The role of vitamin D deficiency on the Covid-19: A systematic review and meta-analysis of observational studies

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Objectives: Although vaccination has started, it seems that Covid-19 will continue to threaten public health for a long time. Therefore, in addition to the vaccine, the use of supplements to support the immune system may also be important. The main purpose of this study is to indicate the possible effect of low serum vitamin D level (25(OH)D<20 ng/mL or 50nmol/L) on the Covid-19 infection and outcomes.

Methods: To accomplish our objectives, we searched on Google Scholar, PubMed, Scopus, Web of Science, and ScienceDirect databases without any language restrictions for articles between 01.01.2020 and 15.12.2020. We performed three meta-analyses to combine the odds ratio values by paying attention to laboratory measurement units for vitamin D and the measured serum 25(OH)D level.

Results: There were 23 eligible studies that were found to be relevant to the relationship between vitamin D and Covid-19 infection/outcomes (n=206861). We applied three meta-analyses called D-CIMA, D-CSMA and D-CMMA for Covid-19 infection, severity, and mortality, respectively. According to obtained result from D-CIMA, one which has low serum vitamin D level are 1.64 times (95% CI=[1.32-2.04], p<0.001) more likely to get Covid-19 infection. In D-CSMA, we found that people with the serum 25(OH)D level below 20ng/mL or 50nmol/L have 2.58 times (95% CI=[1.28-5.19], p=0.008) more risky for the severe Covid-19. We obtained from D-CMMA that low vitamin D level has no effect on Covid-19 mortality (OR=2.42, 95% CI=[0.73-8.04], p=0.148).

Conclusions: According to obtained our main results, vitamin D deficiency (VDD) may increase the risk of Covid-19 infection and the potential for the severity of the disease. Therefore, we recommend the supplemantation of vitamin D for the prevention of Covid-19 and its negative outcomes.

Keywords: Vitamin D deficiency, Covid-19, SARS-CoV-2, Meta-analysis, Systematic review

Introduction

While the Covid-19 progress asymptomatic or with mild symptoms in the majority of the population, it may lead to death by causing serious clinical syndromes such as pneumonia, acute respiratory distress syndrome (ARDS), myocarditis, microvascular thrombosis, and cytokine storm in some groups [1]. Protecting from coronavirus disease which is reported to become more contagious with its mutation, reducing the risk of severity of the disease, and consequently the mortality rate are current problems. Vaccines that have been approved for emergency use have been a gleam of hope in the global struggle against to Covid-19. However, the effects of vaccines on the immune system have not been clearly proven. It is also observed that there are cases that infected by Covid-19 and had a severe illness and died, although they are vaccinated. As we learned from similar viral infections, supplements such as vitamin D to support the immune system are important in addition to vaccination.

The effects of vitamin D on the treatment and complications of Covid-19 and its potential contribution to the reduction of the incidence of the pandemic are among the most frequently researched topics. Vitamin D has antiviral activity and inhibitor effect of virus replication by stimulating the release of cathelicidin and defensin proteins in monocytes and macrophages [2,3]. Vitamin D has an important role in the prevention of respiratory system infections due to its effects such as stimulating the chemotaxis of T-lymphocytes and clearing respiratory pathogens by inducing apoptosis and autophagy in the infected epithelium [4]. It has been reported that the low T-lymphocyte level was found in some groups of Covid-19 patients with severe symptoms [5]. Considering that vitamin D supplement increases the level of T-lymphocytes [6], this supports hypotheses that it could be useful in the treatment of Covid-19.

The severe progress of Covid-19 in some cases is one of the most important problems of the pandemic. The studies have indicated that thrombotic events and cytokine storm increase in severe Covid-19 patients. These are held responsible for leading to death during Covid-19

infection [7-9]. It is well known that vitamin D sufficiency reduces the risk of cytokine storm and regulates thrombotic pathways [10,11]. It has been reported that the vitamin D sufficiency may reduce increased inflammatory markers and cytokine storm during the Covid-19 disease, the VDD may relate to severity and mortality of Covid-19 [12,13]. Consequently, the effect of VDD on the Covid-19 infection/outcomes is an attractive topic.

In the most of current studies about this subject, although it has been focused that the effect of VDD on the infection/severity/treatment of Covid-19, the findings on the relationship between VDD and Covid-19 mortality are limited. Therefore, as reported in literature [13-15], more comprehensive clinical studies are still needed.

There are three meta-analysis studies that one of which is a pre-print article in the literature [16-18]. In these studies, we identified some problems such as the uncertain definition of deficiency of serum vitamin D level (e.g. 25(OH)D <20 or 12 in ng/mL, <50 or 30 in nmol/L), combining of different summary statistics (e.g. Odds Ratio, Risk Ratio, Hazard Ratio, etc.) and performing the meta-analysis of an individual study and giving this result as if it were the pooled result of the meta-analysis. In the published Endocrine Society's Practice Guidelines on Vitamin D, VDD was defined as a 25(OH)D < 20 ng/mL or 50 nmol/L, insufficiency as 21–29 ng/mL and sufficiency as at least 30 ng/mL for maximum musculoskeletal health [19]. This is the first systematic review and meta-analysis that establishes the association between Covid-19 infection/outcomes and VDD according to the common cut-off value (VDD was defined as a 25(OH)D<20 ng/mL or 50 nmol/L) proposed by the advisory bodies for the deficiency. The main purpose of this study is to indicate the association between Covid-19 infection/severity/mortality and VDD and to provide an analytical evidence to the literature for the evaluating of vitamin D supplement in the treatment and prevention protocols to Covid-19.

Methods

Throughout this systematic review and meta-analysis study, we followed the PRISMA guidelines [20] (Supplementary Material 1).

Search strategy

We searched Google Scholar, PubMed, Scopus, Web of Science, and Science Direct databases without any language restriction and publication status limit. Our search keywords are "vitamin D" and "Covid-19", "vitamin D" and "SARS-CoV-2" and "vitamin D" and "Coronavirus disease". The articles that include search keywords in their title and published between 01.01.2020 and 15.12.2020 were chosen (Supplementary Material 2). Additionally, we screened the reference lists of other meta-analysis studies. Two independent researchers (MOK and EP) screened titles, abstracts, and full-texts for inclusion in qualitative and quantitative analysis.

Selection Criteria

The inclusion criteria for eligible studies were as follows: (1) cohort or case-control studies on the association between vitamin D deficiency and Covid-19 disease. (2) vitamin D deficiency according to common definition of the deficiency (25(OH)D<20 ng/mL or 50 nmol/L) as the exposure of interest; (3) studies in which the primary outcome was the occurrence of the risk of Covid-19 infection, severity and mortality (given the number of case as a crosstabulated table). Studies were excluded if any of the following criteria were met: (1) non-human studies; (2) non-observational studies or observational studies without an analytical epidemiologic approach; (3) irrelevant exposure or outcome variables; (4) duplication or unobtainable abstract/full-text; (5) studies that reported risk estimates (rate ratio [RR], odds ratio [OR], or hazard ratio [HR]) and their 95% confidence intervals (CIs) without present the number of cases.

Data extraction

MOK and EP excluded article types that are review, reply, and letter. Also the research articles that have inappropriate or inadequate results for the quantitative analysis were excluded. MOK and EP extracted the data using a standardized data format from studies that give the number of cases related to vitamin D level and Covid-19 infection/outcomes as a cross-tabulated table. We did not use the estimates of summary statistics presented in the studies. Any discrepancies were resolved by consensus. For the qualitative analysis, we elaborated an electronic spreadsheet in which information belongs to studies the following information was recorded: the authors, location, region, study design, sample size, gender, population age, the definition of deficiency and insufficiency of vitamin D, the evaluated outcomes in the studies, whether included in the meta-analysis. Five studies [21-25] in the qualitative analysis were not included in the meta-analysis because they did not contain sufficient information for quantitative analysis.

Data Analysis

We used the Mendeley Desktop application (version 1.19.4;©2008–2018 Mendeley Ltd.) to remove the duplicates and applied the inclusion criteria. To be infected from Covid-19, the severity, and the mortality of Covid-19 were considered as Covid-19 outcomes in the meta-analyses. For the classification of serum levels of vitamin D, there are definitions of VDD and insufficiency according to advisory committees [26]. It can be found that vitamin D laboratory measurement units can be converted to each other in the following way 12 ng/mL=30 nmol/L, 20 ng/mL=50 nmol/L, and 30 ng/mL=75 nmol/L. Although there are classifications and definitions about the deficiency cut-off value in the literature, it was observed that some of the studies did not pay attention to this distinction as given in Table 1. In order to create a subgroup according to a common cut-off value, we chose the serum 25(OH)D level less than 20 ng/mL (50 nmol/L) suggested by [19] for the deficiency.

We extracted data from included studies that classified using the number of cases according to serum vitamin D level and Covid-19 infection/outcomes to calculate combined odds ratio

estimation. We did not use that presented vitamin D levels as mean or median values and summary statistics such as odds ratio (OR), risk ratio (RR), hazard ratio (HR) and incidence rate ratio (IRR), etc. that expressed without the classification according to the number of cases. We created a subgroup from studies that used 25(OH)D<20 ng/mL (or <50 nmol/L). We performed also an overall meta-analysis without considering the differences in the definition of serum 25(OH)D level to compare with the results.

We examined the heterogeneity and the publication bias of included studies using the Cochran Q test, the funnel plots, and Egger's test. It was detected the heterogeneity for all groups according to the Cochran Q test and identified the level of heterogeneity with I^2 index. Therefore, Peto Random Effect Model was performed to estimate combined OR values. We generated forest plots to show the detailed representation of all studies based on the OR effect size and with 95% confidence interval (CI). Moreover, although it was seen that there was the individual publication bias in funnel plots since the publication bias detected in one meta-analysis according to Egger's test, a trim-fill adjustment method was performed. All statistical analyses were done by using RStudio (version 1.2.5019;©2009-2019 RStudio, Inc) with R for Windows 4.0.3.

Assesment of methodological quality

The Newcastle-Ottawa Quality Scale (NOS) [50] (Supplementary Material 3) was used to assess the quality and risk of bias, and it contains eight customized evaluation sheet criteria divided into three groups: selection, comparability, and outcome. The case representativeness, research methodologies, and study outcomes were all reviewed on the assessment sheet. Different criteria were used to measure quality depending on different research designs. For the assessment of quality, a score 3 or 4 stars in the selection domain and 1 or 2 stars in the comparability domain and 2 or 3 stars in the exposure/outcome domain indicates good quality; a score 2 stars in selection domain and 1 or 2 stars in comparability domain and 2 or 3 stars in

exposure/outcome domain indicates fair quality; a score 0 or 1 star in selection domain and 0 or 1 star in comparability domain and 1 or 2 stars in exposure/outcome domain indicates poor quality.

Results

Total record by initial searching is 805 articles according to search keywords and 23 articles from screening the referance lists. After removal of duplicates and excluding studies on the basis of their abstracts or through examining their full text, while the 28 articles were eligible for systematic review, the 23 articles were eligible for meta-analysis (Figure 1). Five studies were excluded from the meta-analysis because they did not report data necessary to calculate ORs. Therefore, only the systematic review was done on these studies. The baseline characteristics of the studies presents in Table 1. The studies were done in Europe (53.5%; 15 studies), in Asia (35.7%; 10 studies), in America (10.7%; 3 studies). Total sample size from 28 studies in systematic review is 2278150. From 26 studies that reported the distribution of gender, the sample size is 1936754 (85%), of which 896824 (46.3%) were men. The summary statistics for the population age were presented different ways such as mean (±SD), and median [min-max or IQR] in 16 studies. In 12 studies, the summary statistics were presented separately for each subgroup (case, control etc.) and they did not report them for the population. Therefore, we cannot provide the mean or median values of age of population from studies, but the light of information that is available, we can say that almost the population consist of adults between 18-85 years. For the level of 25(OH)D, 14 studies used ng/mL, 9 studies used nmol/L, one study used ng/dL as the laboratory measurement unit and 4 studies did not report it. The VDD was defined as 25(OH)D<10 ng/mL in 2 studies; <12 ng/mL in 1 study; <20 ng/mL in 11 studies; <25 nmol/L in 3 studies; <50 nmol/L in 3 studies. One study defined the deficiency as 25(OH)D<20 ng/dL. In one of the studies, the cut off value of serum 25(OH)D level was reported as 34.4 nmol/L. This value was expressed as the cohort median, not as deficiency. The

vitamin D insufficiency was defined as 25(OH)D<20 ng/mL in 1 study; <30 ng/mL in 9 studies; <50 nmol/L in 3 studies and <75 nmol/L in 1 study.

The Covid-19 outcomes that evaluated in the studies are as follows: Single evaluated outcome was infection in 8 studies, severity in 3 studies and mortality in 1 study. Two evaluated outcomes were infection and mortality in 2 studies, severity and mortality in 3 studies, infection and severity in 3 studies, infection and hospitalization in 1 study, hospitalization and severity in 1 study, hospitalization and mortality in 1 study. Among the studies that examined three outcomes, 3 studies reported Covid-19 infection /severity/mortality, 1 study reported Covid-19 infection/hospitalization and severity, 1 study reported Covid-19 hospitalization/severity and mortality. Due to the limitations in the studies, we could not extract data on the all evaluated outcomes. Therefore, in some cases, the examined outcomes in meta-analyses and appropriate samples for the meta-analyses were less than the studies (Table 2).

The assessment of quality of included studies in meta-analyses is evaluated by using Newcastle-Ottawa Quality Assessment Scale (NOS) [50] (Supplemantary Material 3).

The primary hypothesis is that there is a relationship between low vitamin D levels that is defined as 25 (OH) D <20 ng/mL or 50 nmol/L and Covid-19 infection/outcomes. However, since the units of laboratory measurement and levels of measurement are different in the studies included in the meta-analyses, we also applied the overall meta-analysis which included all studies to show how to effect these differences on the findings. The results obtained from overall meta-analyses were presented in supplementary materials. We performed six meta-analyses as follows:

D-CIMA (Vitamin-**D** and **C**ovid-19 **I**nfection **M**eta-**A**nalysis for 25 (OH) D <20 ng/mL or 50 nmol/L, the number of included studies is 8),

D-CIMA$_{Overall}$ (Vitamin-**D** and **C**ovid-19 **I**nfection **M**eta-**A**nalysis for all measurement units, the number of included studies is 11),

D-CSMA (Vitamin-**D** and **C**ovid-19 **S**everity **M**eta-**A**nalysis for 25 (OH) D <20 ng/mL or 50 nmol/L, the number of included studies is 10),

D-CSMA$_{Overall}$ (Vitamin-**D** and **C**ovid-19 **S**everity **M**eta-**A**nalysis for all measurement units, the number of included studies is 14),

D-CMMA (Vitamin-**D** and **C**ovid-19 **M**ortality **M**eta-**A**nalysis for 25 (OH) D <20 ng/mL or 50 nmol/L, the number of included studies is 6), and

D-CMMA $_{Overall}$ (Vitamin-**D** and **C**ovid-19 **M**ortality **M**eta-**A**nalysis for all measurement units, the number of included studies is 9).

The sample sizes used are: 202561 and 203962 included in D-CIMA and D-CIMA$_{Overall}$, 2332 and 3776 included in D-CSMA and D-CSMA$_{Overall}$, 1397 and 1776 included in D-CMMA and D-CMMA $_{Overall}$, respectively. Since more outcomes are examined on the same sample in some included studies in the meta-analyses, there are overlapping samples. Considering these samples, the total sample size is 206861. For the distribution of samples, please see (Supplementary Material 4).

Note that, in 2 studies [28, 34], we could not find the distribution of gender for the included samples in D-CIMA and D-CSMA. Therefore, the size of samples whose gender distribution can be reached was 19615 and of which 8750 (44.6%) were men. As mentioned before, the summary statistics for age have been presented in different ways such as mean (±SD) and median [min-max or IQR]. Therefore, we cannot provide a summary statistic about the age of the population from studies, but in the light of knowledge available information from studies, we can say that almost the population consists of adults (18-85 years).

We included 8 studies in the D-CIMA meta-analysis. All of these studies were reported that there is a significant positive relationship where the risk of infection increases while VDD increases. We also obtain that there is significant positive relation between Covid-19 infection and VDD (OR=1.64 95% CI= [1.32-2.04], p<0.001]. There was no publication bias for 8 studies

according to Egger's Test (p=0.399). For the heterogeneity, I^2=85.4%, 95% CI=[73.2-92.1] and τ^2=0.06, 95% CI=[0.05-1.02] (Cochran Q, p<0.001) were obtained. We generated the forest and funnel plots (Figure 2).

We included 11 studies in the D-CIMA$_{Overall}$ meta-analysis. All of the studies except for one study reported that there is a significant positive relationship between VDD and Covid-19 infection. According to D-CIMA$_{Overall}$ results, the low serum level of vitamin D was positively associated with Covid-19 infection (OR=1.86 95% CI= [1.51-2.30], p<0.001]. There was no publication bias for 11 studies according to Egger's Test (p=0.091). For the heterogeneity, I^2=87.0%, 95% CI=[78.7-92.1] and τ^2=0.08, 95% CI=[0.06-0.76] (Cochran Q, p<0.001) were obtained. We presented the forest and funnel plots (Supplementary Material 5). Considering the D-CIMA and D-CIMA$_{Overall}$ results, we should note that the combined OR values are different. This is a remarkable finding that demonstrates the importance of distinction according to serum vitamin D level.

We included 10 studies in the D-CSMA meta-analysis. All of the studies except for one study were reported that there is a significant positive relationship where the risk of Covid-19 severity increases while VDD increases. We obtain that there is significant positive relation between Covid-19 severity and VDD (OR=2.58 95% CI= [1.28-5.19], p=0.008]. There was no publication bias for 10 studies according to Egger's Test (p=0.054). For the heterogeneity, I^2=91.5%, 95% CI=[86.6-94.7] and τ^2=1.11, 95% CI=[0.45-4.37] (Cochran Q, p<0.001) were obtained. We generated the forest and funnel plots (Figure 3).

We included 14 studies in the D-CSMA$_{Overall}$ meta-analysis. All of the studies except for one study were reported that low serum level of vitamin D was positively associated with Covid-19 severity. There was publication bias for 14 studies according to Egger's Test (p=0.015). Due to publication bias, we applied the trim-fill adjustment method. According to adjusted results, there is no significant relation between Covid-19 severity and low serum level of vitamin D

(OR=1.37 95% CI= [0.80-2.33], p=0.251]. This is also a remarkable finding that demonstrates the importance of distinction according to serum vitamin D level. The forest and funnel plots belong to adjusted method were presented in (Supplementary Material 6). For the heterogeneity for the adjusted method, I^2 =92.7%, 95% CI=[90.0-94.6] and τ^2 =1.32, 95% CI=[0.84-3.64](Cochran Q, p<0.001) were obtained. Moreover, we provide the overall results without applying the trim and fill adjusted method to show that there is a difference in the results (Supplamentary Material 7).

Considering the D-CSMA and D-CSMA$_{Overall}$ results, we should note that conducting all data leads to misleading such as finding that there is no relationship that actually exists. This is also a remarkable finding that demonstrates the importance of distinction according to serum vitamin D level.

We included 6 studies in the D-CMMA meta-analysis. Four studies were reported that there is a significant positive relationship where the Covid-19 mortality increases while VDD increases.In contrast to reported results in 4 studies, we found that there is no significant relation between Covid-19 mortality and VDD (OR=2.42 95% CI= [0.73-8.04], p=0.148]. There was no publication bias for 6 studies according to Egger's Test (p=0.528). For the heterogeneity, I^2=92.3%, 95% CI=[85.9-95.7] and τ^2=1.95, 95% CI=[0.54-13.05] (Cochran Q, p<0.001) were obtained. The forest and funnel plots are presented in Figure 4.

We included 9 studies in the D-CMMA$_{Overall}$ meta-analysis. While 5 of 9 studies reported that the low serum level of vitamin D was positively associated with Covid-19 mortality, the rest of the studies reported that there was no significant relation. We found that the low serum level of vitamin D was not associated with Covid-19 mortality, (OR=2.05 95% CI= [0.79-5.30], p=0.138]. There was no publication bias for 9 studies according to Egger's Test (p=0.669) and the heterogeneity, I^2=90.3%, 95% CI=[83.8-94.2] and τ^2=1.74, 95% CI=[0.44-6.18] (Cochran

Q, p<0.001) were obtained. The forest and funnel plots are presented in (Supplementary Material 8). All of the results obtained from meta-analyses were presented in Table 3.

Discussion

Although vaccination has started in many countries with vaccines that have been approved for emergency use, it seems that Covid-19 will continue to threaten public health for a long time. To protect from coronavirus disease which is reported [51] to become more contagious with its mutation, to reduce the risk of severity of the disease, and consequently to reduce the mortality are current problems. In addition to vaccination to prevent this pandemic, it has also been recommended to use supplements that strengthen the immune system [52,53]. From this point of view, the purpose of this study is to reveal the possible effect of VDD on Covid-19 infection/outcomes due to its antiviral properties and to provide strong evidence to the literature. For this purpose, we provided a systematic review of 28 studies and conducted three meta-analyses of 23 studies, paying attention to laboratory measurement units for vitamin D and the measured serum 25(OH)D level. We should emphasize that it is important to construct the main hypothesis by stating VDD with serum 25(OH)D level. Because it has been observed that studies describe different serum 25(OH)D levels as a deficiency. For instance, considering the serum 25(OH)D level=23ng/mL, one study identified this value as deficiency while another study would define it as insufficiency. Therefore, to handle just the definition of VDD in the studies without paying attention to serum 25(OH)D level may lead to misleading. Our findings have shown how important this discrimination is. Therefore, to the best of our knowledge, this meta-analysis study is the most comprehensive study to date, with the number of included studies, inclusion criteria, sample size, and well-defined hypotheses. However, our study has some limitations. In our study design, we did not receive OR values that were presented in a logistic regression model in included studies to show the pure effect of VDD on the examined outcomes. Because, since the logistic models in the studies were established with different

explanatory variables, the presented OR values cannot represent the same effect. We would like to provide evidence for comorbidities, treatment, and hospitalization with meta-analyzes, but we could not do it because we could not extract data suitable for our study design. In addition, the difficulties encountered arising from study designs of included studies in meta-analyses during the data extraction are presented in Table 3.

According to obtained result from D-CIMA, one which has serum 25(OH)D level below 20ng/mL or 50nmol/L is 1.64 times more likely to get Covid-19 infection. In D-CSMA, we found that people with a serum 25(OH)D level below 20ng/mL or 50nmol/L have 2.58 times more likely to risk having severe Covid-19. According to the result, we obtained by combining the findings of the studies included in D-CMMA, low vitamin D level has no effect on covid-19 mortality. It is also important to discuss the overall meta-analyses results we presented in the supplementary materials with our main findings. When 11 studies were combined without the distinction based on serum 25(OH)D level in D-CIMA$_{Overall}$, OR=1.86 was obtained (Supplementary Material 5). This is a misleading result that can be interpreted as VDD will increase the risk of infection from Covid-19 more. Similarly, when 14 studies were combined without discrimination according to serum 25(OH)D level in D-CSMA$_{Overall}$, no significant relationship was found between VDD and the risk of having severe covid-19 (Supplementary Material 6). This result will cause the existing relationship to be ignored and the effect of vitamin D on preventing severity to be neglected. Although we could not find a significant relationship between VDD and mortality, we think that similar results also would be obtained for D-CMMA. However, due to the fact that the available data was limited, we could not provide enough evidence for the effect of vitamin D on mortality (Figure 4, Supplementary Material 8).

We think that existing meta-analyses on the subject cannot provide reliable and sufficient evidence. Pereira et al. [16] performed a meta-analysis including 21 studies on the 8176 samples

to examine the relationship between Covid-19 infection/severity/hospitalization and mortality and VDD. They found that the relationship between vitamin D and Covid -19 infection is not significant (OR=1.21, 95% CI=[0.83-1.60]), however there were significant relation between VDD with the severity (OR=1.65, 95% CI=[1.30-2.09]), the hospitalization (OR=1.81, 95% CI=[1.42-2.21]) and the mortality (OR=1.82 95% CI=[1.06-2.58]). However, we identified some problems such as incorrect referencing, inconsistencies for the number of included studies throughout the text, the given OR values in the meta-analyses was not in the relevant studies, incorrect presentation of the characteristics of the included studies. As an example, they included in the same meta-analysis Hastie and Pell et al.'s article [23] and corrigendum [54] about Hastie and Mackay et al. [55]. Moreover, it is unclear how they obtained the OR value from the corrigendum where only a correction table for population characteristics is presented. In addition, the IRR value presented by Hastie and Pell et al.[23] for VDD<25nmol/L, Pereira et al. [16] used it as the OR value for VDD<50nmol/L. Considering the other included studies, Meltzer et al. [42] and Darling et al. [22] presented their findings using RR and OR, respectively. It has seemed that Pereira et al. [16] combined different summary statistics such as IRR, OR, and RR. Therefore, the findings obtained from this study are doubtful. Munshi et al. [17] conducted the meta-analysis study combining only 6 studies on a relatively small sample (n=376). They reported that patients with poor prognosis had significantly lower serum levels of vitamin D compared with those with a good prognosis, representing an adjusted standardized mean difference of -0.58 (95% Cl= -0.83 to -0.34, $p < 0.001$). In addition, they presented a subgroup meta-analysis in which they examined the differences in vitamin D according to the regions. Here, it was determined that a subgroup was formed from a single study inappropriately. Chen et al. [18] conducted a meta-analysis including 6 studies on 377265 samples to examine the relationship between Covid-19 infection/hospitalization and mortality and VDD. They found a significant association for the infection (OR=1.47, 95% CI=[1.09-

1.97]) and the hospitalization (OR=1.83, 95% CI=[1.22-2.74]), while they did not find a meaningful relationship for the mortality (OR=2.73, 95% CI=[0.27-27.61]). Although subgroup analyzes were performed according to serum 25(OH)D level <20ng/mL and <30ng/mL, the results were given on the overall estimate. The problem that the subgroup analysis performed with the single study was identified also here. When we consider our findings together with results obtained from these studies, we can say that we put forth the relationship between Covid-19 infection and severity and VDD more strongly.

As we present in the results section, our findings support the literature. According to previously reported results in the literature, vitamin D supplementation has a protective effect against acute respiratory infections [56]. It has been reported that antigen-presenting cells such as macrophages and dendritic cells have a role in the synthesis of the active form of vitamin D and that macrophages and dendritic cells can be affected by vitamin D. Studies have reported that active Vitamin D [1,25$(OH)_2$D] synthesis will be reduced during the case of vitamin D deficiency, so immune responses will be impaired and thus innate immune function will be impaired. In addition, vitamin D shows antimicrobial and antiviral activity by increasing the expression of cathelicidin/defensin. Cathelicidin and defensin contributes to host defense by stimulating the expression of antiviral cytokines and chemokines involved in the recruitment of monocytes/macrophages, natural killer cells, neutrophils, T cells. Cellular production of cathelcidin and defensin depends on the vitamin D receptor and *CYP27B1* the expressions of which are enhanced following interaction of pathogens with membrane PRRs, such as toll-like receptor 2 and toll-like receptor. The above-mentioned mechanism explains the role of vitamin D in combating respiratory viruses [57]. In the early stage of infection, it limits viral entry and replication by increasing cathelicidin /defensin expression in the respiratory epithelium [58]. In Covid-19, pneumonia and ARDS have been held responsible for the severity of the disease. Many studies have reported the protective effects of vitamin D against pneumonia, cytokine

hyperproduction, and many conditions associated with ARDS [59,60]. It has also been reported that VDD is directly associated with the risk of acute respiratory failure [61,62]. In another study executed on rats, it was reported that Vitamin D supplementation caused a decrease in lung damage and regression in disease severity in rats with ARDS [63]. Although there is not enough data for Covid-19, vitamin D has recently been recommended as a drug in the treatment of lung damage in pneumonia caused by influenza A virus [64]. In light of the evidence we have obtained, vitamin D supplements can be recommended to reduce the severity of Covid-19 disease.

The fact that Covid-19 mortality rates differ between countries and that mortality rates are lower in the Southern Hemisphere has attracted attention to the relationship between VDD and death. In a study conducted in European countries, which are located in the Northern Hemisphere and do not have sufficient sunlight in winter, it has been reported that average vitamin D levels are associated with mortality, especially in countries with a high prevalence of VDD such as Italy and Spain where the mortality due to Covid-19 is high [65]. Although there are findings that benefiting from sunlight reduces influenza infection and related mortality, there are no studies in the literature that reveal the effect of vitamin D supplementation and seasonal change on mortality associated with Covid-19 [53,66]. Cytokine storm that causes hyper inflammation and tissue damage is held responsible for the mortality associated with Covid-19 [67]. In the literature, it has been emphasized that vitamin D can be an important agent in preventing cytokine storms and ARDS. Based on this information, it can be said that vitamin D can reduce the mortality rates due to Covid-19. However, current literature could not provide us adequate data to support this hypothesis in our meta-analysis study. We should note that large-scale and multi-center randomized controlled studies are still needed to determine the effectiveness of vitamin D in the treatment of severity of disease and reduce mortality.

Conclusion

Despite the fact that the vaccination has started in many countries, it appears that Covid-19 will continue to threaten public health for a long time. Hence, in addition to the vaccine, the usage of immune-supporting supplements may be beneficial. The main purpose of this study was to indicate the possible effect of low serum vitamin D level (25(OH)D<20 ng/mL or 50nmol/L) on the Covid-19 infection and outcomes. According to our valuable results, VDD may increase the risk of Covid-19 infection and the potential for the severity of the disease. Therefore, vitamin D supplements may be added to prevention and treatment protocols for Covid-19 disease. Note that, current measures to reduce transmission, such as frequent hand washing, wearing a mask, physical separation, air circulation, and avoiding crowded locations or enclosed spaces, continue to operate against new varieties by limiting viral transmission and thereby reducing the virus's ability to mutate. Vaccines are a vital tool in the fight against COVID-19, and employing the tools we already have has significant public health and lifesaving benefits.

Supplementary Materials

Supplementary materials are available at http://www.e-epih.org/.

Conflict of interest

The authors have no conflicts of interest to declare for this study

Role of the funding source

There was no funding source for this study. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit this study for publication.

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Contributors

MOK and EP revealed the study design and the main idea. MOK and EP searched the literature for review. MOK and EP assessed the eligibility and quality of the studies and extracted data. MOK and EP carried out the statistical analysis, prepared tables, figures, and supplementary files. MOK and EP wrote the original draft with support from BY. MOK, EP and BY reviewed and edited. All authors contributed to the interpretation and subsequent edits of the manuscript and agreed on the final version for submission.

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Table 1: The base characteristics of included studies in systematic review and meta-analyses

Author	Location	Region	Study design	Sample size	Gender (Male n%)	Population age Mean(±SD) or Median[Min-Max or IQR]	The definition of VDD and/or VDIS[b]	The evaluated outcomes[c] in the study	Included in the meta-analysis
Abdollahi et. al. [27]	Iran	Asia	Case – Control	402	132 (32.8)	47.1 (±15.32)	VDD<10 ng/mL VDIS<30 ng/mL	I	Yes
Alipio et. al. [28]	Southern Asian Countries	Asia	Cohort	212	NR[a]	NR	VDD<20 ng/mL VDIS<30 ng/mL	S	Yes
Baktash et. al. [29]	UK	Europe	Cross-Sectional	105	57 (54.2)	81 [65-102]	VDD<30 nmol/L	I-M	Yes
Campi et. al. [30]	Italy	Europe	Cohort	361	243 (67.0)	66 [54 – 78]	VDD<50 nmol/L	I-S-M	Yes
Cereda et. al. [31]	Italy	Europe	Cohort	129	70 (54.3)	77 [65 – 85]	VDD<20 ng/mL VDIS<30 ng/mL	S-M	Yes
D'Avolio et. al. [21]	Switzerland	Europe	Case-Control	1484	682 (45.9)	NR	NR	I	No
Darling et. al. [22]	UK	Europe	Case – Control	1303	713 (54.7)	57.7 (±8.7)	NR	I	No
De Smet et. al. [32]	Belgium	Europe	Cross-Sectional	2903	1108 (38.1)	NR	VDD<20 ng/mL	I-S	Yes

Hastie and Pell et. al. [23]	UK	Europe	Cohort	341484	NR	NR	VDD<25 nmol/L VDIS<50 nmol/L	I-M	No
Hernandez et. al. [33]	Spain	Europe	Case – Control	413	253 (61.2)	NR	VDD<20 ng/mL	I-S-M	Yes
Im et.al. [24]	South Korea	Asia	Case – Control	200	84 (42.0)	52.3 (±20.3)	VDD<20 ng/dL	I-S	No
Israel et. al. [34]	Israel	Asia	Case – Control	576455	271601 (47.1)	NR	VDD<50 nmol/L	I	Yes
Karahan et. al. [35]	Turkey	Europe	Cross-Sectional	149	81 (54.3)	63.5 (±15.3)	VDD<20 ng/mL VDIS<30 ng/mL	S-M	Yes
Katz et. al. [25]	USA	America	Case – Control	987849	455458 (46.1)	NR	NR	I	No
Li et. al. [36]	UK	Europe	Case – Control	353299	161298 (45.6)	67.7 (±8.1)	VDD<25 nmol/L VDIS<50 nmol/L	I-H-S	Yes
Livingston et. al. [37]	UK	Europe	Cohort	104	39 (37.5)	68.5 (18.3)	VDSL<34.4 nmol/L	I	Yes
Luo et. al. [38]	China	Asia	Cross – Sectional	895	405 (45.2)	NR	VDD<30 nmol/L	I-S-M	Yes
Macaya et. al. [39]	Spain	Europe	Cohort	80	35 (43.7)	NR	VDD<20 ng/mL	S	Yes
Maghbooli et. al. [40]	Iran	Asia	Cross – Sectional	235	144 (61.3)	58.7 (±15.2)	VDD<20 ng/mL VDIS<30 ng/mL	H-S-M	Yes

Mardani et. al. [41]	Iran	Asia	Cross-Sectional	123	65 (52.8)	42.1(±14,9)	VDD<10 ng/mL VDIS<30 ng/mL	I	Yes
Meltzer et. al. [42]	USA	America	Case – Control	489	123 (25.0)	49.2 (±18.4)	VDD<20 ng/mL	I	Yes
Mendy et.al. [43]	USA	America	Cohort	689	365 (53.0)	49.5 [35.2 – 67.5]	NR	H-S	Yes
Merzon et. al. [44]	Israel	Asia	Case – Control	7807	3234 (41.4)	41.4 (±NR)	VDD<20 ng/mL VDIS<30 ng/mL	I-H	Yes
Panagiotou et. al. [45]	UK	Europe	Cohort	134	73 (54.4)	68.7 (±14.0)	VDD<25 nmol/L VDIS<50 nmol/L	S	Yes
Radujkovic et. al. [46]	Germany	Europe	Cohort	185	95 (51.0)	60 [49 – 70]	VDD<12 ng/mL VDIS<20 ng/mL	S-M	Yes
Raharusuna et al. [47]	Indonesian	Asia	Cohort	780	380 (48.7)	54.5 (NR)	VDD<20 ng/mL VDIS<30 ng/mL	M	Yes
Vasiliou et. al. [48]	Greece	Europe	Cohort	39	31 (79.4)	61.5 (±13.2)	VDD<20 ng/mL VDIS<30 ng/mL	H-M	Yes
Ye et. al. [49]	China	Asia	Case – Control	142	55 (38.7)	NR [0.1-85]	VDD<50 nmol/L VDIS<75 nmol/L	I-S	Yes

[a] NR: Not Reported
[b] VDD: Vitamin D deficiency, VDIS: Vitamin D InSuffiency, VDSL: Vitamin D Serum Level
[c] H: Hospitalization, I: Infection, S: Severity, M: Mortality

Table 2: Limitations and additional characteristics for included studies

Study	Sample size in the study	The sample size included in meta-analyses			Evaluated outcomes in the study	Evaluated outcomes in the meta-analyses	Findings in the study	Limitations for extracting data
		D-CIMA	D-CSMA	D-CMMA				
Abdollahi et. al. [27]	402	402	–	–	I	I	I(+)	VDD is defined as <10ng / mL, but the sample distribution for VDD is not suitable for statistical analysis. Statistical comparison was made for VDIS <30ng/mL. Therefore, the data belong to VDIS were included in the meta-analysis, and the measurement unit was assigned as "Other".
Alipio et. al. [28]	212	–	212	–	S	S	S(+)	Clinical outcomes of Covid-19 cases were classified as mild, ordinary, severe, and critical in the study. When we extracted data, we chose mild and ordinary cases as non-severe, severe, and critical cases as severe. Age and gender information of the population was not available.
Baktash et. al. [29]	105	–	–	70	I-M	M	I(+); M(-)	There is uncertainty in the definition of comparison groups for Covid-19 infection. We sent an email to the corresponding author, but we could not receive a

								reply. Therefore, Covid-19 infection data were not included in the D-CIMA.
Campi et. al. [30]	361	361	103	103	I-S-M	I-S-M	I(+); S(+); M(+)	There was no limitation for extracting data
Cereda et. al. [31]	129	–	129	129	S-M	S-M	S(+); M(+)	Clinical outcomes were severe pneumonia, admission to intensive care units (ICU), and in-hospital mortality. ICU admission data was not appropriate for statistical analysis (zero observed case). Therefore, we extracted data from severe pneumonia for the severity cases.
D'Avolio et. al. [21]	1484	–	–	–	I	–	I(+)	The definition of VDD was not available. There was no adequate information deal with descriptive statistics. The VDD was used as median [IQR] and the findings were presented on the graphs. Since we extracted the data based on the number of cases, we did not include it in meta-analysis.
Darling et. al. [22]	1303	–	–	–	I	–	I(-)	In the study, the date of the data received from UK Biobank for the Covid-19 (-) control group was long ago. There was no VDD definition and they used quartiles values of VDD. Since we extracted the data based on the number of cases, we did not include it in the meta-analysis.

De Smet et. al. [32]	2903	2903	186	–	I-S	I-S	I(+); S(+)	In the study, chest CT was received for all Covid-19 patients to determine the disease stage. They classified the patients as stage 1(early stage), stage 2(progressive stage), and stage 3 (peak stage). We chose severity cases from stage 3 and non-severe cases from stage 1-2.
Hastie and Pell et. al. [23]	341484	–	–	–	I-M	–	I(-); M(-)	There was no adequate information deal with descriptive statistics. The findings were presented by using HR and IRR values. Since we extracted the data based on the number of cases, we did not include it in the meta-analysis.
Hernandez et. al. [33]	413	–	197	197	I-S-M	S-M	I(+); S(-); M(-)	For the Covid-19 infection, the VDD was presented as mean (±SD). Since the number of cases was not reported, we could not include it in D-CIMA.
Im et.al. [24]	200	–	–	–	I-S	–	I(+); S(+)	In the study, ng/dL was used as the laboratory measurement unit of the serum 25(OH)D ~~25 (OH) D~~ level and the definition of VDD as 25(OH)D<20 ng/dL. We converted 20 ng/dL to 0.2 ng/mL. Since we thought there was an inconsistency, we could not include it in meta analyzes.

Israel et. al. [34]	576455	187234	–	–	I	I	I(+)	Age matching was made between the case and control groups, but the single summary statistics for the population were not presented.
Karahan et. al. [35]	149	–	149	149	S-M	S-M	S(+); M(+)	There was no limitation for extracting data
Katz et. al. [25]	987849	–	–	–	I	–	I(+)	There was no adequate information deal with descriptive statistics. The findings were presented by using OR value. Since we extracted the data based on the number of cases, we did not include it in the meta-analysis.
Li et. al. [36]	353299	3502	1082	–	I-H-S	I-S	I(+); H(+); S(+)	They defined the hospitalization as one record of origin (whether the patient was tested positive or not). In the study, hospitalized, confirmed and severe Covid-19 cases were compared with community control. We think that the study design is wrong. For this reason, we extracted data for the infection from the patients who came to the hospital with the suspicion of COVID-19 and the confirmed Covid-19 and for the severity from the patients who confirmed Covid-19 and the severe ones.
Livingston et. al. [37]	104	104	–	–	I	I	I(-)	The definition of VDD was not available. Statistical comparison was made for 25(OH)D <34.4 nmol/L

								Therefore, the data belong to this distinction were included in the meta-analysis, and the measurement unit was assigned as “Other”.
Luo et. al. [38]	895	895	335	74	I-S-M	I-S-M	I(+); S(+); M(-)	There was no limitation for extracting data
Macaya et. al. [39]	80	–	80		S	S	S(+)	There was no limitation for extracting data
Maghbooli et. al. [40]	235	–	235	235	H-S-M	S-M	H(+); S(+); M(+)	There was no limitation for extracting data. Although the authors defined VDD as 25(OH)D<20ng/mL, the data set that can be extracted from the article belongs to 25(OH)D=30ng/ML. Therefore, it was included in the overall analysis.
Mardani et. al. [41]	123	123	–	–	I	I	I(+)	We extracted data from their supplementary file according to 25(OH)D<20 ng/mL
Meltzer et. al. [42]	489	489	–	–	I	I	I(+)	There was no limitation for extracting data
Mendy et. al. [43]	689	–	689	–	H-S	S	H(+); S(+)	The definition of VDD was not available. Therefore, the measurement unit was assigned as “Other” in the D-CSMA
Merzon et. al. [44]	7807	7807	–	–	I-H	I	I(+); H(+)	There was no limitation for extracting data
Panagiotou et. al. [45]	134	–	134	–	S	S	S(+)	They classified the patients as admitted to the Intensive Therapy Unit (ITU) and to non-ITU wards. When we extracted data, we chose the admitted to non-ITU wards cases as non-severe and the admitted

								to ITU cases as severe. Statistical comparison was made for VDIS <50 nmol/L. Therefore, the data belong to VDIS were included in the D-CSMA.
Radujkovic et. al. [46]	185	–	185	–	S-M	S	S(+); M(+)	They classified the patients as inpatients and outpatients. When we extracted data, we chose the outpatients as non-severe and the inpatients as severe according to their definition.
Raharusuna et al. [47]	780	–	–	780	M	M	M(+)	There was no limitation for extracting data
Vassiliou et. al. [48]	39	–	–	39	H-M	M	H(-); M(-)	There was no limitation for extracting data
Ye et. al. [49]	142	142	60	–	I-S	I-S	I(+); S(+)	There was no limitation for extracting data
Total	2278150	203962	3776	1776				

(+) indicates that the association of low-level vitamin D and Covid-19 infection(I) /severity(S) /mortality (M) /hospitalization (H) are statistically significant; (-) indicates that the association of low-level vitamin D and Covid-19 infection/severity/mortality/hospitalization are not statistically significant. D-CIMA:Vitamin-D and Covid-19 Infection Meta-Analysis, D-CSMA: Vitamin-D and Covid-19 Severity Meta-Analysis, D-CMMA: Vitamin-D and Covid-19 Mortality Meta-Analysis.

Table 3: p-values of tests of published bias, heterogeneity and meta-analyses findings and bias scores for Egger's test

	Tests of Published Bias	Test of Heterogeneity	Quantifying Heterogeneity	Findings of Meta-Analyses	
Meta-Analyses	**Egger's Test**	**Cochran Q Test**	**I^2 index (95% CI) τ^2 value (95% CI)**	**Peto's Random Effect Model**	
	p	**p**		**OR (95% CI)**	**p**
D-CIMA for the serum 25(OH)D level<20ng/mL and 50nmol/L	0.399	**<0.001**	85.4% (73.2 – 92.1) 0.06 (0.05 – 1.02)	1.64 (1.32 – 2.04)	**<0.001**
D-CIMA$_{Overall}$ for the serum 25(OH)D level with all different measurement units	0.091	**<0.001**	87.0% (78.7 – 92.1) 0.08 (0.06 – 0.76)	1.86 (1.51 – 2.30)	**<0.001**
D-CSMA for the serum 25(OH)D level<20ng/mL and 50nmol/L	0.054	**<0.001**	91.5% (86.6 – 94.7) 1.11 (0.45 – 4.37)	2.58 (1.28 – 5.19)	**0.008**
D-CSMA$_{Overall}$ for the serum 25(OH)D level with all different measurement units	**0.015**	**<0.001**	92.7% (90.0 – 94.6)* 1.32 (0.84 – 3.64)*	1.37 (0.80 – 2.33)*	0.251
D-CMMA for the serum 25(OH)D level<20ng/mL and 50nmol/L	0.528	**<0.001**	92.3% (85.9 – 95.7) 1.95 (0.54 – 13.05)	2.42 (0.73 – 8.04)	0.148
D-CMMA$_{Overall}$ for the serum 25(OH)D level with all different measurement units	0.669	**<0.001**	90.3% (83.8 – 94.2) 1.74 (0.44 – 6.18)	2.05 (0.79 – 5.30)	0.138

***Trim fill method applied**

The values for p<0.05 present in bold. D-CIMA:Vitamin-D and Covid-19 Infection Meta-Analysis, D-CSMA: Vitamin-D and Covid-19 Severity Meta-Analysis, D-CMMA: Vitamin-D and Covid-19 Mortality Meta-Analysis. OR: Odds Ratio, CI: Confidence Interval.

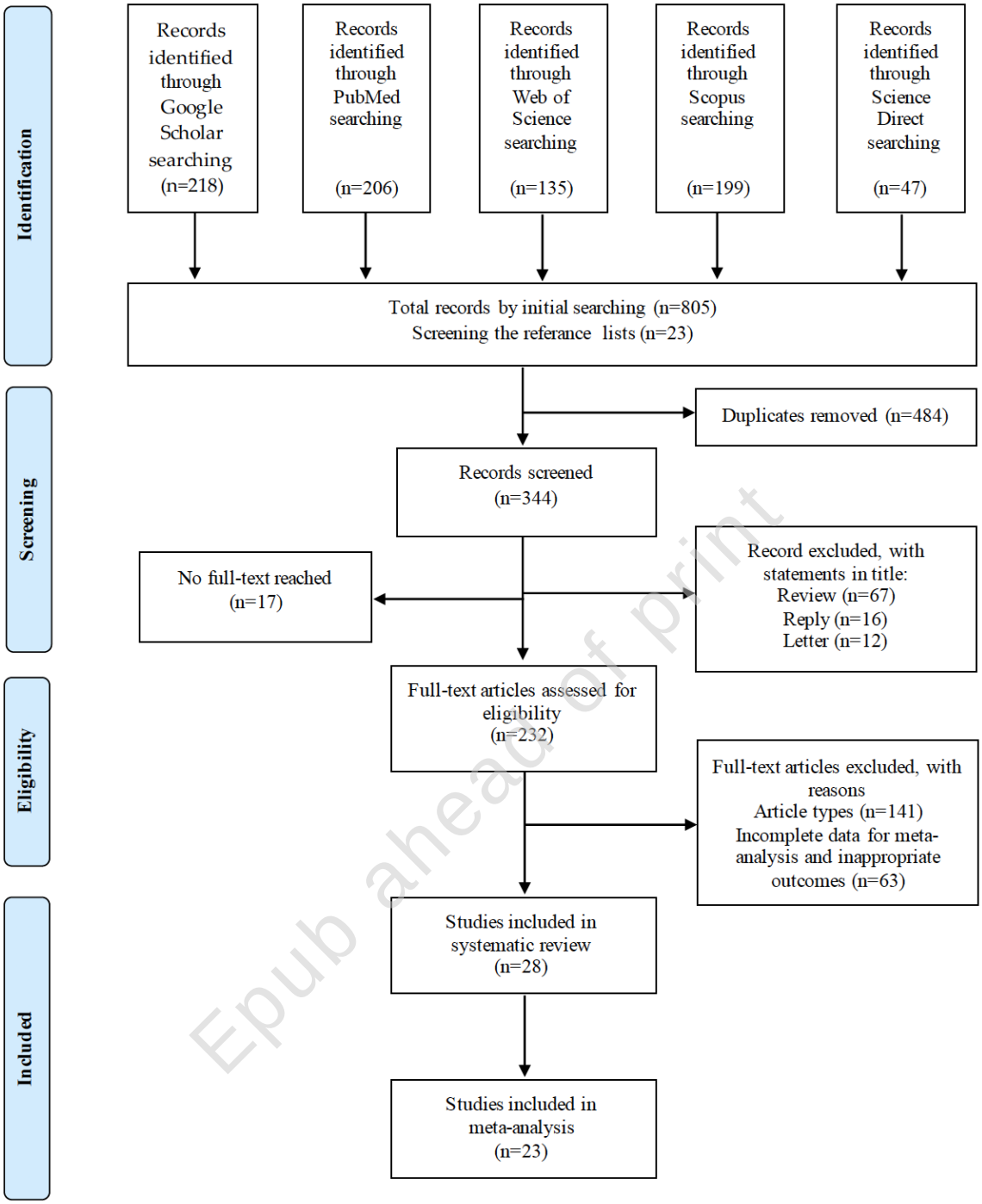
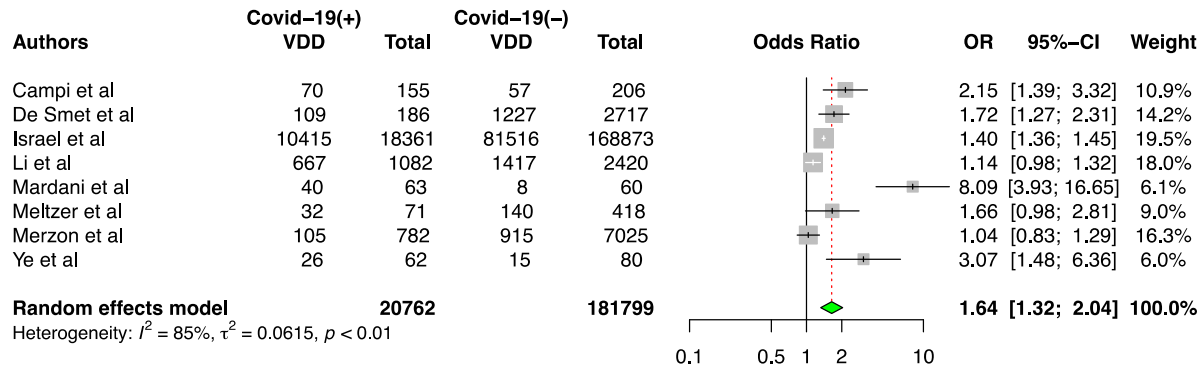


Figure 1: Flowchart of the study selection process



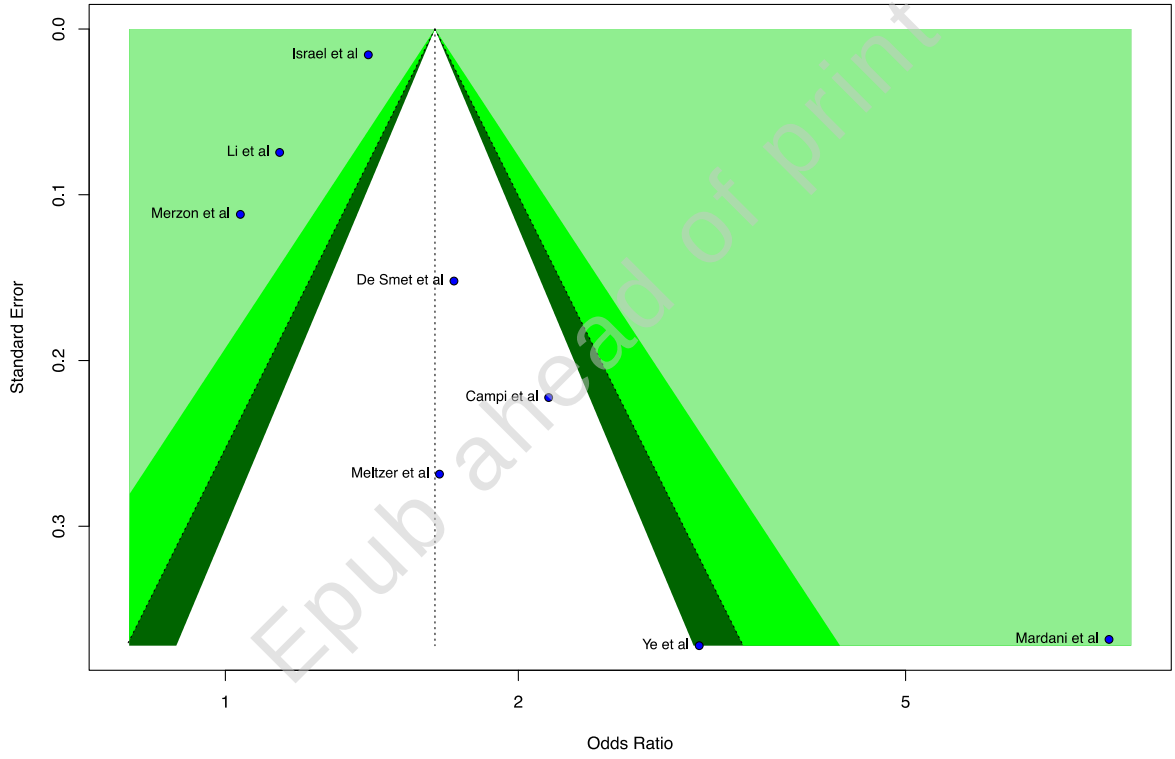
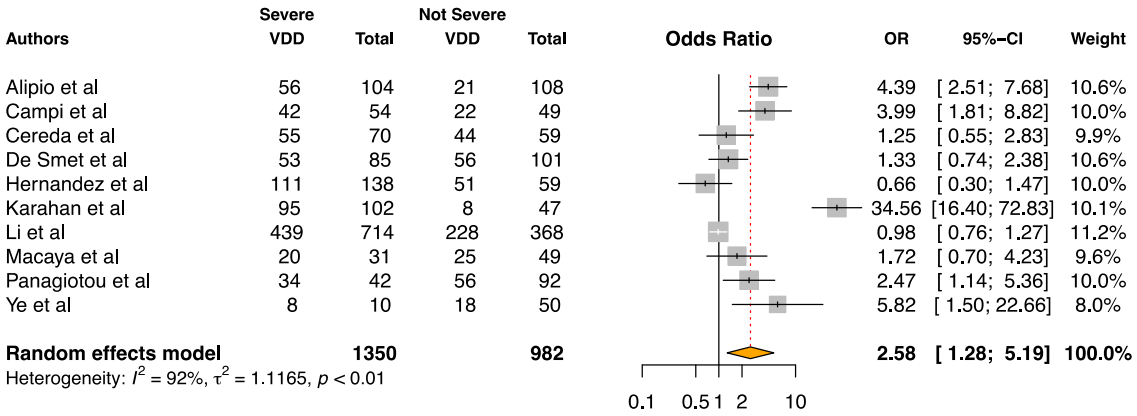


Figure 2: Forest plot of random effect meta-analysis and contour enhanced funnel plot to assess causes of funnel plot asymmetry for vitamin-D and Covid-19 infection meta-analysis for serum 25(OH)D level less than 20 ng/mL or 50 nmol/L. For the confidence interval, the lightgreen area indicates p<0.01, the green area indicates 0.01≤p<0.05, and the darkgreen area indicates 0.05≤p<0.1. Egger's test p=0.399.



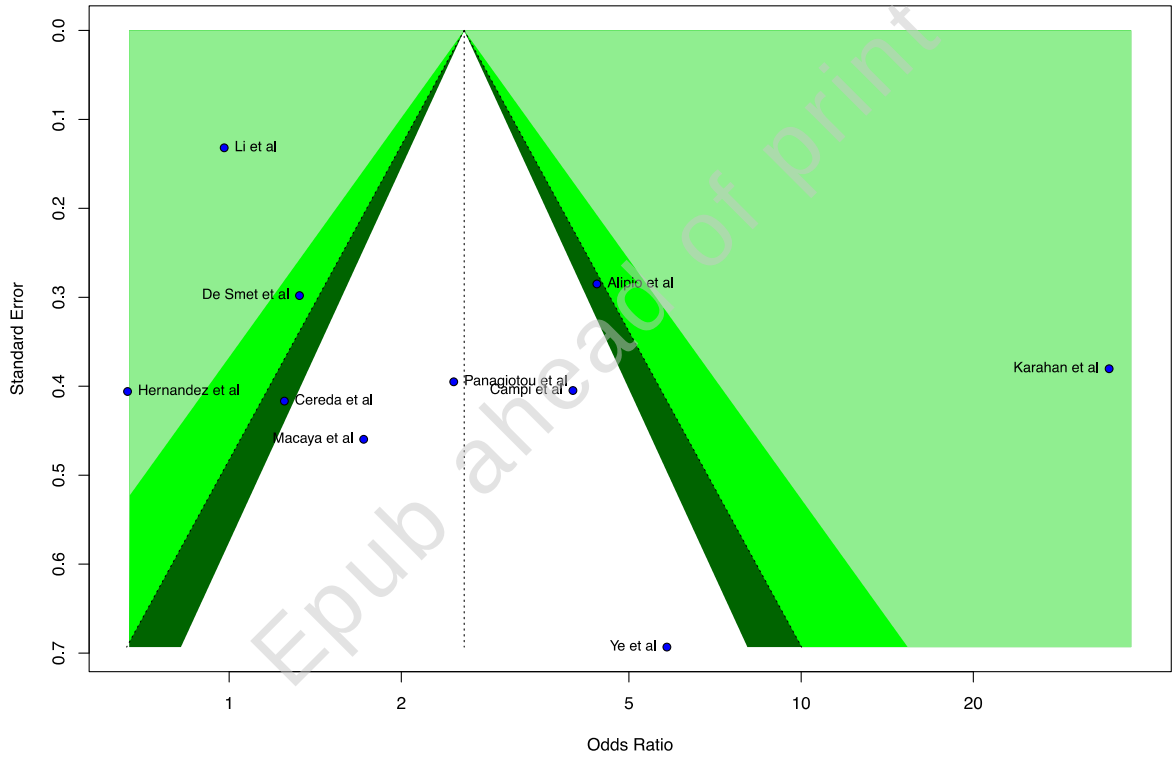


Figure 3: Forest plot of random effect meta-analysis and contour enhanced funnel plot to assess causes of funnel plot asymmetry for vitamin-D and Covid-19 severity meta-analysis for serum 25(OH)D level less than 20 ng/mL or 50 nmol/L. For the confidence interval, the lightgreen area indicates p<0.01, the green area indicates 0.01≤p<0.05, and the darkgreen area indicates 0.05≤p<0.1. Egger's test p=0.054.

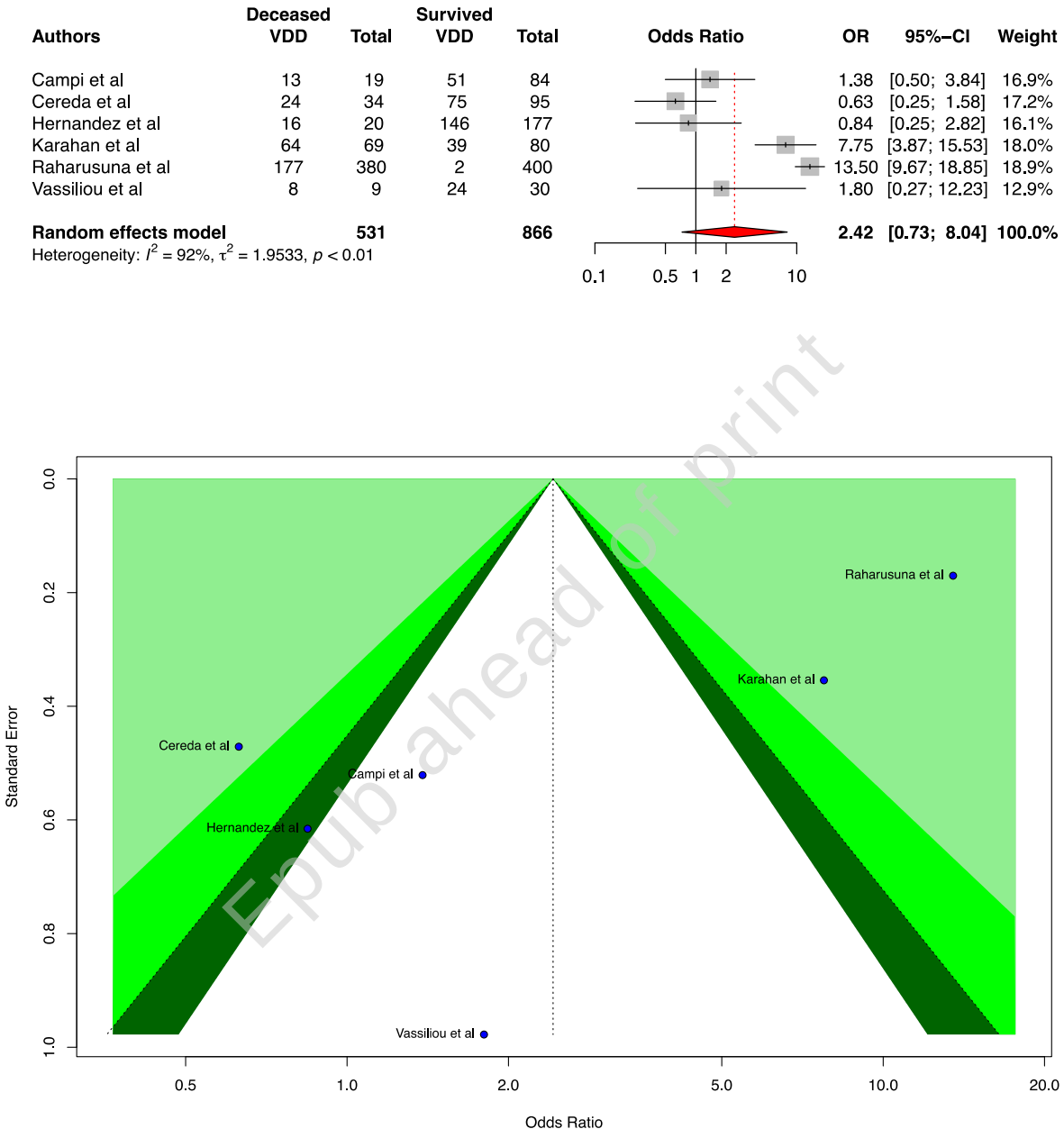


Figure 4: Forest plot of random effect meta-analysis and contour enhanced funnel plot to assess causes of funnel plot asymmetry for vitamin-D and Covid-19 mortality meta-analysis for serum 25(OH)D level less than 20 ng/mL or 50 nmol/L. For the confidence interval, the lightgreen area indicates p<0.01, the green area indicates 0.01≤p<0.05, and the darkgreen area indicates 0.05≤p<0.1. Egger's test p=0.528.