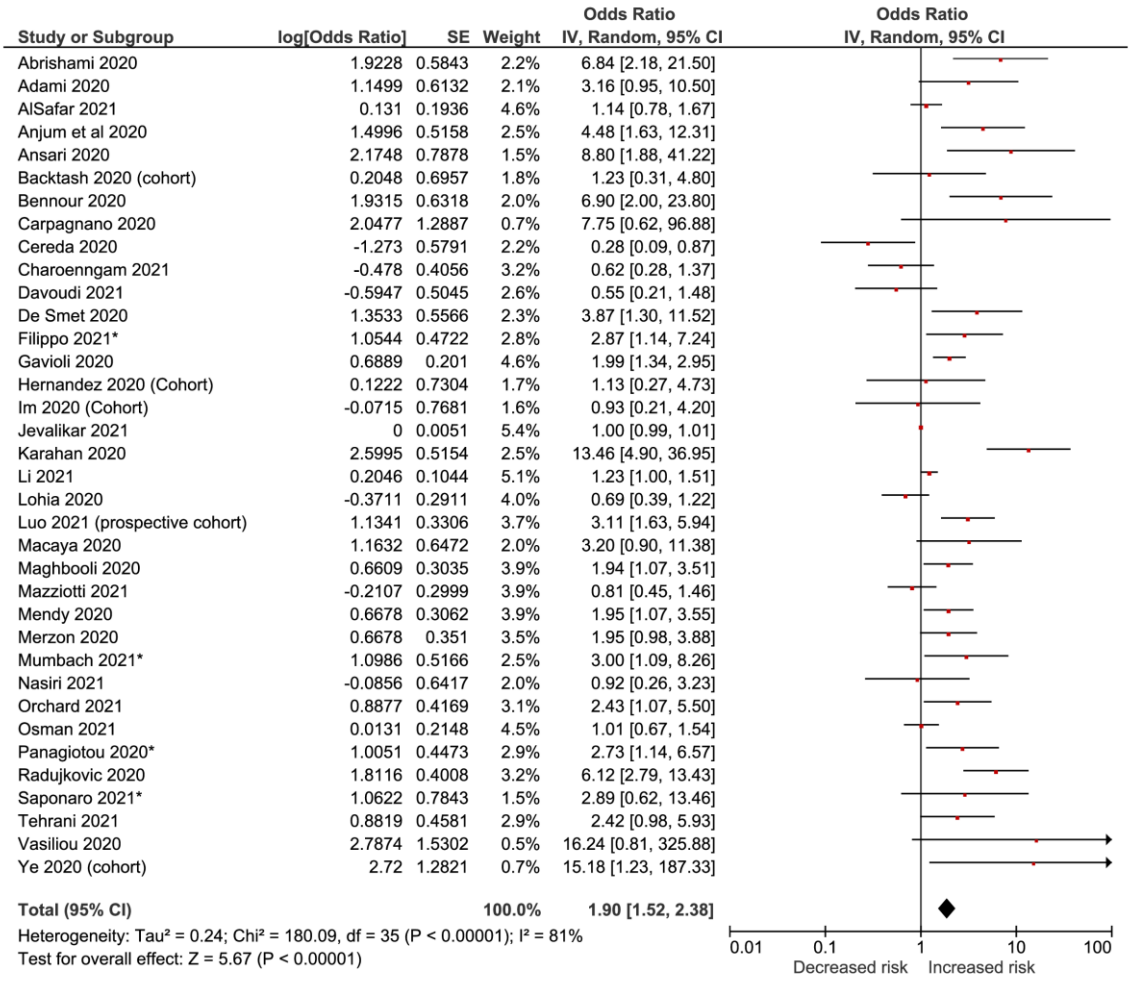

Figure 6

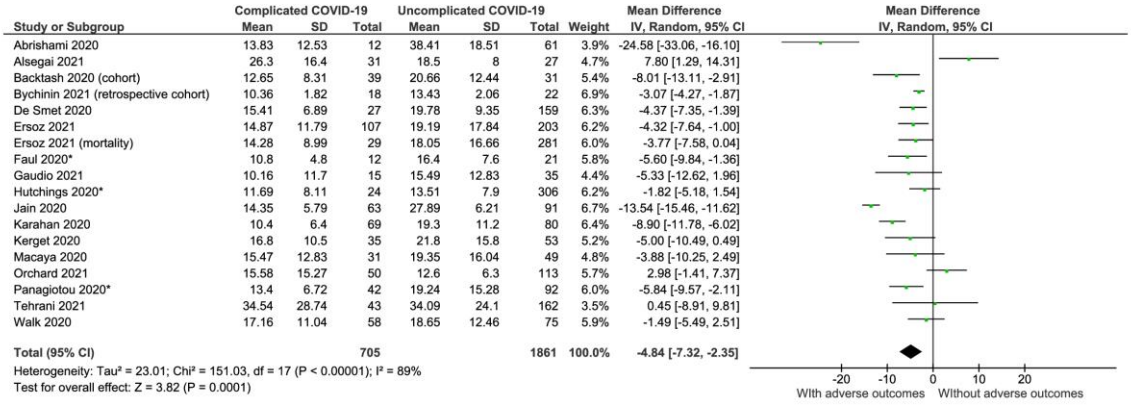

Figure 7

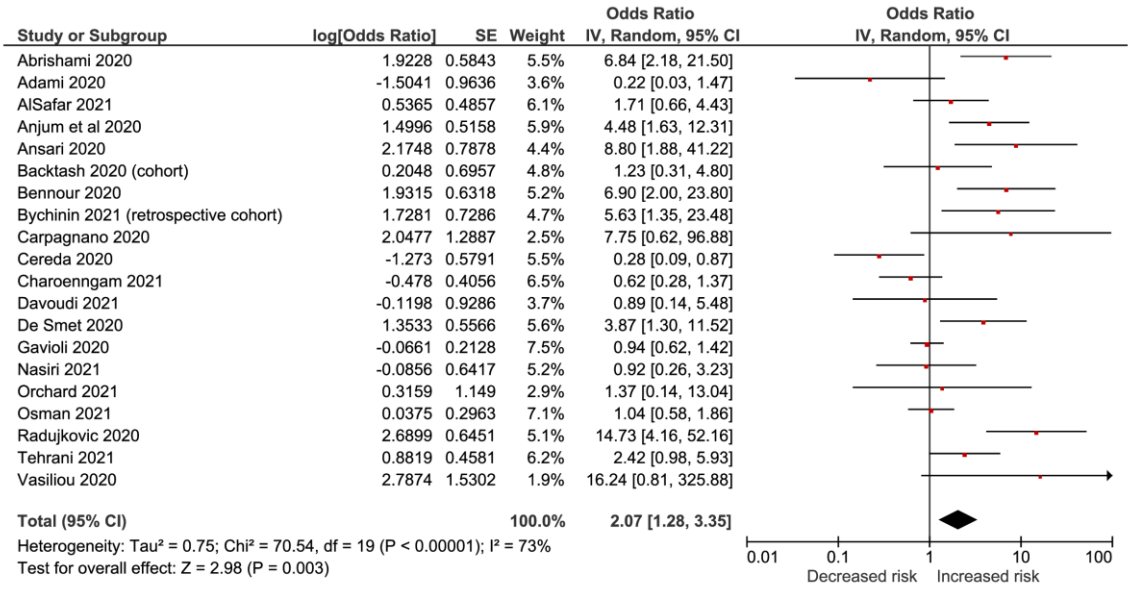

Figure 8