25-hydroxyvitamin D concentrations and clostridium difficile infection

A meta-analysis

Furuya-Kanamori, Luis; Wangdi, Kinley; Yakob, Laith; McKenzie, Samantha J; Doi, Suhail A R; Clark, Justin; Paterson, David L.; Riley, Thomas V; Clements, Archie C

Published in:
Journal of Parenteral and Enteral Nutrition

DOI:
10.1177/0148607115623457

Brief Communication

25-Hydroxyvitamin D concentrations and *Clostridium difficile* infection – a meta-analysis

Running title: Vitamin D deficiency and CDI

Authors: Luis Furuya-Kanamori MEpi[1]; Kinley Wangdi MSc(Trop Med)[1]; Laith Yakob DPhil[2]; Samantha J. McKenzie PhD[3]; Suhail A.R. Doi PhD, FRCP[1]; Justin Clark BA[4]; David L. Paterson PhD, FRACP, FRCPA[5]; Thomas V. Riley PhD, FASM, FAAM, FRCPath, FFSc(RCPA)[6]; Archie C.A. Clements PhD[1]

Authors' affiliation:

[1] Research School of Population Health, The Australian National University, Canberra, Australian Capital Territory, Australia

[2] London School of Hygiene and Tropical Medicine, Department of Disease Control, London, United Kingdom

[3] Institute for Teaching and Learning Innovation, The University of Queensland, St. Lucia, Queensland, Australia

[4] Faculty of Health Sciences and Medicine, Bond University, Gold Coast, Queensland, Australia

[5] The University of Queensland, UQ Centre for Clinical Research, Herston, Queensland, Australia

[6] Microbiology & Immunology, The University of Western Australia and Department of Microbiology, PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre, Nedlands, Western Australia, Australia

Correspondence to: Luis Furuya-Kanamori, The Australian National University, Research School of Population Health, Building 62 Mills Road, Canberra, ACT 2601, Australia; email: luis.furuya-kanamori@anu.edu.au; Telephone: +61 2 6125 9206; Fax + 61 2 6125 5608

Abstract

Background: Well-known risk factors for *Clostridium difficile* infection (CDI) are exposure to antibiotics and gastric acid suppressants. Recent studies have provided some evidence of an association between hypovitaminosis D and the risk of CDI. Therefore, this meta-analysis aimed to pool all the existing evidence to investigate the association between 25-hydroxyvitamin D (25[OH]D) and CDI.

Methods: A systematic search was conducted in three databases (PubMed, Embase and Web of Sciences) for epidemiological studies that examined the association between mean 25(OH)D concentrations and CDI as well as between 25(OH)D status and CDI severity or recurrence. 25(OH)D status was defined as "lower" or "higher" at a threshold concentration of <20 or ≥20ng/ml, respectively. Pooled effect sizes were computed using the inverse variance heterogeneity model of meta-analysis.

Results: Eight publications (n = 4479 patients) were included in the meta-analysis. The mean concentration of 25(OH)D in patients with CDI was 3.54 ng/ml (95%CI: 0.39 to 6.89ng/ml) lower than in patients without CDI. Patients with lower 25(OH)D status had a higher odds (OR = 1.61; 95%CI: 1.02 to 2.53) of developing severe CDI compared to those with a higher 25(OH)D status. No significant association was found between 25(OH)D status and CDI recurrence.

Conclusion: The results of this meta-analysis suggest that lower mean concentrations of 25(OH)D were associated with CDI. A lower 25(OH)D status increased the odds of severe CDI but not of CDI recurrence.

Keywords: *Clostridium difficile*; infection; recurrence; severity; vitamin D; 25-hydroxyvitamin D.

Clinical Relevancy Statement

Clostridium difficile infection (CDI) is the leading cause of antibiotic-associated nosocomial diarrhea. Recent studies have reported contradictory evidence of hypovitaminosis D as a novel risk factor for CDI; therefore, the current meta-analysis was conducted to examine the association between 25-hydroxyvitamin D (25[OH]D) concentrations and CDI. The results of the pooled estimates reveal a lower mean concentration of 25(OH)D in subjects with CDI and an increased odds of severe CDI in patients with a lower 25(OH)D status.

Introduction

Clostridium difficile is a Gram-positive, spore-forming anaerobic bacillus and worldwide it is the main cause of infectious diarrhea in hospitalized patients. The incidence and severity of *C. difficile* infection (CDI) has increased in the last decades mainly due to the emergence of hypervirulent strains.[1] It is estimated that the additional CDI attributable length of stay in acute-care facilities ranges from 2.8 to 6.4 days with an estimated cost per CDI case of up to US$ 15 397.[2] The economic burden to the United States of America (USA) healthcare system attributable to CDI in 2008 was estimated at US$ 4.8 billion.[3]

Traditionally, CDI has been associated with exposure to antimicrobials and gastric acid suppressant medications; however, a recent study has reported an association between higher concentrations of 25-hydroxyvitamin D (25[OH]D) and reduction in risk of CDI in patients with inflammatory bowel disease.[4] Furthermore, Abdelfatah et al.[5] found a protective effect against severe cases of CDI in patients with concentrations of 25(OH)D > 20ng/ml. In contrast, van der Wilden et al.[6] did not find an association between 25(OH)D concentrations and CDI severity.

Given the heavy burden on health systems imposed by CDI and the current contradictory evidence around 25(OH)D and CDI, a meta-analysis was conducted to assess the impact of 25(OH)D status on CDI.

Methods

Search strategy and eligibility criteria

A systematic search with no language restrictions was undertaken in three medical and life sciences databases (PubMed, Embase and Web of Sciences) from their inception to August 2015. Search terms included were "*Clostridium difficile*" and "vitamin D"; the specific keywords and connectors for each database are listed in the Supplementary material.

The inclusion of studies was restricted to published (full-text or conference abstracts) epidemiological studies in humans that reported concentrations of 25(OH)D in an extractable format. The studies were included if they reported mean 25(OH)D concentrations or data around the CDI related outcomes of severity or recurrence. Studies that reported findings in animal model were excluded. No exclusion criteria were considered for indirect methods to detect CDI cases such as ICD codes, as these have proven to be highly specific for CDI.[7] Similarly, no restrictions about CDI severity scores were considered as the majority of the score indices have a good sensitivity and specificity.[8]

Study selection and data extraction

Two researchers (LFK and KW) independently assessed all the citations by titles and abstracts followed by a full-text review of all potentially relevant studies. Data from the included studies were then independently extracted in a spreadsheet by the same two researchers. The recorded fields included study identifiers (authors, publication year); study characteristics (design, setting, inclusion criteria, sample size); mean 25(OH)D concentrations and outcome measurement (CDI, CDI severity, CDI recurrence). 25(OH)D status of "lower" or "higher" were defined based on concentrations <20 ng/ml and ≥20ng/ml.[9] The extracted data were then cross-checked by the two researchers and any

discrepancies during the selection of studies or data extraction were resolved through discussion and consensus.

Statistical analyses

The effect sizes for the difference in mean 25(OH)D concentrations across CDI diagnosis status and the odds ratios (OR) for the association between 25(OH)D status and CDI severity or CDI recurrence were pooled using the inverse variance heterogeneity (IVhet) model.[10] Statistical heterogeneity among studies was assessed by both the Cochran's Q and I^2 index; heterogeneity was defined as low (I^2 < 25%), moderate (I^2 = 26-50%) and high (I^2 > 50%). While I^2 is the percentage of variability that is due to between-study heterogeneity, $1 - I^2$ is the percentage of variability that is due to sampling error. The latter, it is affected by study size; thus, when the studies become very large, the sampling error tends to 0 and I^2 tends to 1.[11] Such heterogeneity may not be clinically relevant and studies with relatively large I^2 in this situation may still be usefully pooled if other measures such as Q or τ^2 remain relatively small and clinically relevant heterogeneity is unlikely to be present. Additionally, the model used to pool effect sizes (IVhet model) takes account of the uncertainty due to heterogeneity and adjusts the confidence interval adequately,[10] which does not happen with the random effects model, thus again justifying pooling in the face of heterogeneity documented using the I^2 index.

The meta-analyses were conducted using MetaXL v3.0 (EpiGear International, Sunrise Beach, Australia).

Results

Yield of search strategy

The search strategy identified 147 records in the three databases after removal of duplicate records. Of these, 121 papers were excluded based on a review of title and abstract. Full texts of the remaining 26 studies were reviewed and 8 articles were selected and included in the final analyses (see Figure 1).

Characteristics of included studies

All the studies were conducted in healthcare settings in the USA. Half of the studies were conducted prospectively,[4,6,12,13] one of which only enrolled patients with inflammatory bowel disease.[4] Among the included studies, three reported 25(OH)D concentrations by *C. difficile* diagnosis outcome (infected versus non-infected).[4,14,15] Three studies[5,6,16] assessed the association between 25(OH)D status (<20 ng/ml versus ≥20 ng/ml) and CDI severity (mild versus severe). Finally, four studies[5,12,13,16] examined the association between 25(OH)D status (<20 ng/ml versus ≥20 ng/ml) and CDI recurrence (see Table 1).

Quantitative synthesis

The pooled mean difference in 25(OH)D concentrations between patients with and without CDI was -3.54 ng/ml (95%CI: -6.89 to -0.39ng/ml) and thus mean 25(OH)D was lower in patients with CDI. Patients with lower 25(OH)D status were at higher odds of developing severe CDI compared to those with higher 25(OH)D status (OR = 1.61; 95%CI: 1.02 to 2.53). No significant difference was found between patients with lower versus higher 25(OH)D status in terms of CDI recurrence (OR = 1.26; 95%CI: 0.56 to 2.83; see Figure 2). Moderate (I^2 = 48%) and high (I^2 = 63%) heterogeneity was observed for the mean difference in 25(OH)D concentrations across CDI status group and the OR for 25(OH)D status and CDI

153 recurrence, respectively. No heterogeneity (I^2 = 1%) more than expected due to chance was
154 observed for the OR for 25(OH)D status and CDI severity. Despite the different degrees of
155 heterogeneity, the confidence intervals under the IVhet model adequately account for the
156 uncertainty due to heterogeneity and retain nominal coverage. Due to the limited number of
157 studies included in each meta-analysis, visual inspection of the funnel plots was not possible
158 to assess the presence of publication bias.

Discussion

Our findings provide some evidence that lower mean concentrations of 25(OH)D were present in subjects diagnosed with CDI and that CDI severity was associated with a lower 25(OH) status. Paradoxically, pooled estimates did not reveal an association between 25(OH)D concentrations and CDI recurrence, one possible explanation for this finding may be differences in the duration of follow-up time used by the researchers. For instance, when CDI recurrence was defined as "within 30 days", Arramraju et al.[12] and Wang et al.[13] found a significant association between lower 25(OH)D status and recurrence; however, when CDI recurrence was evaluated in a longer follow-up period of 56 and 90 days, Abdelfatah et al. [5] and Wong et al. [16] did not find an association. We must point out however that study considerations may have had a role in this discrepancy. For example Wang et al[13] categorized patients who died as a 'non-resolution' of CDI, which may have led to an overestimation of failure to resolve *C. difficile*, as the exact cause of mortality in each patient was unknown. Additionally, other factors such as exposure to certain antibiotics (cephalosporins, aminopenicillins and clindamycin), proton pump inhibitor use, increased patients age and number of previous admissions[17] may have impacted on the CDI recurrence pooled estimate as controlling for these covariates was not possible.

The findings align with those reported by Youssef and colleagues who described an association between 25(OH)D deficiency (< 20 ng/ml) and other hospital-associated infections such as bacterial sepsis and methicillin-resistant *Staphylococcus aureus* colonization.[18] The findings are also in line with those hypothesized beneficial effect of vitamin D supplementation on the reduction of surgical site infections as well as catheter-associated urinary tract infections.[18] Additionally, ecological studies have reported an inverse relationship between ultraviolet B rays exposure (a major promotor of vitamin D synthesis) and CDI mortality[19] or influenza cases complicated by pneumonia.[20] Our findings therefore

add to the growing body of evidence identifying a potential role of lower 25(OH)D status in infectious disease susceptibility.

Although the mechanisms by which 25(OH)D may act as an immunomodulator for CDI are not fully understood, possible explanations are available. Vitamin D plays a vital role in innate (nonspecific) immune response through the stimulation of nitric oxide,[21] cathelicidins[22] and β-defensin 2[23] production in macrophage lysosomes and epithelial cells. Furthermore, vitamin D also modulates cell-mediated immunity via the differentiation of naïve T cells into regulatory $CD4^+$ T lymphocytes.[24] The immunomodulatory activity of vitamin D has also been described in patients with autoimmune disease (multiple sclerosis, systemic lupus erythematosus and rheumatoid arthritis) in which supplementation of vitamin D resulted in a reduction in disease severity.[25-27]

This is the first meta-analysis that examines a potentially new risk factor for CDI; however, several limitations were noted that warrant future research. First, the strains of *C. difficile* ribotypes infecting individuals differ by country/region and certain *C. difficile* ribotypes are associated with different outcomes (e.g., recurrence, severity, mortality). The studies included here were all conducted in the northeast or midwest regions of the USA and *C. difficile* ribotypes was not taken into account. Second, due to the limited number of studies identified, subgroup analysis by the source of CDI (healthcare- versus community-acquired) was not possible. Finally, no studies were identified that examined the effect of 25(OH)D in asymptomatic *C. difficile* colonized individuals. Given that this group of people are a potential source of CDI and may contribute to the transmission of the pathogen, further epidemiological studies are required in order to investigate the role of 25(OH)D in this particular group of people. In view of the safety of vitamin D supplements and their potential to favorably influence the outcome or burden of CDI, we recommend the implementation of

randomized controlled trials to examine the effect of vitamin D supplementation in the reduction of CDI occurrence and CDI severity.

Conflict of Interest

None declared.

Acknowledgement

The authors would like to thank Dr. Wallace Wang for kindly provide us with additional data from his study.

LFK is funded by an Endeavour Postgraduate Scholarship (#3781_2014), an Australian National University Higher Degree Scholarship, and a Fondo para la Innovación, Ciencia y Tecnología Scholarship (#095-FINCyT-BDE-2014). ACAC is funded by an Australian National Health and Medical Research Council Senior Research Fellowship (#1058878).

References

1. Lessa FC, Gould CV, McDonald LC. Current status of *Clostridium difficile* infection epidemiology. *Clin Infect Dis* 2012; 55: S65-S70.

2. O'Brien JA, Lahue BJ, Caro JJ, Davidson DM. The emerging infectious challenge of *Clostridium difficile*-associated disease in Massachusetts hospitals: clinical and economic consequences. *Infect Control Hosp Epidemiol* 2007; 28: 1219-27.

3. Dubberke ER, Olsen MA. Burden of *Clostridium difficile* on the healthcare system. *Clin Infect Dis* 2012; 55: S88-S92.

4. Ananthakrishnan AN, Cagan A, Gainer VS, et al. Higher plasma vitamin D is associated with reduced risk of *Clostridium difficile* infection in patients with inflammatory bowel diseases. *Aliment Pharmacol Ther* 2014; 39: 1136-42.

5. Abdelfatah M, Nayfe R, Moftakhar B, et al. Low vitamin D level and impact on severity and recurrence of *Clostridium difficile* infections. *J Investig Med* 2015; 63: 17-21.

6. van der Wilden GM, Fagenholz PJ, Velmahos GC, Quraishi SA, Schipper IB, Camargo CA, Jr. Vitamin D status and severity of *Clostridium difficile* infections: a prospective cohort study in hospitalized adults. *JPEN J Parenter Enteral Nutr* 2015; 39: 465-70.

7. Jones G, Tarlight N, Boelle PY, et al. Accuracy of ICD-10 codes for surveillance of *Clostridium difficile* infections, France. *Emerg Infect Dis* 2012; 18: 979-81.

8. Fujitani S, George WL, Murthy AR. Comparison of clinical severity score indices for *Clostridium difficile* infection. *Infect Control Hosp Epidemiol* 2011; 32: 220-8.

9. Thacher TD, Clarke BL. Vitamin D Insufficiency. *Mayo Clinic Procs* 2011; 86: 50-60.

10. Doi SAR, Barendregt JJ, Khan S, Thalib L, Williams GM. Advances in the meta-analysis of heterogeneous clinical trials I: The inverse variance heterogeneity model. *Contemp Clin Trials* 2015 [Epub ahead of print].

11. Rucker G, Schwarzer G, Carpenter J, Schumacher M. Undue reliance on I2 in assessing heterogeneity may mislead. *BMC Med Res Methodol* 2008; 8: 79.

12. Arramraju S, Shalomov A, John BK, Rubin M. Higher resolution rate of clostridia difficile enteritis in hospitalized patients with normal vitamin D levels. *Gastroenterology* 2010; 138: S580.

13. Wang WJ, Gray S, Sison C, et al. Low vitamin D level is an independent predictor of poor outcomes in *Clostridium difficile*-associated diarrhea. *Therap Adv Gastroenterol* 2014; 7: 14-9.

14. Quraishi SA, Litonjua AA, Moromizato T, et al. Association between prehospital vitamin D status and hospital-acquired *Clostridium difficile* infections. *JPEN J Parenter Enteral Nutr* 2015; 39: 47-55.

15. Sahay T, Ananthakrishnan AN. Vitamin D deficiency is associated with community-acquired *Clostridium difficile* infection: a case-control study. *BMC Infect Dis* 2014; 14: 661.

16. Wong KK, Lee R, Watkins RR, Haller N. Prolonged *Clostridium difficile* infection may be associated with vitamin D deficiency. *JPEN J Parenter Enteral Nutr* 2015 [Epub ahead of print].

17. Zilberberg M, Reske K, Olsen M, Yan Y, Dubberke E. Risk factors for recurrent *Clostridium difficile* infection (CDI) hospitalization among hospitalized patients with an initial CDI episode: a retrospective cohort study. *BMC Infect Dis* 2014; 14: 306.

18. Youssef DA, Ranasinghe T, Grant WB, Peiris AN. Vitamin D's potential to reduce the risk of hospital-acquired infections. *Dermatoendocrinol* 2012; 4: 167-75.

19. Govani SM, Waljee AK, Stidham RW, Higgins PDR. Increasing ultraviolet light exposure is associated with reduced mortality from *Clostridium difficile* infection. *United European Gastroenterol J* 2015; 3: 208-14.

20. Grant WB, Giovannucci E. The possible roles of solar ultraviolet-B radiation and vitamin D in reducing case-fatality rates from the 1918–1919 influenza pandemic in the United States. *Dermatoendocrinol* 2009; 1: 215-9.

21. Rockett KA, Brookes R, Udalova I, Vidal V, Hill AV, Kwiatkowski D. 1,25-Dihydroxyvitamin D3 induces nitric oxide synthase and suppresses growth of *Mycobacterium tuberculosis* in a human macrophage-like cell line. *Infect Immun* 1998; 66: 5314-21.

277 22. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide
278 (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in
279 myeloid cells by 1,25-dihydroxyvitamin D3. *Faseb J* 2005; 19: 1067-77.

280 23. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a
281 direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004; 173: 2909-12.

282 24. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic
283 concepts. *J Steroid Biochem Mol Biol* 2005; 97: 93-101.

284 25. Cantorna MT, Mahon BD. Mounting evidence for vitamin D as an environmental
285 factor affecting autoimmune disease prevalence. *Exp Biol Med* 2004; 229: 1136-42.

286 26. Pelajo CF, Lopez-Benitez JM, Miller LC. Vitamin D and autoimmune rheumatologic
287 disorders. *Autoimmun Rev* 2010; 9: 507-10.

288 27. Agmon-Levin N, Theodor E, Segal RM, Shoenfeld Y. Vitamin D in systemic and
289 organ-specific autoimmune diseases. *Clin Rev Allergy Immunol* 2013; 45: 256-66.

290

Table 1.- Characteristics of the included studies.

Authors	Setting	Study design	Inclusion criteria	Patients' mean (SD) age in years	Sample size (25(OH)D <20 / ≥20 ng/ml)	Outcome measured
Abdelfatah et al., 2015 [5]	Akron General Medical Center, Akron, OH, USA	Case-control study (2007-2013)	Hospitalized patients with positive *C. difficile* toxin assay and recorded 25(OH) D concentration	68.7 (16.7)	271 (133 / 138)	Severity of CDI and recurrence of CDI associated with 25(OH)D status
Ananthakrishnan et al., 2014 [4]	Massachusetts General Hospital and Brigham and Women's Hospital, Boston, MA, USA	Cohort study	Patients with inflammatory bowel disease and recorded plasma 25(OH) D concentration	60.5 (16.9) / 48.7 (18.0) †	3188 (20.4[12.8] / 27.1[12.7])*	Development of CDI associated with 25(OH)D concentrations
Arramraju et al., 2010 [12]	New York Hospital Queens, Flushing, NY, USA	Cohort study (2008-2009)	Admitted patients with positive *C. difficile* toxin assay	NR	62 (34 / 28)	Resolution of CDI (no recurrence) associated with 25(OH)D status
Quraishi et al., 2015 [14]	Massachusetts General Hospital and Brigham and Women's Hospital, Boston, MA, USA	Retrospective cohort study (1993-2006)	Patients aged ≥18 years with documented 25(OH)D concentration prior admission. Patients without vitamin D supplementation or prior CDI.	63 (18)	568 (17[10] / 19[12])*	Development of hospital-acquired CDI associated with 25(OH)D concentrations
Sahay and Ananthakrishnan 2014 [15]	Massachusetts General Hospital, Boston, MA, USA	Case-control study (2010-2013)	Patients with positive *C. difficile* toxin assay and recorded 25(OH)D concentration	62 (19)	116 (28.5[15.4] / 33.8[12.8])*	Community-acquired CDI associated with 25(OH)D concentrations
van der Wilden et al., 2015 [6]	Massachusetts General Hospital, Boston, MA, USA	Cohort study (2011-2013)	Admitted patients with confirmed CDI	62 (19)	100 (43 / 57)	Severity of CDI associated with 25(OH)D status
Wang et al., 2014 [13]	New York Hospital Queens, Flushing, NY, USA	Cohort study (2008-2009)	Hospitalized patients with positive *C. difficile* toxin assay	75 (17)	62 (38 / 24)	Mortality and CDI recurrence associated with 25(OH)D status
Wong et al., 2015 [16]	Akron General Medical Center, Akron, OH, USA	Case-control study (2007-2012)	Hospitalized patients diagnosed with CDI and recorded 25(OH)D concentration within 3 months of CDI	68 (15.7) / 71 (4.4) ‡	112 (56 / 56)	Severity of CDI and recurrence of CDI associated with 25(OH)D status

CDI: Clostridium difficile infection; *SD*: standard deviation; *NR*: not reported
† mean (standard deviation) age for patients with CDI / mean (standard deviation) age for patients without CDI
‡ mean (standard deviation) age for patients with 25(OH)D < 20ng/ml / mean (standard deviation) age for patients with 25(OH)D ≥ 20ng/ml
* mean[standard deviation] of the patients with CDI / mean[standard deviation] of the patients without CDI

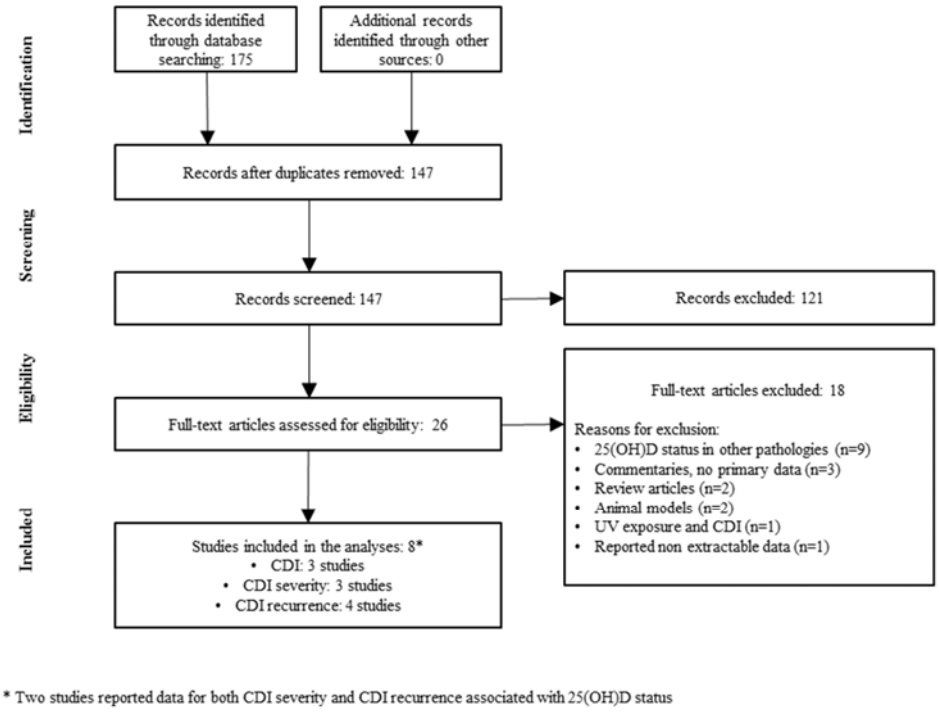
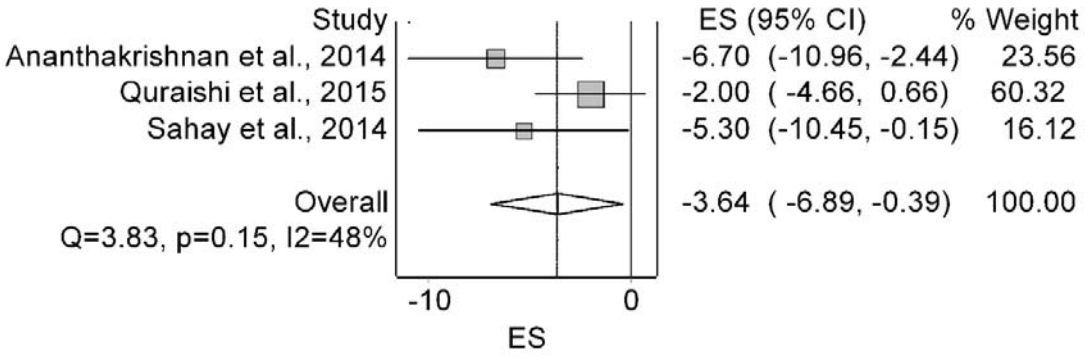
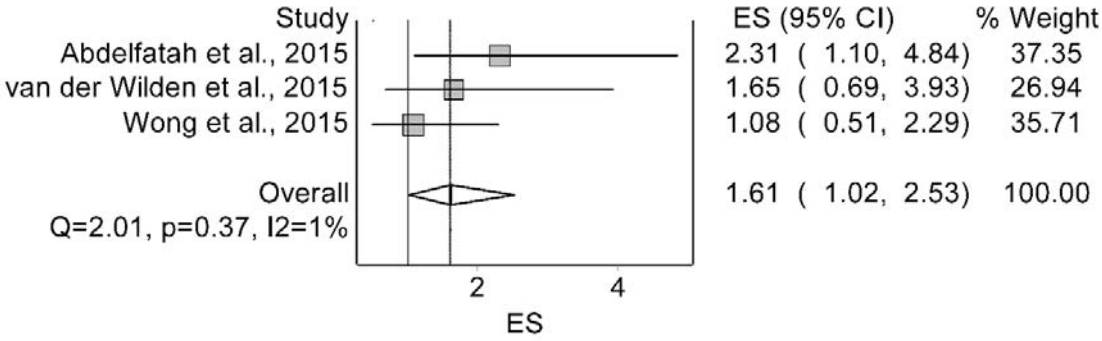


Figure 1.- Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow-diagram.

A



B



C

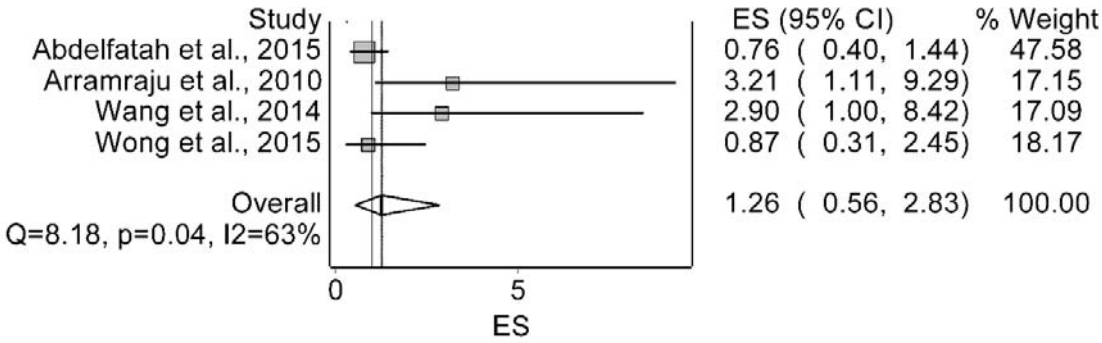


Figure 2.- Forest plots depicting (A) the weighted mean difference in 25(OH)D concentrations across CDI status groups; (B) the odds ratio for 25(OH)D status and CDI severity; and (C) the odds ratios for 25(OH)D status and CDI recurrence.