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## Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

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Complete List of Authors:	Zhang, Lin; Peking Union Medical College Hospital, Clinical Laboratory; University of Melbourne School of Population and Global Health, Centre of Epidemiology and Biostatistics Zou, Huachun; Sun Yat-sen University, School of Public Health Zhao, Yang; The University of Melbourne, Melbourne School of Population and Global Health; WHO Collaborating Centre on Implementation Research for Prevention & Control of NCDs, Hu, Chunlei; Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Department of Gastrointestinal Surgery, Peking University Shenzhen Hospital Atanda, Adejare; Morgan State University, School of Community Health and Policy Qin, Xuzhen; Peking Union Medical College Hospital, Clinical Laboratory Jia, Peng; University of Twente, Faculty of Geo-Information Science and Earth Observation (ITC); International Initiative on Spatial Lifecourse Epidemiology (ISLE) Jiang, Yu; Chinese Academy of Medical Sciences & Peking Union Medical College, School of public health Qi, Zhihong; Peking Union Medical College Hospital, Clinical Laboratory
Keywords:	Colorectal cancer, Colorectal adenoma, Vitamin D, 25-hydroxyvitamin D, Asia, Meta-analysis

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# Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

Lin Zhang,<sup>1,2,3,4,12</sup> Huachun Zou,<sup>5,6,7</sup> Yang Zhao,<sup>8</sup> Chunlei Hu,<sup>9</sup> Adejare Atanda,<sup>10</sup> Xuzhen Qin,<sup>1</sup> Peng Jia,<sup>11,12</sup> Johnny Y. Jiang,<sup>2</sup> Zhihong Qi<sup>1</sup>

<sup>1</sup> Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>2</sup> Peking Union Medical College School of Public Health, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>3</sup> Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, Victoria 3100, Australia

<sup>4</sup> The University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Parkville, Victoria 3100, Australia

<sup>5</sup> School of Public Health (Shenzhen), Sun Yat-sen University, Shenzhen 518107, Guangdong, China

<sup>6</sup> School of Public Health, Sun Yat-sen University, Guangzhou 510085, Guangdong, China

<sup>7</sup> Kirby Institute, University of New South Wales, Sydney 2052, NSW, Australia

<sup>8</sup> School of Population and Global Health, The University of Melbourne & WHO Collaborating Centre on Implementation Research for Prevention & Control of NCDs, Parkville, Victoria 3100, Australia

<sup>9</sup> Department of Gastrointestinal Surgery, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, China

<sup>10</sup> School of Community Health and Policy, Morgan State University, Baltimore, Maryland, USA

<sup>11</sup> Faculty of Geo-Information Science and Earth Observation (ITC), University of Twente, Enschede 7500, The Netherlands

<sup>12</sup> International Initiative on Spatial Lifecourse Epidemiology (ISLE)

## \*Correspondence to:

Prof. Zhihong Qi

Clinical Laboratory, Peking Union Medical College Hospital  
Chinese Academy of Medical Sciences & Peking Union Medical College  
Shuai Fu Yuan 1#, DongCheng District, Beijing 100730, China

Tel.: + 86 10 69159740

Email: cizeng1010@sina.com

Prof. Johnny Y Jiang

Chinese Academy of Medical Sciences & Peking Union Medical College,  
DongDan SanTiao 9, DongCheng District, Beijing 100730, China

Tel.: + 86 10 65105916

Email: wingedsky@gmail.com

**Transparency declaration**

The Corresponding Authors affirm that this manuscript is an accurate account of the study proposed with no important aspects omitted, and that any discrepancies from the study as planned and registered will be explained upon the completion of the proposed study.

**Ethical approval**

Ethics approval is not required for this study because it is a systematic review.

**Details of funding**

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**Conflict of interest**

The authors have declared that no competing interests exist.

**Statement of independence of researchers form funders**

Not applicable.

**Patient involvement**

No patients were involved in the design or analysis of this study.

**Data sharing statement**

Not applicable

## ABSTRACT

### Objectives

We conducted this dose-response meta-analysis to assess the association between blood circulating Vitamin D level and colorectal cancer risk in Asian populations.

### Methods

Relevant studies were identified by searching published literature in MEDLINE, EMBASE, and Web of Science until 31 January 2019, with no restrictions. All studies that reported odds ratio (OR) with 95% confidence interval (CI) for the association of interest were included. Two authors independently extracted data and assessed the quality of included studies. Study-specific ORs were pooled using a random-effects model. A dose-response meta-analysis was performed with generalized least squares regression.

### Results

The eight included studies encompassed a total of 2,916 cases and 6,678 controls. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D levels was 0.75 [95% CI, 0.58-0.97] up to 36.5 ng/mL in Asian population. There was heterogeneity among the studies ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ). The dose-response meta-analysis indicated a significant linear relationship ( $P_{\text{non-linearity}}=0.11$ ). An increment of 16 ng/mL in blood circulating Vitamin D level corresponded to an OR of 0.79 [95% CI, 0.64-0.97].

### Conclusions

The results of this meta-analysis indicate that blood circulating Vitamin D level is associated with decreased risk of colorectal cancer in Asian countries. This dose-response meta-analysis further shows that the strength of this association among Asian populations is similar to that among western populations. Our study suggested that Asian population should improve nutritional status and maintain a higher level of blood circulating Vitamin D.

### Keywords:

Colorectal cancer; Colorectal adenoma; Vitamin D; 25-hydroxyvitamin D; Asia; Meta-analysis

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**Word Count (main text):** 5,313

## Article summary

### Article focus

- The present systematic review aims to explore if there is a decreased colorectal cancer risk associated with blood circulating Vitamin D.

### Key messages

- Meta-analysis of 8 observational studies from Asian countries showed blood circulating Vitamin D level was associated with decreased risk of colorectal cancer in Asian countries.
- This dose-response meta-analysis further showed that the strength of this association among Asian populations was similar to that among western populations.

### Strengths and limitations of this study

- Our study seeks to extend previous work by including a number of new studies and by distinguishing Asian population explicitly.
- The number of included studies is not sufficient to provide a robust estimate, so the results should be interpreted in the context of the limitations of the available data.
- Heterogeneous definitions of blood circulating Vitamin D categories were used across studies. The variability in definitions could limit comparability between studies.
- Our study included seven case-control studies; the study design implies that the measurement of blood circulating Vitamin D is measured in individuals already diagnosed with colorectal cancer. Therefore, results from case-control studies need to be interpreted cautiously because of the potential for reverse causation.
- Time of blood sampling in relation to outcome ascertainment also varied among studies. Such cross-sectional measurements may not accurately reflect an individual's Vitamin D status across time.

## INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer and second in terms of mortality, with over 1.8 million new cases and 881,000 deaths worldwide in 2018, accounting for about 1 in 10 cancer cases and deaths.<sup>1</sup> Some Asian countries where the incidence of colorectal cancer was historically low, such as Japan, Israel, Singapore, China, and the Philippines, are experiencing rising incidence rates over the past decades. In 2012, Japan (Miyagi Prefecture Cancer Registry) presented the highest colorectal cancer incidence in the world for men (62 per 100,000 persons) and women (37 per 100,000 persons).<sup>2</sup> Observational studies have identified several risk factors associated with an increased incidence of colorectal cancer including lifestyle factors (e.g., obesity, physical inactivity, smoking, and heavy alcohol use) and non-modifiable factors (e.g., aging, personal and family history of colorectal cancer or adenoma).<sup>3</sup> Other observational studies conducted in western countries suggested blood circulating 25-hydroxyvitamin D (25(OH)D) (also named as Vitamin D) has a protective role in the development of colorectal cancer.<sup>4-8</sup> Some meta-analyses have consistently reported that there was an inverse association of plasma Vitamin D concentration in the blood with incidence and mortality of colorectal cancer.<sup>9-15</sup>

The prevalence of Vitamin D deficiency has increased in recent decades.<sup>16 17</sup> In a recent population-based study of Asian adults, approximately 75% had suboptimal Vitamin D concentrations.<sup>18</sup> The Endocrine Society Clinical Practice Guideline defines Vitamin D deficiency as 25(OH)D level <20 ng/mL and insufficiency as 21 to 29 ng/mL.<sup>19</sup> Feldman et al.<sup>20</sup> reported anti-neoplastic actions of Vitamin D, particularly in colorectal cancer.<sup>9</sup> Touvier et al.<sup>12</sup> reported that improving Vitamin D levels could be beneficial in reducing colorectal cancer incidence. Data from a cohort of healthy women shows that plasma Vitamin D levels were inversely related to the occurrence and death from colorectal cancer.<sup>21</sup> In the Nurses' Health Study, total circulating Vitamin D was associated with lower risk of colorectal cancer in white women.<sup>22</sup> A recent international study of 17 cohorts in western population found that Vitamin D deficiency was associated with increased colorectal cancer risk, and Vitamin D above sufficiency levels was associated with 19% to 27% lower risk.<sup>23</sup> Compared with western countries, there was inconsistent conclusion about the relationship between blood circulating Vitamin D level and colorectal cancer risk in studies of Asian countries,<sup>24-30</sup> given that lifestyle, ethnic and environmental factors are different between Asian and western countries.

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3 We hypothesized that the association between blood circulating Vitamin D and colorectal  
4 cancer in Asian countries is distinct from western countries. Thus, this review aimed to  
5 summarize epidemiological evidence regarding blood circulating Vitamin D level and  
6 colorectal cancer risk in Asian countries. This study underlines the public health importance  
7 of reaching and maintaining an optimal vitamin D status in Asian populations and may help  
8 to guide clinical and nutritional practice in Asians countries.  
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## 14 **METHODS**

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18 We performed the systematic review according to a predetermined protocol and reported in  
19 accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses  
20 Protocols (PRISMA-P) guidelines.<sup>31</sup> Two reviewers (L.Z. and Z.H.Q) independently  
21 undertook the literature search, assessment for eligibility, data extraction, and qualitative  
22 assessment. Any inconsistencies between the two reviewers were reviewed by a third  
23 reviewer (J.Y.) and resolved by consensus.  
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### 30 **Eligibility criteria**

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33 *Participants:* Our study draws participants from the Asian population in Asia countries,  
34 according to the list of sovereign states and dependent territories in Asia by the United Nation  
35 (<https://unstats.un.org/unsd/methodology/m49/>), with 48 countries located in five regions  
36 (Central Asia, Eastern Asia, Southern Asia, Southeastern Asia, and Western Asia).  
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38 Populations of Asian origin but who live in western countries were excluded.

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42 *Exposure:* The exposure is blood circulating 25-hydroxyvitamin D (25(OH)D) level which is  
43 commonly measured to assess and monitor Vitamin D status in individuals. Most studies only  
44 report the total level and do not distinguish D2 and D3 forms of the vitamin. In our meta-  
45 analysis, we consider the total level of Vitamin D as the exposure.  
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49 *Comparators (controls):* In order to be eligible for inclusion, studies must compare outcomes  
50 in a group of exposed individuals with highest category of blood circulating Vitamin D level  
51 and a group of unexposed individuals with lowest category of blood circulating Vitamin D  
52 level.  
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56 *Outcome:* Studies included in the review have a diagnosis of colorectal cancer or colorectal  
57 adenoma, clinically confirmed by colonoscopy or pathology.  
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3 *Study design:* We target observational studies (case-control, cross-sectional and cohort).  
4 Studies conducted post-1980 and reported in English were considered eligible. Animal  
5 studies were not included.  
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10 Studies were excluded if they: 1) were reviews, editorial, case report, or guideline articles; 2)  
11 did not explicitly state the blood circulating Vitamin D level and its association with  
12 colorectal cancer risk; 3) allowed controls to have a previous disease history of cancer; 4)  
13 focused on western populations or Asian populations living in western countries; or 5)  
14 investigated the blood circulating Vitamin D level and its association with survival of  
15 colorectal cancer. By consensus among all three reviewers (L.Z., Z.H.Q, and J.Y), if data  
16 sources were duplicated in more than one study, only the original study was included in the  
17 meta-analysis.  
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### 24 25 **Search strategy**

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28 We conducted a literature search using Medline, Embase, and Web of Science to search all  
29 relevant articles that reported the association plasma or serum Vitamin D level and the risk of  
30 colorectal neoplasia in Asian countries, published from January 1980 to 31 January 2019.  
31 The Medical Subject Heading (MeSH) terms were used in conjunction with the following  
32 keywords to search for the studies: (colorectal neoplasm or colon neoplasm or colorectal  
33 cancer or colon cancer) AND (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-  
34 hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-  
35 hydroxylase or Vitamin D) AND (Asia\* or Afghanistan or Armenia or Azerbaijan or Bahrain  
36 or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or  
37 Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan  
38 or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or  
39 North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi  
40 Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or  
41 Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan  
42 or Vietnam or Yemen). Full search strings are presented in **Table S1**. References from any  
43 relevant articles, editorials, conference abstracts, letters, and reviews were also thoroughly  
44 reviewed to identify any additional relevant studies. Full manuscripts of every article with a  
45 relevant title and abstract were then reviewed for eligibility.  
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## Data extraction and qualitative assessment

Two reviewers (L.Z. and Z.H.Q) independently extracted the following study-level characteristics from each eligible study: first author, year of publication, type of study, country where the study was conducted, selection criteria, the numbers of cases and controls (for case-control studies or cross-sectional studies) and the numbers of total participants and incident cases (for cohort studies), population characteristics (sex and age), follow-up period (for cohort studies), sample size, levels of Vitamin D in both case and control group, measures and ranges of Vitamin D, adjusted variables, and risk estimates with corresponding 95% CI for each category. For studies that reported both crude and adjusted estimates of the association between blood circulating Vitamin D and risk of colorectal cancer or adenoma, we used the adjusted estimates for the meta-analysis. For studies that reported several adjusted estimates of association, we used the estimates adjusted for the most variables.

We applied the Newcastle-Ottawa Scale (NOS) quality assessment tool to evaluate the quality of the selected observational studies. This tool was used to measure the key aspects of the methodology in selected studies with regard to design quality and the risk of biased estimates based on three design criteria: 1) selection of study participants; 2) comparability of study groups; and 3) assessment of outcome and exposure with a star system (with a maximum of 9 stars). We judged studies that received a score of 7-9 stars to be at low risk of bias, studies that scored 4-6 stars to be at medium risk, and those that scored 3 or less to be at high risk of bias. A funnel plot was used to assess the publication bias. Any disagreement on the data extraction and quality assessment of the studies were resolved through comprehensive discussion (L.Z., Z.H.Q, and J.Y).

## Statistical analysis

Study-specific OR estimates were combined using a random-effects model, which considers both within-study and between-study variation's and their corresponding 95% CIs were extracted directly from articles where available, with adjusted ORs extracted preferentially over unadjusted ORs. The dose-response analysis was utilized to assess the relationship between blood circulating Vitamin D level and colorectal cancer risk using the generalized least squares (GLS) method since this method could resolve the inconsistency issue of different Vitamin D levels analyzed in included studies.<sup>32 33</sup> For the dose-response meta-analysis of blood circulating Vitamin D levels, we used the method proposed by previously published studies to compute the trend from the correlated log OR estimates across categories of Vitamin D levels.<sup>34</sup> This analytical method collected the distribution of cases and controls, median values of blood circulating Vitamin D levels, and corresponding OR estimates in each category for each study. The assigned value of the lowest category was designated as a reference level. If the study did not provide median values of blood Vitamin D, the midpoint of the upper and lower boundaries in each category was assigned. For the open-ended exposure categories, the length of the open-ended interval was assumed to be the same as that of the adjacent interval. We examined a potential non-linear dose-response relationship between blood circulating Vitamin D level with colorectal cancer risk by modelling Vitamin D levels using random-effect restricted cubic splines with three knots at percentiles 25%, 50%, and 75% of the distribution (spline model). A *P*-value for nonlinearity was calculated by testing the null hypothesis that the regression coefficient of the second spline was equal to zero by Wald-type test of nonlinear hypotheses.<sup>34</sup> A small *P*-value (<0.05) of Wald-type test indicates departure from linearity. To test and verify the non-linear model, a meta-analysis comparing the appropriate open categories (or highest category) of exposure to the lowest category was performed. The non-linear dose-response relationship was also verified by several representative point values and the risk estimates of a subgroup analysis based on the range of exposure.

The statistical heterogeneity among studies was evaluated using Cochran's *Q* test and *I*<sup>2</sup> statistic, with values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively.<sup>35</sup> The criterion for identifying heterogeneity was a *P* value less than 0.05 for the *Q* test. If substantial heterogeneity was detected, we performed univariate meta-regression analyses to explore the proportion of between-study variance explained by

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3 study quality, participant characteristics, and study characteristics. We were unable to  
4 perform a multivariate meta-regression analysis as only a small number of included studies  
5 reported information for all study-level factors. We also performed subgroup analyses  
6 comparing pooled association estimates and heterogeneity with stratification by year of  
7 publication, participants sex, outcome type, the subregion of Asia, blood sample type, and  
8 range of Vitamin D levels (The range is the difference in the midpoint between the highest  
9 and lowest categories of blood circulating Vitamin D in each study). An estimation of  
10 publication bias was evaluated by the Beggs funnel plot, in which the SE of log (OR) of each  
11 study was plotted against its log (OR). An asymmetrical plot suggests possible publication  
12 bias. Egger's linear regression test assessed funnel plot asymmetry, a statistical approach to  
13 identify funnel plot asymmetry on the natural logarithm scale of the ORs. All statistical  
14 analyses were performed using Stata software (version 14.2; StataCorp LP, College Station,  
15 Texas). All of the *P* values were two-sided, and *P* <0.05 was considered as statistically  
16 significant.  
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## RESULTS

### Selection of studies

A detailed PRISMA flow diagram<sup>31</sup> of literature search and inclusion is shown in **Figure 1**. A total of 611 studies were initially identified with this literature search (250 from Medline, 272 from Embase and 89 from Web of Science), but 114 studies were excluded due to duplication and 465 were excluded after screening the titles and abstracts. Then 24 studies were excluded after a full-text review (details shown in **Table S2**). Finally, a total of eight studies were identified as eligible for this meta-analysis.

### Study characteristics

The eight studies of blood circulating Vitamin D level were included with a total of 2,916 cases and 6,678 controls (**Tables 1 and 2**). The eight studies included in the meta-analysis were published between 2007 and 2018, with seven studies from Eastern Asia in which four are from Japan,<sup>24 25 27 30</sup> two from Korea,<sup>26 28</sup> and one from China;<sup>36</sup> and one study is from Western Asia.<sup>29</sup> Regarding the study design, seven were case-control,<sup>24-29 36</sup> and one was a nested case-cohort study.<sup>30</sup> Of these eight studies, four studies provided the main endpoint of colorectal cancer<sup>24 29 30 36</sup> and four studies provided the main endpoint of colorectal adenoma.<sup>25-28</sup>

### Meta-analysis and dose-response analysis

The multivariable-adjusted ORs for each study and the combination of all eight studies for the highest versus lowest categories of blood circulating Vitamin D levels are shown in **Figure 2**. The mean blood circulating Vitamin D level of the included study population was 20.21 ng/mL, with an SD of 7.92 ng/mL, the minimal concentration of 3.65 ng/mL, and the maximal concentration of 36.5 ng/mL. Results from the studies on blood circulating Vitamin D levels in relation to colorectal cancer risk were inconsistent, with both inverse and positive associations reported. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D level was 0.75 [95% CI, 0.58-0.97], which indicate a higher blood circulating Vitamin D level had a significant inverse association with risk of colorectal cancer. There was statistically significant heterogeneity among the studies ( $I^2=53.9\%$ ,  $P=0.034$ ).

We evaluated the non-linear dose-response relationship between blood circulating Vitamin D

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3 level and colorectal cancer risk. A 16 ng/mL increment (about 2 SDs=15.84 ng/mL) in blood  
4 circulating Vitamin D level conferred an OR of 0.79 [95% CI, 0.64-0.97], which meant the  
5 risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in blood Vitamin D,  
6 and the reduction was statistically significant. A moderate heterogeneity existed ( $I^2=53.9\%$ ,  
7  $P_{\text{heterogeneity}}=0.034$ ) in the overall analysis of blood circulating Vitamin D level, without a  
8 significant non-linear dose-response relationship ( $P_{\text{non-linearity}}=0.11$ ), suggesting that the non-  
9 linear dose-response relationship does not depart from linearity. Similar trends were observed  
10 with linear and spline models (**Figure 3**).  
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### 19 **Subgroup analysis**

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21 When we stratified the analysis according to blood sample type, the pooled ORs of serum  
22 sample and plasma for the highest versus lowest categories of blood circulating Vitamin D  
23 levels were 0.52 [95% CI, 0.34-0.80] and 0.77 [95% CI, 0.59-1.00] respectively. The results  
24 showed that there was a substantial risk reduction (48%) for blood serum Vitamin D level  
25 associated with risk of colorectal cancer. There was no evidence of significant statistical  
26 heterogeneity among studies ( $I^2=22.67\%$ ,  $P=0.21$ ). We then performed subgroup analysis of  
27 blood circulating Vitamin D range in each study. The pooled ORs was 0.93 [95% CI, 0.70-  
28 1.25] for studies with range  $\leq 15$  ng/mL and 0.62 [95% CI, 0.47-0.83] for studies with range  
29  $>15$  ng/mL. There was no evidence of statistical heterogeneity among studies ( $I^2=28.48\%$ ,  
30  $P=0.08$ ). Stratifying by year of publication, the pooled ORs of colorectal cancer for the  
31 highest versus lowest categories of blood circulating Vitamin D level was 0.76 [95% CI,  
32 0.49-1.19] for studies published before 2014, and 0.74 [95% CI, 0.53-1.03] for studies  
33 published after 2014. There was no significant statistical heterogeneity among studies of the  
34 Vitamin D level ( $I^2=60.47\%$ ,  $P=0.76$ ). When stratifying by outcome, the pooled ORs were  
35 0.67 [95% CI, 0.40-1.14] for studies when the outcome was colorectal adenoma and 0.83  
36 [95% CI, 0.66-1.06] when the outcome was colorectal cancer respectively with no  
37 significantly statistical heterogeneity among studies ( $I^2=59.48\%$ ,  $P=0.73$ ). When we stratified  
38 the studies by sex, the pooled ORs were 0.59 [95% CI, 0.13-2.74] for studies with estimates  
39 for women, and 1.13 [95% CI, 0.81-1.58] for men respectively with no significantly  
40 statistical heterogeneity among studies ( $I^2=45.89\%$ ,  $P=0.70$ ). We also conducted analyses that  
41 were stratified according to the region of the population. The pooled ORs was 0.75 [95% CI,  
42 0.57-1.00] for Eastern Asia and 0.69 [95% CI, 0.36-1.34] for Western Asia. There was no  
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3 significantly statistical heterogeneity among studies ( $I^2=59.79\%$ ,  $P=0.87$ ). The above  
4 subgroup meta-analyses are shown in **Figure S1** and summarized in **Table 3**.  
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### 8 **Qualitative assessment and publication bias**

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11 The NOS tool was used to conduct a qualitative assessment of the selected studies to review  
12 the quality of the studies and detect possible bias. Of the eight studies, five studies were at  
13 low risk of bias was 8-9 stars.<sup>24-27 30</sup> The three studies at medium risk (5-7 stars) mainly due  
14 to the bias from representativeness of cases or controls, control definition, and response  
15 rate.<sup>29 36 37</sup> ( shown in **Table S3**.) The Funnel plot and Eggers statistical test indicated no  
16 evidence of publication bias in the studies included in the meta-analysis ( $P=0.338$ ) (**Figure**  
17 **S2**.)  
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## DISCUSSION

Colorectal cancer is one of the cancers with high morbidity and mortality in the world. The one-carbon metabolism pathway requires adequate Vitamin D, and this raises the possibility that Vitamin D may have an essential role in the risk of colorectal cancer. Many epidemiological studies from Europe and the United States believe that increasing the concentration of circulating Vitamin D can reduce the morbidity and mortality of colorectal cancer.<sup>38</sup> However, the association between blood circulating Vitamin D level and risk of colorectal cancer in the Asian population is still under debate due to a lack of sufficient evidence. This systematic review highlights the inconsistencies among studies addressing the role of blood circulating Vitamin D and colorectal cancer risk in the Asian population. Our systematic review identified eight studies of 2916 cases and 6678 controls that addressed the relationship between blood circulating Vitamin D level and colorectal cancer risk. This meta-analysis found that 25% reduced risk of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D level (OR=0.75, 0.58-0.97) up to 36.5 ng/mL, which indicated higher blood circulating Vitamin D level had a significant inverse association with risk of colorectal cancer in Asian population. Our meta-analysis results showed that the negative correlation between Vitamin D and the risk of colorectal cancer is similar to that of European and American population studies<sup>11 12 21 22 39-42</sup> and consistent with the a meta-analysis from Ekmekcioglu C et al.<sup>13</sup> revealed a pooled relative risk (RR) of 0.62 (0.56–0.70) for colorectal cancer when comparing individuals with the highest category of 25(OH)D with those in the lowest.

Our results found a 16 ng/mL increment in blood circulating Vitamin D level conferred an OR of 0.79 [95% CI, 0.64-0.97], which meant the risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in blood Vitamin D, and the reduction was statistically significant. Our study also suggested a linear dose-response relationship ( $P_{\text{non-linearity}} = 0.11$ ) - several studies conducted in western populations reveal a similar protective dose-response association of blood circulating Vitamin D and colorectal cancer risk. For example, a meta-analysis reported a 26% lower risk of colorectal cancer per 10 ng/mL increment in blood circulating Vitamin D levels.<sup>11</sup> Most experts define Vitamin D deficiency as a Vitamin D level of less than 20 ng/mL.<sup>43 44</sup> Vitamin D concentration of 21 to 29 ng/mL can be considered to indicate a relative insufficiency of Vitamin D, and a level of 30 ng/mL or higher can be considered to indicate sufficient Vitamin D.<sup>45</sup> With the use of such definitions, it has been estimated that many people have Vitamin D deficiency or insufficiency.<sup>43 44</sup>



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3 Previous research has implied the association of Vitamin D deficiency with an increased  
4 incidence of bone fractures.<sup>46</sup> There have also been data showing Vitamin D deficiency to be  
5 associated with cancer,<sup>47-49</sup> diabetes,<sup>50</sup> cognitive impairment,<sup>51</sup> and all-cause mortality.<sup>52</sup>  
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7 Among colorectal cancer patients, the prevalence of Vitamin D deficiency was much higher  
8 (nearly 90%) than among patients with other chronic diseases.<sup>53</sup> Humans obtain Vitamin D  
9 from exposure to sunlight, from natural diets, fortified diets, and medicines.<sup>46</sup> It is possible  
10 that the dose-response between Vitamin D concentrations and colorectal cancer risk is  
11 different between Asian and European populations due to ethnic, anthropometric, dietary, and  
12 environmental factors.<sup>12</sup> Lifestyle and diet can promote the development of early-onset colon  
13 lesions by regulating growth factors that interact with inflammatory pathways.<sup>41</sup> An  
14 association between Vitamin D status and reduced risk of colorectal cancer has been found in  
15 ethnically diverse populations.<sup>5</sup> Vitamin D interacts with calcium to enhance the reduction of  
16 colon cancer risk.<sup>54-56</sup> Studies have shown that Vitamin D and calcium may interact and that  
17 both are needed in reducing cancer risk.<sup>57</sup> However, even after adjusting for calcium intake in  
18 some studies,<sup>6,58</sup> Vitamin D was associated with a lower risk. The independent effects of  
19 Vitamin D are supported, but the combined effects of the Vitamin D and calcium may be  
20 greater than the sum of their independent effects.<sup>59</sup> Vitamin A has an antagonistic effect on  
21 Vitamin D<sup>58</sup> and taking both at the same time can lead to decreased calcium absorption. Still,  
22 Vitamin A is often combined with Vitamin D in supplements. Epidemiological evidence links  
23 the incidence of colorectal cancer to lifestyle, smoking, physical activity, alcohol  
24 consumption, and sleep.<sup>60</sup> It has also been linked to reduced fruit and vegetable consumption  
25 and increased consumption of red meat. Dairy products, fish and other foods and cooking  
26 methods also play an essential role.<sup>61</sup> In addition, some drugs such as non-steroidal anti-  
27 inflammatory drugs and cyclooxygenase inhibitors are also involved.<sup>62</sup> Women on estrogen  
28 therapy, for example, did not reduce their risk of colorectal cancer by taking Vitamin D and  
29 calcium supplements.<sup>63</sup> Increased dietary fiber intake reduces the risk of colorectal cancer,  
30 and obesity and low physical activity reduce plasma 25(OH)D concentration, thereby  
31 increasing the risk of colorectal cancer.<sup>42</sup> For every 1 kg/m<sup>2</sup> increase in BMI of colorectal  
32 cancer patients, serum Vitamin D level decreased significantly (0.46 ng/mL).<sup>64</sup> Most  
33 variation in Vitamin D levels usually comes from exposure to the sun, which is an essential  
34 source of Vitamin D for people who get more from fish, even in Japan.<sup>65</sup>  
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36 This meta-analytic comparison revealed a statistically significant beneficial effect of blood  
37 circulating Vitamin D for colorectal cancer. However, the diversity of the studies and the  
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3 presence of moderate heterogeneity in our study ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ) in the overall  
4 analysis of blood circulating Vitamin D levels may preclude making meaningful conclusions  
5 from the pooled analysis because the pooled estimate may not reflect the true underlying  
6 effect. Much of the heterogeneity was not explained through subgroup analyses in our study.  
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10 The potential reasons for the heterogeneity in the strength of the association by Asian  
11 subregions may include many factors. Firstly, the type of food consumption and vitamin  
12 supplements varied according to the specific dietary habits and lifestyle in each subregion of  
13 Asia. A systemic review studied correlations between various diet types, food or nutrients  
14 and colorectal cancer risk among Asian populations, and suggested that red meats, processed  
15 meats, preserved foods, saturated/animal fats, cholesterol, high sugar foods, spicy foods,  
16 tubers or refined carbohydrates have been found to have a positive association with colorectal  
17 cancer risk.<sup>66</sup> Besides diet, other personal and lifestyle factors (e.g., obesity, smoking and  
18 drinking habit) may alter the strength of the association and contribute to the heterogeneity of  
19 the association in Asian countries. Our study revealed no evidence of publication bias, and  
20 most of the studies included in our meta-analysis verified the diagnoses of colorectal cancer  
21 for cases. Histologic confirmation of cancer diagnoses for cases was an optimal validation for  
22 the case-control design in our meta-analysis.  
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33 Possible confounders for the association between colorectal cancer and blood circulating  
34 Vitamin D levels include sex, age, family history of colorectal cancer, smoking, alcohol  
35 drinking, body mass index, and diabetes. Most studies in our meta-analysis provided risk  
36 estimates that were adjusted for age,<sup>24 26-30 36</sup> sex,<sup>24 26 27 29 30 36</sup> body mass index,<sup>24-30 36</sup>  
37 smoking,<sup>24-30 36</sup> drinking,<sup>24-26 29 36</sup> physical activity,<sup>30</sup> alcohol consumption,<sup>24-30</sup> and family  
38 history of colorectal cancer,<sup>24 25 29</sup> fewer were adjusted for folate,<sup>24 37</sup> energy intake,<sup>24 27</sup>  
39 hypertension,<sup>36</sup> vitamin D binding protein,<sup>36</sup> blood collection,<sup>24 27</sup> and adjusted for vitamin  
40 supplement use.<sup>24</sup> Therefore, for these studies, the observed reduced risk of colorectal cancer  
41 associated with a Vitamin D level is less likely to be confounded by these known  
42 confounders.  
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51 Our analysis had the following limitations. First, the number of included studies is not  
52 sufficient to provide a robust estimate of association of blood circulating Vitamin D level and  
53 colorectal cancer risk, so the analysis and results should be interpreted in the context of the  
54 limitations of the available data. Second, heterogeneous definitions of blood circulating  
55 Vitamin D categories were used across studies. The variability in definitions could limit  
56 comparability between studies. Third, our study included seven case-control studies; the  
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3 study design implies that the measurement of blood circulating Vitamin D is measured in  
4 individuals already diagnosed with colorectal cancer. Therefore, results from this study  
5 design need to be interpreted cautiously because of the potential for reverse causation. Time  
6 of blood sampling in relation to outcome ascertainment also varied among studies. Such  
7 cross-sectional measurements may not accurately reflect an individual's Vitamin D status  
8 across time. Forth, some studies included in our meta-analysis did not adjust for some  
9 potentially relevant confounders which may have led to residual confounding and may  
10 explain some of the observed heterogeneity. Fifth, difference in the method used for  
11 measuring blood circulating Vitamin D levels may also be a source of heterogeneity between  
12 the included studies. Sixth, in the dose-response analysis, the literature selected listed the  
13 median Vitamin D value, instead of the original Vitamin D value, which could also lead to  
14 inaccurate results. Seventh, Although we conducted the investigation of quality levels of the  
15 observational studies in conducting this meta-analysis by the NOS quality assessment tool,  
16 Bae<sup>67</sup> suggested that it is more reasonable to control for quality level by performing  
17 subgroup analysis according to study design rather than by using high-quality based on the  
18 NOS tool. In this context, however, we did not perform a subgroup analysis according to  
19 study design since the included 8 studies were case-control and nested case-cohort studies.  
20 Finally, a recent meta-analysis investigated the association between blood circulating  
21 Vitamin D levels and survival of colorectal cancer and found that the pooled hazard ratios  
22 (95% confidence intervals) were 0.68 [0.55–0.85] and 0.67 [0.57–0.78] for overall and  
23 colorectal cancer-specific survival comparing highest versus lowest categories of blood  
24 Vitamin D, respectively.<sup>15</sup> However, our study did not explore the association of the blood  
25 circulating Vitamin D levels and colorectal cancer mortality in Asian countries. In future  
26 studies, we would like to explore this association in Asian countries.

## 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 **CONCLUSION**

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48 The blood circulating Vitamin D is inversely associated with colorectal cancer prevalence in  
49 the Asian population. Our findings on the inverse association between blood circulating  
50 Vitamin D and the risk of colorectal cancer in Asian population suggest that Asian should  
51 improve their nutritional status and maintain a higher blood circulating Vitamin D level. This  
52 meta-analysis also provides valuable information for future research of association between  
53 blood circulating Vitamin D and colorectal cancer risk in Asian populations. Further studies  
54 may focus on evaluating more detailed Vitamin D levels with colorectal cancer. A  
55 multinational, population-based study in Asian countries may resolve the issue of  
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3 heterogeneity to generate detailed information on blood circulating Vitamin D level and risk  
4 of colorectal cancer.  
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### 9 **Authors Contributions**

10  
11 LZ: Study concept and design; acquisition of data; statistical analysis; interpretation of data;  
12 drafting and critical review of the manuscript for important intellectual content; approval of  
13 the final version of the manuscript.  
14

15  
16 HCZ: Interpretation of data; drafting and critical review of the manuscript for important  
17 intellectual content; approval of the final version of the manuscript.  
18

19  
20 YZ: Interpretation of data; drafting and critical review of the manuscript for important  
21 intellectual content; approval of the final version of the manuscript.  
22

23  
24 CH: Drafting and critical review of the manuscript for important intellectual content;  
25 approval of the final version of the manuscript.  
26

27  
28 AA: Drafting and critical review of the manuscript for important intellectual content;  
29 approval of the final version of the manuscript.  
30

31  
32 XQ: Interpretation of data; drafting and critical review of the manuscript for important  
33 intellectual content; approval of the final version of the manuscript.  
34

35  
36 PJ: Interpretation of data; drafting and critical review of the manuscript for important  
37 intellectual content; approval of the final version of the manuscript.  
38

39  
40 JYJ: Study concept and design; acquisition of data interpretation of data; drafting and critical  
41 review of the manuscript for important intellectual content; approval of the final version of  
42 the manuscript.  
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44  
45 ZHQ: Study concept and design; acquisition of data; interpretation of data; drafting and  
46 critical review of the manuscript for important intellectual content; approval of the final  
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## Figure legends/captions

**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

**Figure 2.** Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D. F, female; M, male;

**Figure 3.** Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modeled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line). Lines with short dashes represent the linear trend.

**Table 1.** Summary of characteristic for 8 studies included in the meta-analysis

Author(year)	Study design	Country	Study period	Outcome	Outcome ascertainment	Sex	Sample size (n); characteristics				Blood sample type	Vitamin D testing method
							Case control	or	No.	Male (%)		
Budhathoki et al. (2018) <sup>30</sup>	Nested case-cohort	Japan	1990-2009	colorectal cancer	NA	All	case control	637	52	56.2(7.5)	plasma	chemiluminescent enzyme immunoassay
Choi et al. (2015) <sup>37</sup>	case-control	Korea	2011-2012	colorectal adenoma	colonoscopy	All	case control	112	51	60.3 (5.3)	serum	chemiluminescent enzyme immunoassay
						Men	case control	112	51	59.5 (5.4)	serum	
						Women	case control	57	100	NA	serum	
							case control	57	100	NA	serum	
Hong et al. (2012) <sup>26</sup>	case-control	Korea	2009-2010	colorectal adenoma	colonoscopy	All	case control	143	68	58.7 (6.0)	serum	direct competitive electrochemiluminescence immunoassay
						control	143	68	58.7 (6.0)			
Otani et al. (2007) <sup>24</sup>	case-control	Japan	1990-2003	colorectal cancer	pathologically confirmed	All	case control	323	52	NA	plasma	competitive protein-binding assay
						Men	case control	621	52	NA		
						Women	case control	163	100	56.9		
							case control	324	100	56.9		
Takahashi et al. (2010) <sup>25</sup>	case-control	Japan	1997-2004	colorectal adenoma	colonoscopy	Men	case control	656	100	52.0 (1.4)	plasma or serum	radioimmunoassay method
						control	648	100	51.8 (1.5)			
Yamaji et al. (2012) <sup>27</sup>	case-control	Japan	2004-2005	colorectal adenoma	colonoscopy	All	case control	737	67	NA	plasma	radioimmunoassay method
Ying et al. (2015) <sup>36</sup>	case-control	China	2010-2014	colorectal cancer	colonoscopy	All	case control	703	65	NA	plasma	direct competitive enzyme-linked immunosorbent assay
						control	212	NA	63(56-73)			
Yurekli et al. (2015) <sup>29</sup>	case-control	Turkey	2012	colorectal cancer	colonoscopy	All	cases control	96	67	57.8(10.0)	serum	NA
						control	195	54	51.2(12.9)			

**Abbreviations:** SD, Standard deviation; NA, Not available;

All\*: all participants, including men and women

**Table 2.** Summary of risk estimate for 8 studies included in the meta-analysis

Author(year)	sex	Vitamin D level cut points (ng/ml)	Vitamin D medium level (ng/ml)	Number of cases	Number of controls	N	OR	LCI	UCI	Adjusted variables
Budhathoki et al. (2018) <sup>30</sup>	All	1st Q, 12.5-16.5 ng/ml	14.7	134	1004	1138	1			age, sex, BMI, smoking, alcohol use, physical activity, family history of cancer, and history of diabetes
		2nd Q, 17.6-21.6 ng/ml	19.9	165	1000	1165	1.08	0.84	1.39	
		3rd Q, 21.2-25.6 ng/ml	21.3	160	1016	1176	0.96	0.74	1.26	
		4th Q, 25.8-33.0 ng/ml	26.5	178	1024	1202	0.95	0.73	1.23	
Choi et al. (2015) <sup>37</sup>	All	1st Q, 10.3 ng/ml	10.3	37	28	65	1			age, sex, BMI, alcohol drinking, smoking status, folate intake; women: additional menopausal status and hormone replacement use
		2nd Q, 14.3 ng/ml	14.3	26	28	54	0.72	0.31	1.66	
		3rd Q, 17.9 ng/ml	17.9	28	28	56	0.73	0.29	1.82	
		4th Q, 24.2 ng/ml	24.2	21	28	49	0.49	0.19	1.27	
	Men	1st Q, 11.1 ng/ml	11.1	15	14	29	1			
		2nd Q, 14.6 ng/ml	14.6	13	14	27	1.31	0.36	4.82	
		3rd Q, 17.7 ng/ml	17.7	17	15	32	1.26	0.4	8.12	
		4th Q, 22.7 ng/ml	22.7	12	14	26	1.8	0.19	8.12	
	Women	1st Q, 9.5 ng/ml	9.5	16	14	30	1			
		2nd Q, 13.5 ng/ml	13.5	22	14	36	0.89	0.26	3.1	
		3rd Q, 18.4 ng/ml	18.4	8	14	22	0.54	0.13	2.16	
		4th Q, 25.4 ng/ml	25.4	9	13	22	0.22	0.04	1.15	
Hong et al. (2012) <sup>26</sup>	All	1st Q, <14.3 ng/ml	10.1	31	42	73	1			age, sex, BMI, smoking, alcohol drinking, physical activity, corrected calcium level
		2nd Q, 14.3-18.5 ng/ml	16.4	29	42	71	0.87	0.43	1.74	
		3rd Q, 18.6-23.8 ng/ml	21.2	41	31	72	0.4	0.2	0.82	
		4th Q, >=23.9 ng/ml	29.1	42	28	70	0.38	0.18	0.8	
Otani et al. (2007) <sup>24</sup>	Men	1st Q, <22.9 ng/ml	18.3	43	74	117	1			age, sex, BMI, smoking, alcohol consumption, physical exercise, Vitamin supplement use, family history of colorectal cancer, total energy intake, dietary fiber intake, folate intake, calcium intake, Vitamin D intake, n-3 fatty acid intake, red meat intake, fish intake
		2nd Q, 22.9-27.5 ng/ml	25.2	40	85	125	0.76	0.42	1.4	
		3rd Q, 27.6-32.0 ng/ml	29.8	36	85	121	0.76	0.39	1.5	
		4th Q, >32.1 ng/ml	36.5	44	80	124	0.73	0.35	1.5	
	Women	1st Q, <18.7 ng/ml	15.2	41	77	118	1			
		2nd Q, 18.7-22.2 ng/ml	20.5	34	73	107	1	0.55	1.9	
		3rd Q, 22.3-26.9 ng/ml	24.6	44	71	115	1.2	0.65	2.3	
		4th Q, >27.0 ng/ml	31.6	41	76	117	1.1	0.5	2.3	
Takahashi et al. (2010) <sup>25</sup>	Men	1st Q, <22 ng/ml	20	128	142	270	1			BMI, hospital, rank in the Self Defense Forces, smoking, alcohol drinking, parental history of colorectal cancer, physical activity, type of blood sample, the month of blood drawing
		2nd Q, 23-25 ng/ml	24	162	156	318	1.21	0.86	1.7	
		3rd Q, 26-29 ng/ml	27.5	223	208	431	1.21	0.87	1.69	
		4th Q, >30 ng/ml	33	143	142	285	1.25	0.85	1.84	
Yamaji et al. (2012) <sup>27</sup>	All	1st Q, 14-19 ng/ml	16.5	145	129	274	1			age, sex, BMI, screening periods, the season of blood collection, smoking, alcohol drinking, family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, height, daily energy intake
		2nd Q, 20-23 ng/ml	21.5	132	128	260	0.86	0.6	1.24	
		3rd Q, 24-26 ng/ml	25	157	145	302	0.91	0.64	1.29	
		4th Q, 27-31 ng/ml	29	175	144	319	1.03	0.73	1.46	
		5th Q, 31-34 ng/ml	32.5	128	157	285	0.64	0.45	0.92	
Ying et al. (2015) <sup>36</sup>	All	1st Q, <7.29 ng/ml	3.65	80	53	133	1			age, sex, BMI, smoking, drinking, history of diabetes, hypertension, vitamin D binding protein
		2nd Q, 7.29-14.61 ng/ml	10.95	49	53	102	0.62	0.35	1.12	
		3rd Q, 14.61-28.84 ng/ml	21.73	46	53	99	0.67	0.38	1.20	
		4th Q, >=28.84 ng/ml	35.96	37	53	90	0.53	0.29	0.98	
Yurekli et al.(2015) <sup>29</sup>	All	1st Q, <8.6 ng/ml	7.1	24	48	72	1			age, sex, BMI, smoking, alcohol intake
		2nd Q, 8.6-10.1 ng/ml	9.35	20	53	73	0.48	0.24	0.98	
		3rd Q, 10.1-14.5 ng/ml	12.3	25	48	73	0.51	0.25	1.03	
		4th Q, >=14.5 ng/ml	18.9	27	46	73	0.69	0.36	1.36	

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3 **Abbreviations:** NA, Not available; OR: odds ratio; LCI: 95% CI's lower confidence interval; UCI: 95% CI's upper confidence interval; BMI: body mass  
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5 All\*: all participants, including men and women  
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**Table 3.** Summary of subgroup analysis for the associations of blood circulating Vitamin D and the risk colorectal cancer and adenoma by year of publication, outcome, participants sex, the subregion of Asia, and blood sample type.

Subgroup analysis	Number of estimates	Random effect The summary OR (95% CI)	Ratio of ORs (95% CI)	$I^2$ (%)	$P$ value
<u>Year of publication</u>					
Before 2014	4	0.76 (0.49-1.19)			
2014 onwards	4	0.74 (0.53-1.03)	0.9 (0.44-1.87)	60.47	0.76
<u>Outcome</u>					
Colorectal adenoma	4	0.67 (0.40-1.14)			
Colorectal cancer	4	0.83 (0.66-1.06)	1.11(0.54-2.28)	59.48	0.73
<u>Sex</u>					
Women	2	0.59 (0.13-2.74)			
Men	3	1.13(0.81-1.58)	1.46 (0.03-63.5)	45.89	0.70
<u>Subregion</u>					
Eastern Asia	7	0.75 (0.57-1.00)			
Western Asia	1	0.69 (0.36-1.34)	0.92 (0.28-2.99)	59.79	0.87
<u>Blood sample type</u>					
Serum	3	0.52 (0.34-0.80)			
Plasma	4	0.77 (0.59-1.00)	1.49 (0.73-3.03)	22.67	0.21
<u>Range</u>					
≤15 ng/mL	4	0.93 (0.70-1.25)			
>15 ng/mL	4	0.62 (0.47-0.83)	0.65(0.39-1.07)	28.48	0.08

Abbreviations: OR, Odds ratio;  $I^2$ , Percent residual variation due to heterogeneity; CI, Confidence interval.

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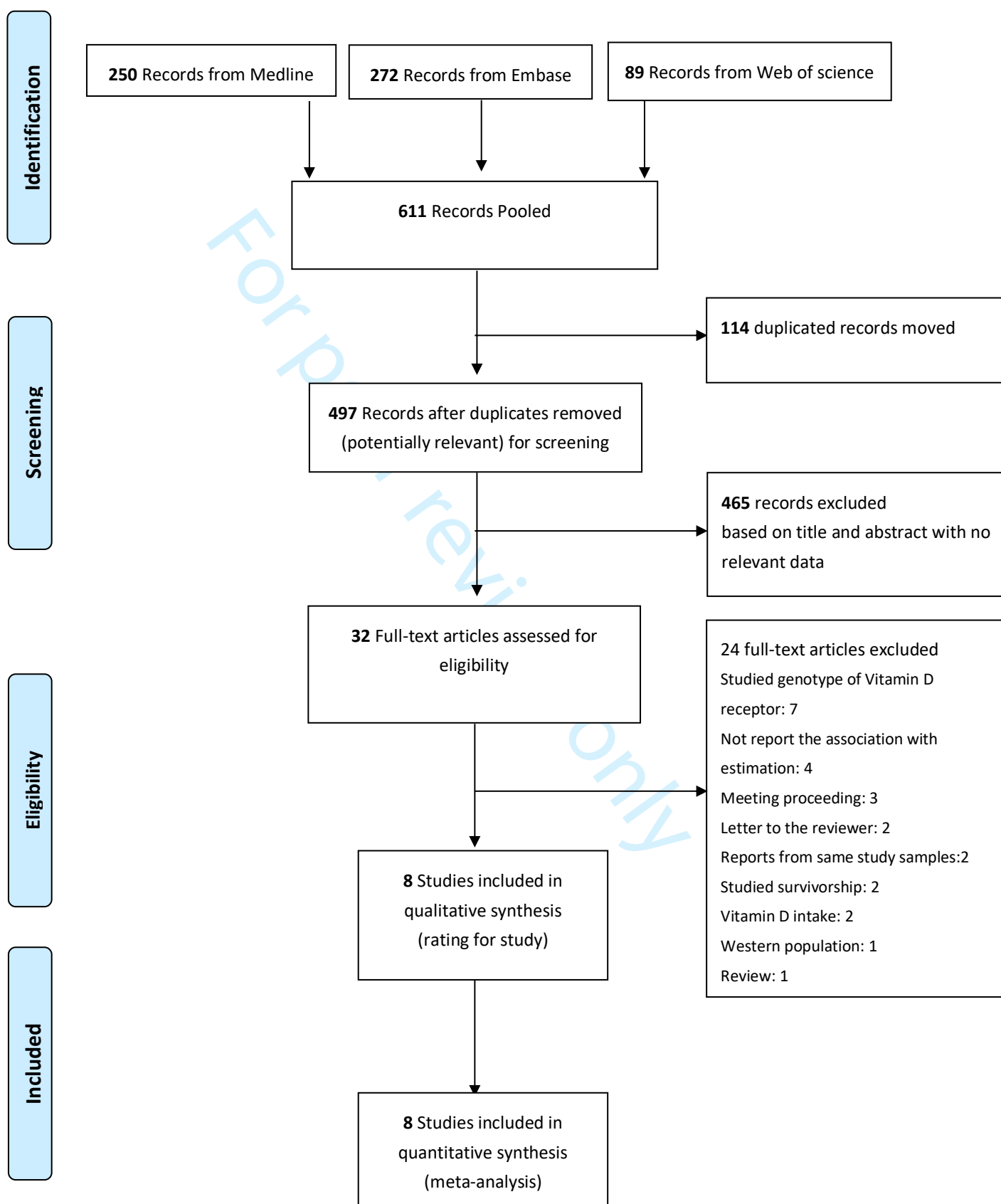
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**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

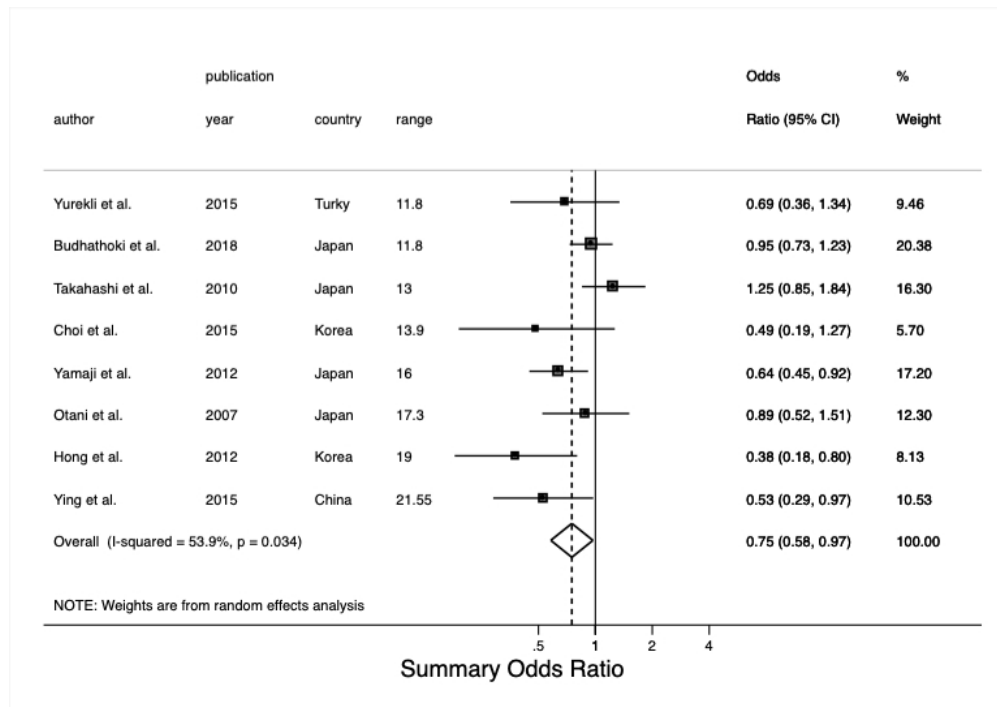


Figure 2. Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D. F, female; M, male;



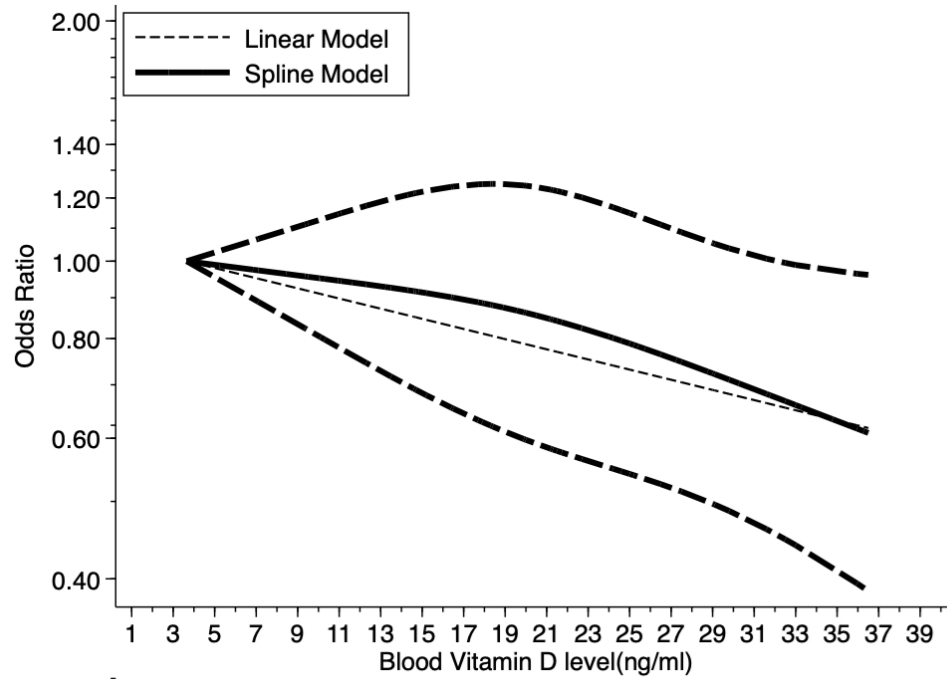


Figure 3. Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modeled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line,  $P_{\text{non-linearity}}=0.11$ ). Lines with short dashes represent the linear trend.

## Supplementary Material

**Table S1.** Literature search results from Medline, Embase, and Web of Science.

	Medline	Results
1	exp colorectal neoplasms/	187315
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	2688871
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	80293
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1074938
5	1 or 2	2690742
6	3 and 4 and 5	258
7	limit 6 to English language	250

	Embase	Results
1	exp colorectal neoplasms/	26250
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1075151
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	137427
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or	1562807

	Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	
5	1 or 2	1075151
6	3 and 4 and 5	279
7	limit 6 to English language	272

	Web of science	Results
1	TS= (colorectal neoplasm or colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*)	460,541
2	TS= (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D)	99,758
3	TS=(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen)	2,184,572
4	#1 AND #2 AND #3	106
5	(#4) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)	89

**Table S2.** List and details of the excluded studies from the meta-analysis

Reason for exclusion	Author	Year	Study title	Journal
Studied genotype of Vitamin D receptor(n=7)	Wong HL, et al.	2003	Vitamin D receptor start codon polymorphism and colorectal cancer risk: Effect modification by dietary calcium and fat in Singapore Chinese	Carcinogenesis
	Li C, et al.	2009	Vitamin D receptor gene polymorphisms and the risk of colorectal cancer in a Chinese population	Digestive Diseases and Sciences
	Kang JW et al.	2015	Role of vitamin D and local vitamin D receptor expression in colon carcinogenesis	Journal of Gastroenterology and Hepatology
	Takehige N, et al.	2015	Associations between vitamin D receptor (VDR) gene polymorphisms and colorectal cancer risk and effect modifications of dietary calcium and vitamin D in a Japanese population	Asian Pac J Cancer Prev
	Aumpansub P, et al.	2016	Strong association of single nucleotide polymorphisms of vitamin D receptor gene, BSML, and colorectal cancer in Asian population	Gastroenterology
	Budhathoki S, et al.	2016	Vitamin D receptor gene polymorphism and the risk of colorectal cancer: A nested case-control study	PLoS ONE
	Gong C, et al.	2017	Dietary factors and polymorphisms in vitamin D metabolism genes: the risk and prognosis of colorectal cancer in northeast China	Scientific Reports
Not report the association with estimation(n=4)	Mizoue T, et al.	2005	Dietary patterns and colorectal adenomas in Japanese men - The self-defense forces health study.	American Journal of Epidemiology
	Atoum MF, et al.	2014	Association between circulating vitamin D, the TaqI vitamin D receptor gene polymorphism and colorectal cancer risk among Jordanians. Asian Pacific journal of cancer prevention	Asian Pacific Journal of Cancer Prevention
	Kumagai Y, et al.	2014	Dietary patterns and colorectal cancer risk in Japan: the Ohsaki Cohort Study.	Cancer Causes & Control
	Hessami Arani S, et al.	2017	Rising rates of colorectal cancer among younger iranians: Is diet to blame?	Current Oncology
Meeting proceeding (n=3)	Grant WB, et al.	2011	Ecological study findings regarding vitamin D and cancer.	Anticancer Research
	Li K, et al.	2014	The association between serum vitamin D concentrations and colorectal cancer.	Clinical Chemistry and Laboratory Medicine
	Ozer C, et al.	2015	The relationship between serum 25-hydroxy vitamin D levels and insulin resistance in breast and colon cancer.	Clinical Chemistry and Laboratory Medicine
Letter to the reviewer (n=2)	Dunnigan MG, et al.	1990	Serum 25-hydroxyvitamin D and colon cancer.	Lancet
	Qu B, et al.	2017	Role of Circulating and Supplemental Calcium and Vitamin D in the Occurrence and Development of Colorectal Adenoma or Colorectal Cancer.	J Clin Gastroenterol
Reports from same study sample (n=2)	Byeon JS, et al.	2007	Colorectal neoplasm in asymptomatic Asians: a prospective multinational multicenter colonoscopy survey.	Gastrointestinal Endoscopy
	Shin A, et al.	2011	Site-specific risk factors for colorectal cancer in a korean population.	PLoS ONE
Studied survivorship (n=2)	Wesa KM, et al.	2015	Serum 25-hydroxy vitamin D and survival in advanced colorectal cancer: a retrospective analysis.	Nutr Cancer
	Woo KW, et al.	2015	Vitamin D deficiency in Hong Kong advanced cancer patients: Result of first 50 patients.	Annals of Oncology
Vitamin D intake (n=2)	Mizoue T, et al.	2008	Calcium, dairy foods, vitamin D, and colorectal cancer risk: The Fukuoka colorectal cancer study.	Cancer Epidemiology Biomarkers and Prevention
	Ishihara J, et al.	2008	Dietary calcium, vitamin D, and the risk of colorectal cancer.	American Journal of Clinical Nutrition
Western population (n=1)	Sy AM, et al.	2013	Association between serum vitamin D levels and colonic carcinomatous polyps.	Journal of Gastrointestinal Cancer
Review (n=1)	Grant WB, et al.	2009	Ecological Studies Of Ultraviolet B, Vitamin D And Cancer Since 2000.	Annals of Epidemiology

**Table S3.** Newcastle-Ottawa Scale for assessing the quality of studies in the systematic review

Author (Year)	Country	Region	Selection				Comparability		Exposure			Total Score
			S1	S2	S3	S4	C1	C2	E1	E2	E3	
Budhathoki et al. (2018)[1]	Japan	Eastern Asian	★	★	★	★	★	★	★	★		8
Choi et al. (2015)[2]	Korea	Eastern Asian	★				★	★	★	★		5
Hong et al. (2012)[3]	Korea	Eastern Asian	★	★	★	★	★	★	★	★		8
Otani et al. (2007)[4]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Takahashi et al. (2010)[5]	Japan	Eastern Asian	★	★	★	★		★	★	★	★	8
Yamaji et al. (2012)[6]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Ying et al. (2015)[7]	China	Eastern Asian	★			★	★	★	★	★	★	7
Yurekli et al.(2015)[8]	Turkey	Western Asian	★				★	★	★	★		5

**Guidelines for review***Selection:*

S1, Case definition adequacy: ★a) requires independent validation (>1 person/record/time/process to extract information or reference to primary record sources such as colonoscopy or medical/hospital records); b) record linkage or self-report with no reference to primary record; c) no description

S2, Representativeness of the cases: ★a) consecutive or representative series of cases; b) potential for selection biases or not stated

S3, Selection of controls: ★a) community controls; b) hospital controls, within same community as cases; c) no description

S4, Definition of controls: ★a) no history of colorectal cancer or adenoma; b) no description of the source

*Comparability:*

C1, ★ Study adjusted for confounder, such as age and sex (the most important factors);

C2, ★ Study adjusted for any additional confounders (1> additional factors, e.g., BMI, drinking or smoking)

*Exposure:*

E1, Ascertainment of exposure: ★a) secure record (e.g., medical records); ★b) structured interview were blind to case/control status; c) interview not blinded to case/control status; d) written self-report or medical record only; e) no description

E2, Same method of ascertainment for cases and controls: ★a) yes; b) no

E3, Non-response rate: ★a) the same rate for both groups; b) non-respondents described; c) rate different and no designation

**Figure S1.** Subgroup meta-analysis, including stratified by publication year (before 2014 or 2014 onwards, **S1A**), outcome (colorectal cancer or colorectal adenoma, **S1B**), sex (women, men or both, **S1C**), subregion (Eastern Asia and Western Asia, **S1D**), sample type (serum or plasma, **S1E**), range ( $\leq 15$  or  $>15$  ng/mL **S1F**).

**Figure S1A**

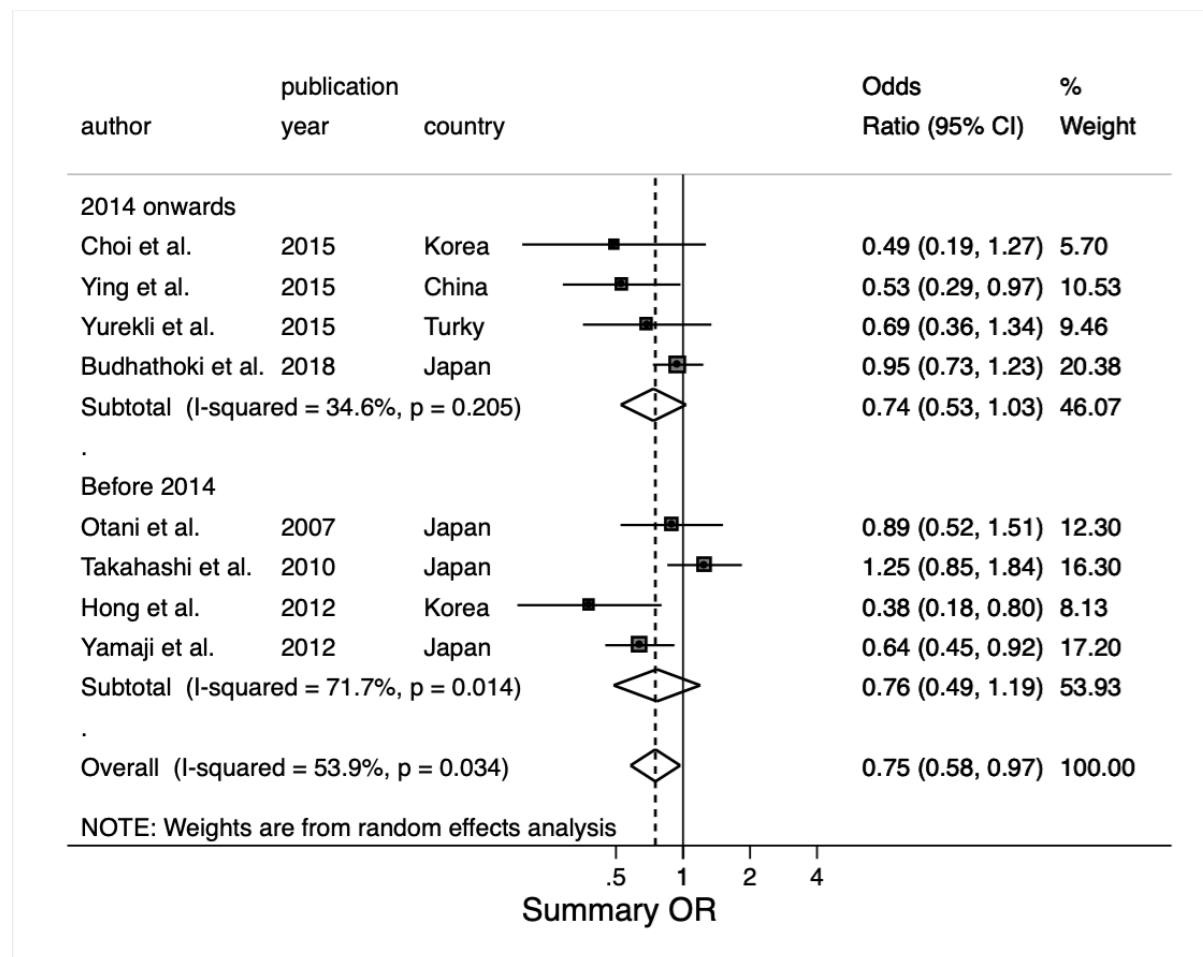


Figure S1B

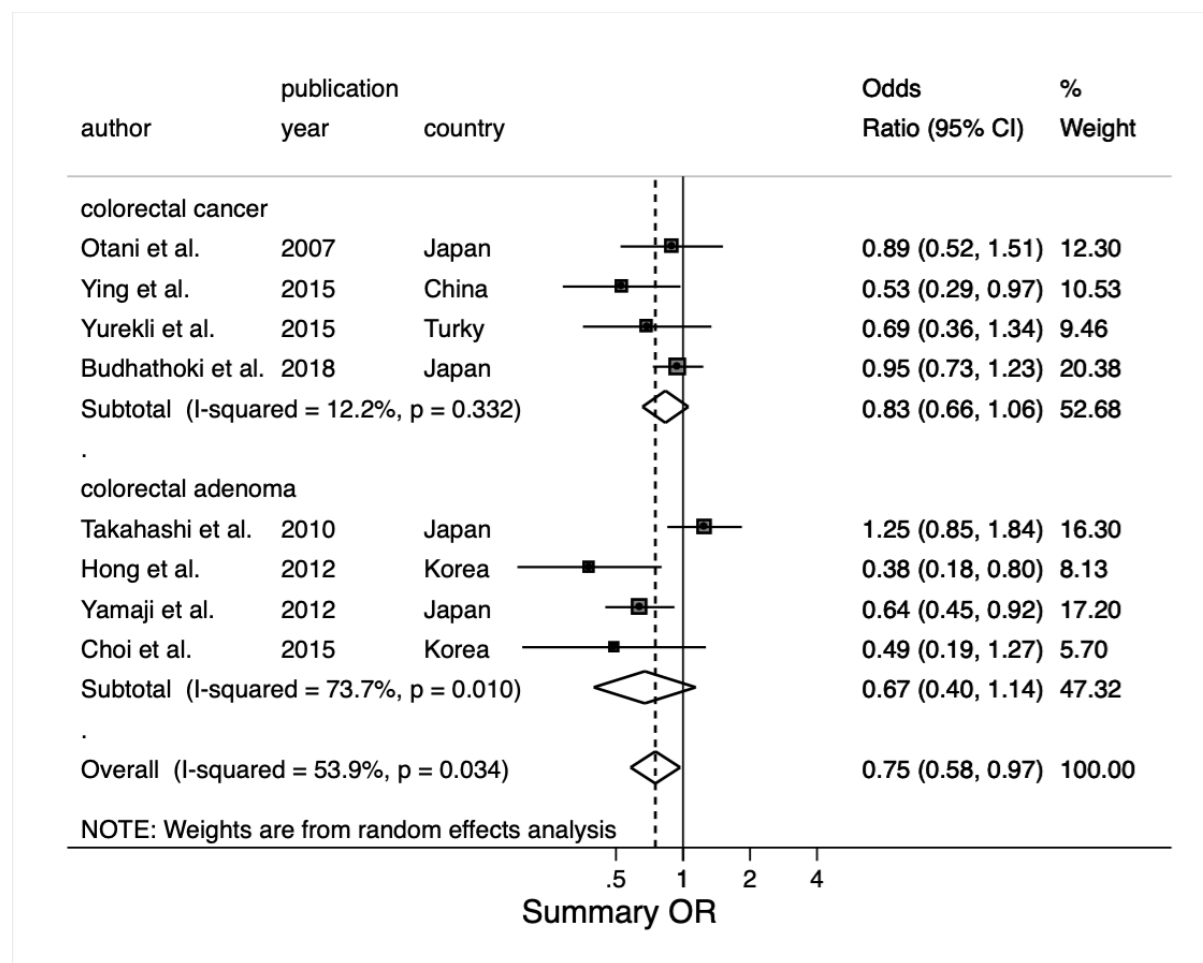


Figure S1C

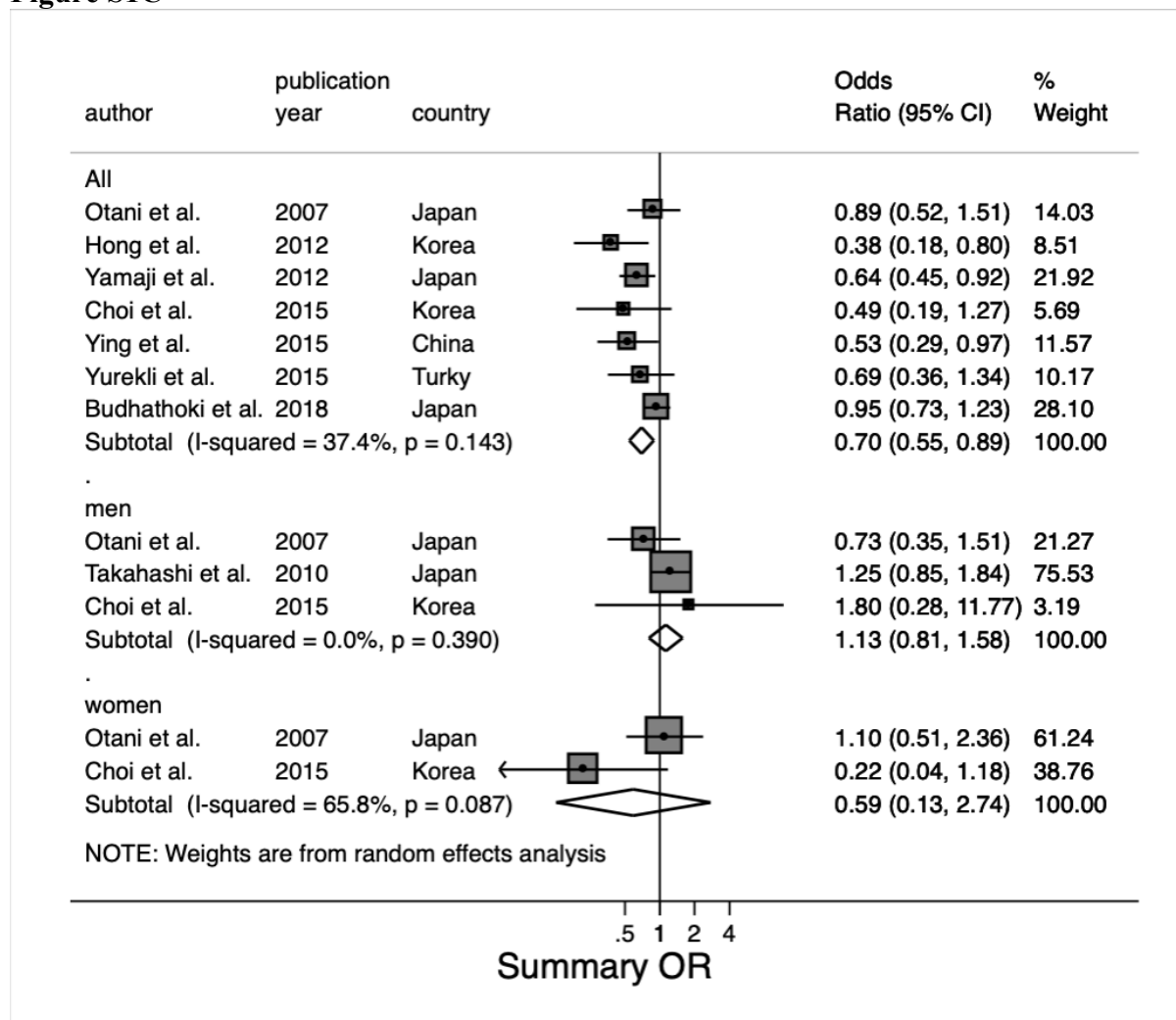




Figure S1D

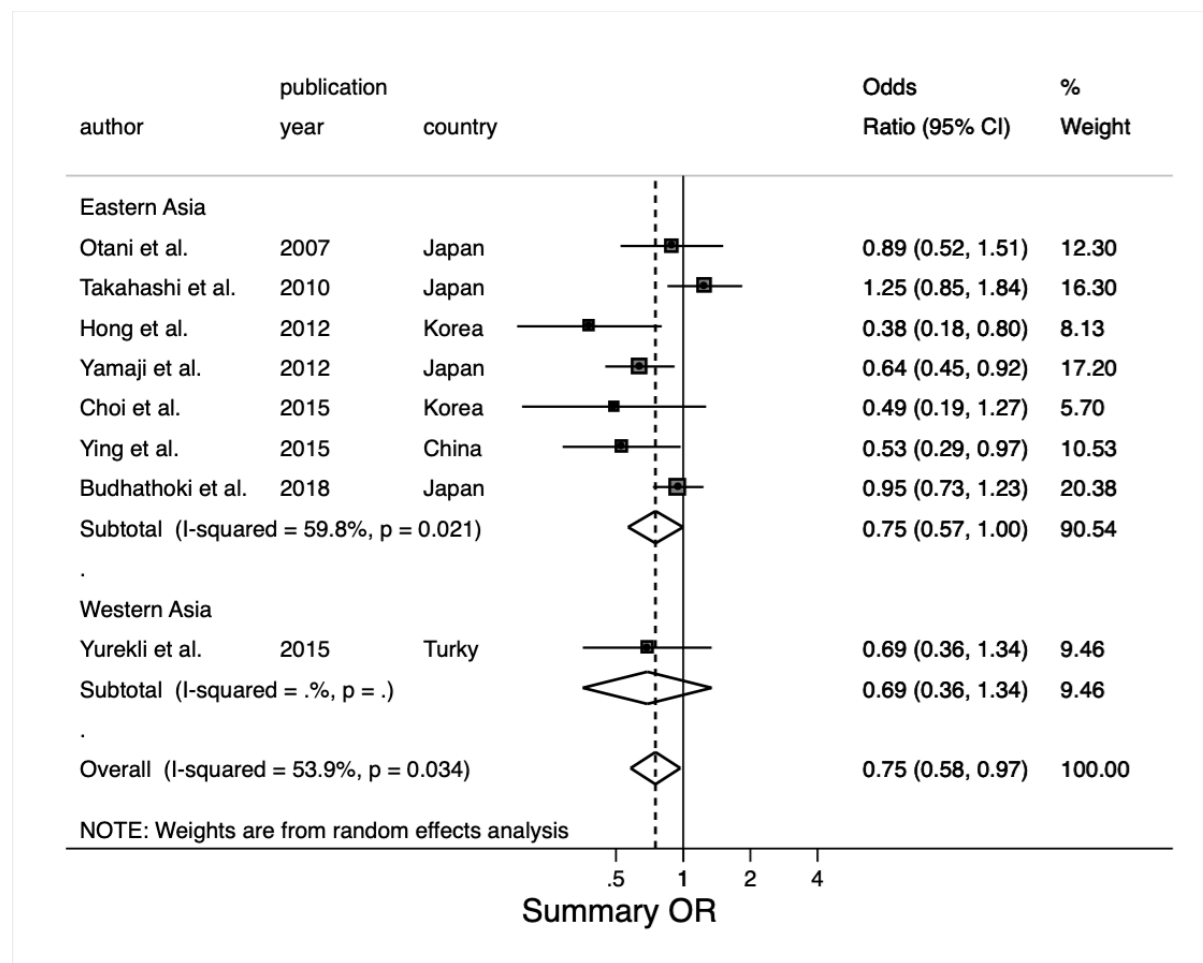


Figure S1E

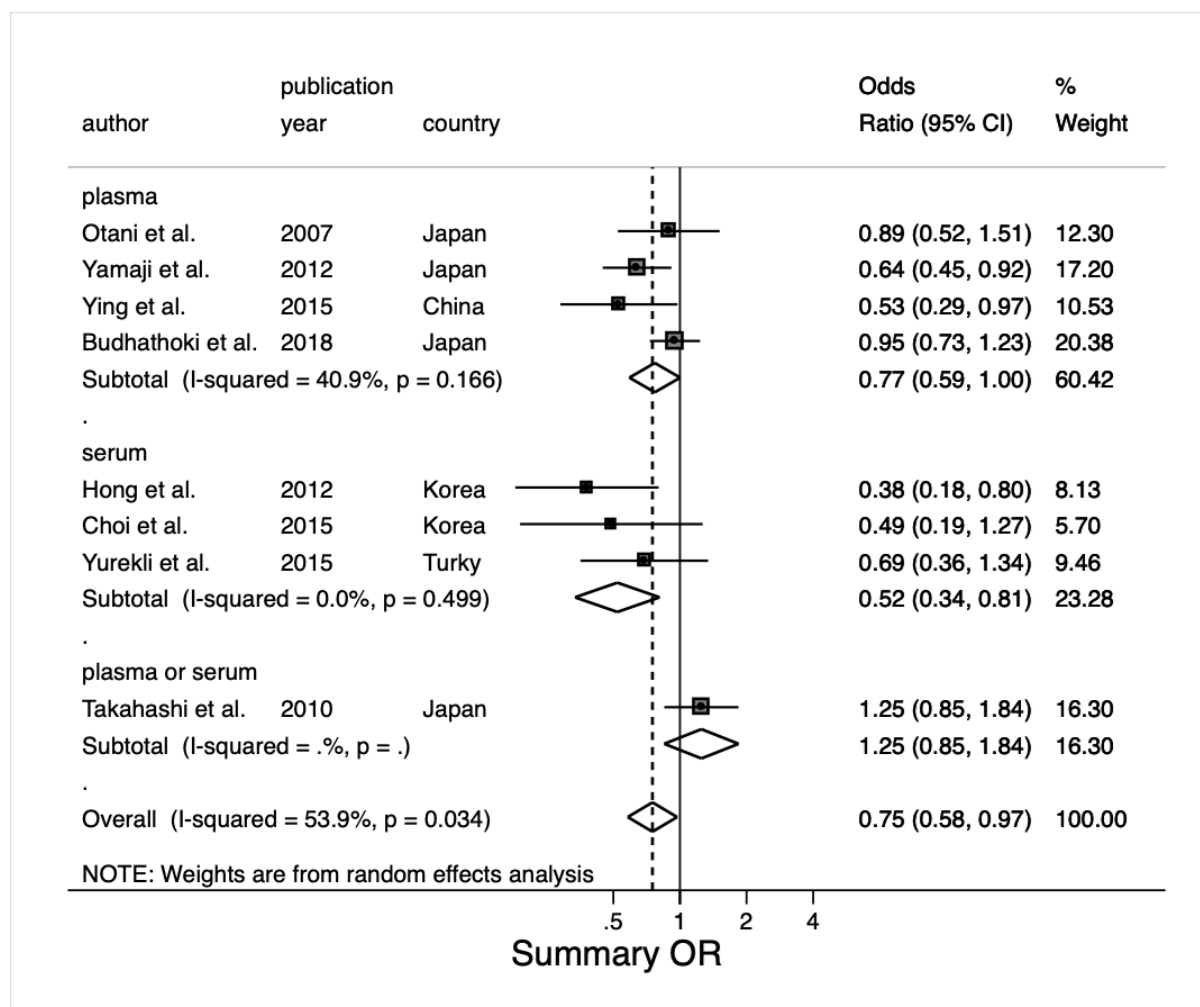
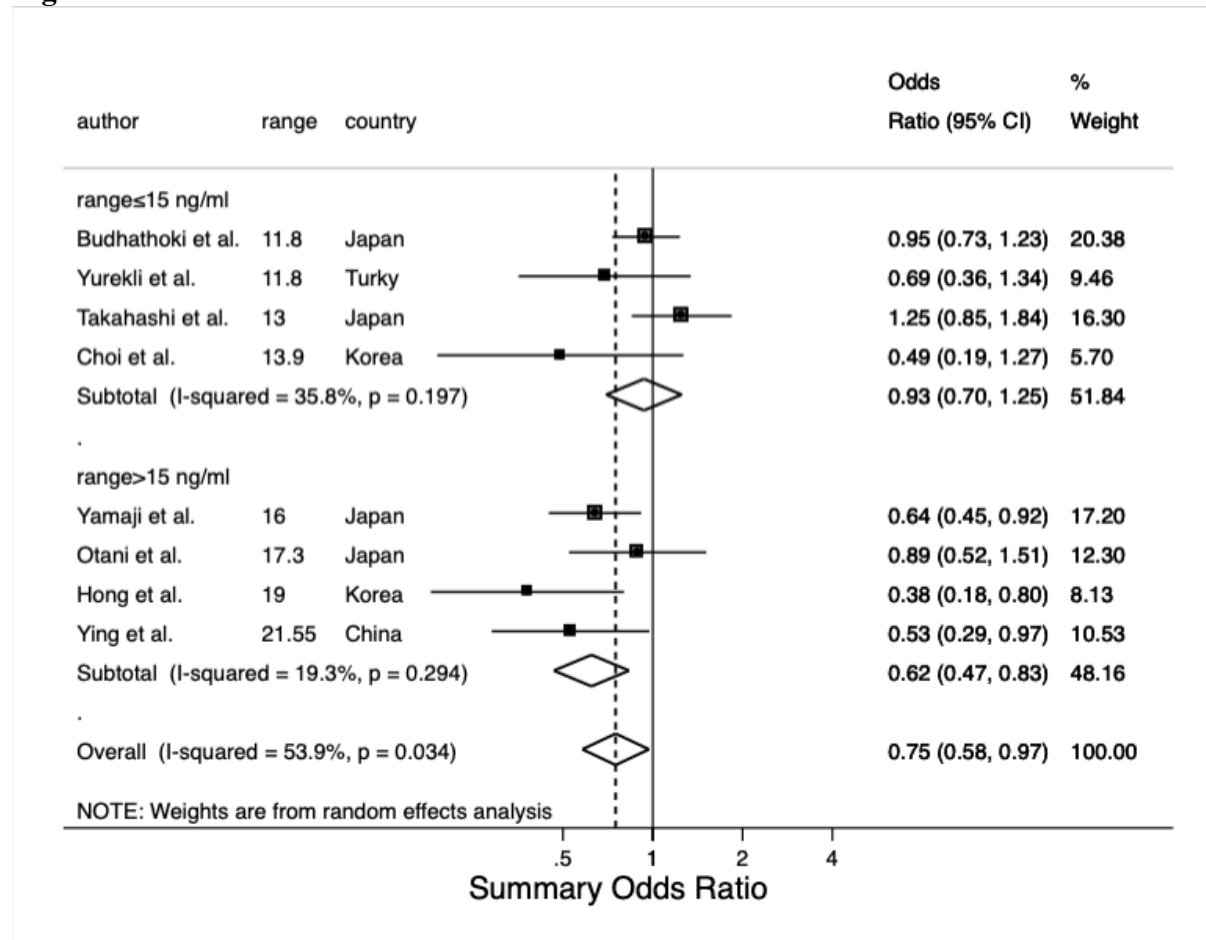
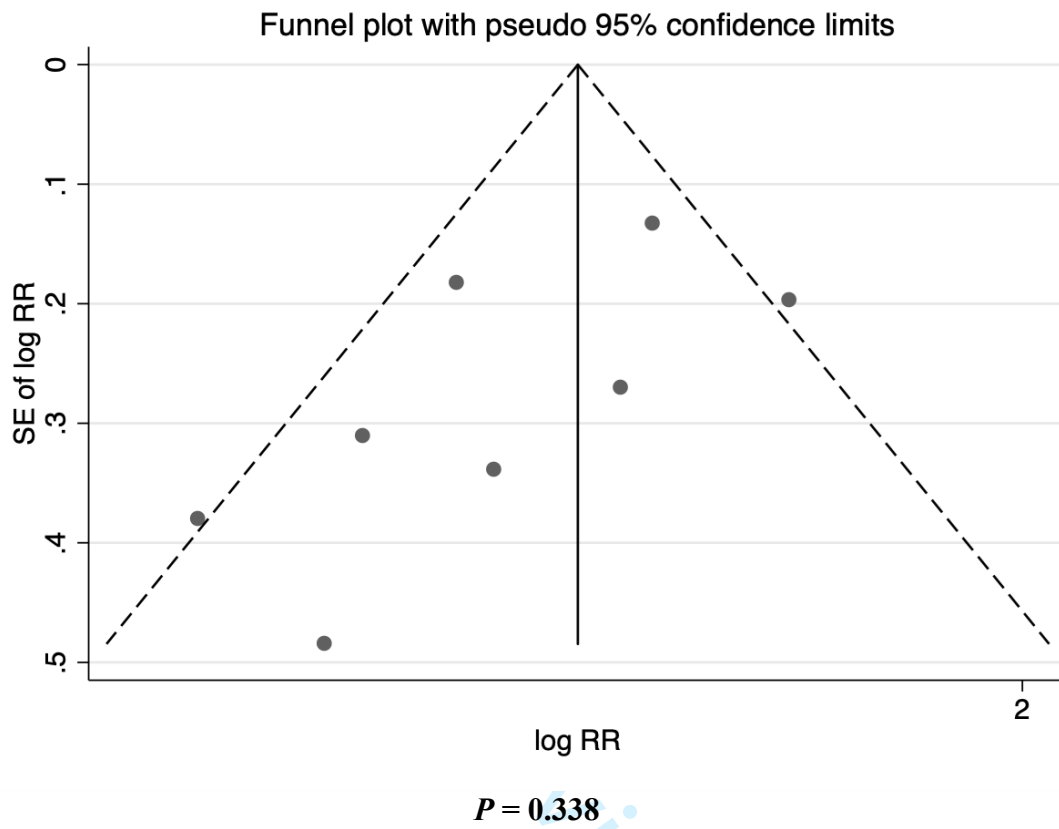


Figure S1F



**Figure S2.** Funnel plot for all studies included in the meta-analysis and between blood circulating Vitamin D and the colorectal cancer risk in Asian countries





# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 6
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Page 5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Page 7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I <sup>2</sup> ) for each meta-analysis.	Page 9



# PRISMA 2009 Checklist

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 10
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 11 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 11, Table 1 and Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 11 and Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 11 and 12
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 12 and 13
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 14 to Page 16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 18
<b>FUNDING</b>			
For peer review only - <a href="http://bmjopen.bmj.com/site/about/guidelines.xhtml">http://bmjopen.bmj.com/site/about/guidelines.xhtml</a>			



# PRISMA 2009 Checklist

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Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Page 2
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From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).

Page 2 of 2

For peer review only

# BMJ Open

## Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030513.R1
Article Type:	Original research
Date Submitted by the Author:	06-Sep-2019
Complete List of Authors:	Zhang, Lin; Peking Union Medical College Hospital, Clinical Laboratory; University of Melbourne School of Population and Global Health, Centre of Epidemiology and Biostatistics Zou, Huachun; Sun Yat-sen University, School of Public Health Zhao, Yang; The University of Melbourne, Melbourne School of Population and Global Health; WHO Collaborating Centre on Implementation Research for Prevention & Control of NCDs, Hu, Chunlei; Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Department of Gastrointestinal Surgery, Peking University Shenzhen Hospital Atanda, Adejare; Morgan State University, School of Community Health and Policy Qin, Xuzhen; Peking Union Medical College Hospital, Clinical Laboratory Jia, Peng; University of Twente, Faculty of Geo-Information Science and Earth Observation (ITC); International Initiative on Spatial Lifecourse Epidemiology (ISLE) Jiang, Yu; Chinese Academy of Medical Sciences & Peking Union Medical College, School of public health Qi, Zhihong; Peking Union Medical College Hospital, Clinical Laboratory
<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Oncology, Public health
Keywords:	Colorectal cancer, Colorectal adenoma, Vitamin D, 25-hydroxyvitamin D, Asia, Meta-analysis

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# Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

Lin Zhang,<sup>1,2,3,4,12</sup> Huachun Zou,<sup>5,6,7</sup> Yang Zhao,<sup>8</sup> Chunlei Hu,<sup>9</sup> Adejare Atanda,<sup>10</sup> Xuzhen Qin,<sup>1</sup> Peng Jia,<sup>11,12</sup> Yu Jiang,<sup>2</sup> Zhihong Qi<sup>1</sup>

<sup>1</sup> Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>2</sup> Peking Union Medical College School of Public Health, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>3</sup> Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, Victoria 3100, Australia

<sup>4</sup> The University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Parkville, Victoria 3100, Australia

<sup>5</sup> School of Public Health (Shenzhen), Sun Yat-sen University, Shenzhen 518107, Guangdong, China

<sup>6</sup> School of Public Health, Sun Yat-sen University, Guangzhou 510085, Guangdong, China

<sup>7</sup> Kirby Institute, University of New South Wales, Sydney 2052, NSW, Australia

<sup>8</sup> School of Population and Global Health, The University of Melbourne & WHO Collaborating Centre on Implementation Research for Prevention & Control of NCDs, Parkville, Victoria 3100, Australia

<sup>9</sup> Department of Gastrointestinal Surgery, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, China

<sup>10</sup> School of Community Health and Policy, Morgan State University, Baltimore, Maryland, USA

<sup>11</sup> Faculty of Geo-Information Science and Earth Observation (ITC), University of Twente, Enschede 7500, The Netherlands

<sup>12</sup> International Initiative on Spatial Lifecourse Epidemiology (ISLE)

## \*Correspondence to:

Prof. Zhihong Qi

Clinical Laboratory, Peking Union Medical College Hospital  
Chinese Academy of Medical Sciences & Peking Union Medical College  
Shuai Fu Yuan 1#, DongCheng District, Beijing 100730, China

Tel.: + 86 10 69159740

Email: cizeng1010@sina.com

Prof. Yu Jiang

Chinese Academy of Medical Sciences & Peking Union Medical College,  
DongDan SanTiao 9, DongCheng District, Beijing 100730, China

Tel.: + 86 10 65105916

Email: wingedsky@gmail.com

**Transparency declaration**

The Corresponding Authors affirm that this manuscript is an accurate account of the study proposed with no important aspects omitted, and that any discrepancies from the study as planned and registered will be explained upon the completion of the proposed study.

**Ethical approval**

Ethics approval is not required for this study because it is a systematic review.

**Details of funding**

This study was supported by National Key R&D Program of China (No. 2018YFC1315000/2018YFC1315003).

**Details of the role of the study sponsor**

Lin Zhang is supported by Peking Union Medical College Hospital 100 Elite Scholarship and Melbourne International Research Scholarship, The University of Melbourne. Peng Jia, Director of the International Initiative on Spatial Lifecourse Epidemiology (ISLE), thanks Lorentz Center, the Netherlands Organization for Scientific Research, the Royal Netherlands Academy of Arts and Sciences, the Chinese Center for Disease Control and Prevention, the West China School of Public Health in Sichuan University, for funding the ISLE and supporting ISLE's research activities.

**Conflict of interest**

The authors have declared that no competing interests exist.

**Statement of independence of researchers form funders**

Not applicable.

**Patient involvement**

No patients were involved in the design or analysis of this study.

**Data sharing statement**

Not applicable

## ABSTRACT

### Objectives

We conducted this dose-response meta-analysis to assess the association between blood circulating Vitamin D levels and colorectal cancer risk in Asian population.

### Design

This study was a systematic review and dose-response meta-analysis for observational studies which investigated the relationship between blood circulating Vitamin D levels and colorectal cancer risk in Asian population.

### Data Sources

Relevant studies were identified by searching literature in MEDLINE, EMBASE, and Web of Science until 31 January 2019. Eligibility criteria: original studies published in peer-reviewed journals investigating association between blood circulating Vitamin D levels and the risk of colorectal cancer and/or adenoma in Asian countries.

### Data extraction and synthesis:

Two authors independently extracted data and assessed the quality of included studies. Study-specific ORs were pooled using a random-effects model. A dose-response meta-analysis was performed with generalized least squares regression. We applied the Newcastle-Ottawa Scale (NOS) quality assessment tool to evaluate the quality of the selected observational studies.

### Results

The eight included studies encompassed a total of 2,916 cases and 6,678 controls. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D levels was 0.75 [95% CI, 0.58-0.97] up to 36.5 ng/mL in Asian population. There was heterogeneity among the studies ( $I^2=53.9%$ ,  $P_{\text{heterogeneity}}=0.034$ ). The dose-response meta-analysis indicated a significant linear relationship ( $P_{\text{non-linearity}}=0.11$ ). An increment of 16 ng/mL in blood circulating Vitamin D level corresponded to an OR of 0.79 [95% CI, 0.64-0.97].

### Conclusions

The results of this meta-analysis indicated that blood circulating Vitamin D level is associated with decreased risk of colorectal cancer in Asian countries. This dose-response meta-analysis further shows that the strength of this association among Asian population is similar to that among Western population. Our study suggested that Asian population should improve nutritional status and maintain a higher level of blood circulating Vitamin D.

### Strengths and limitations of this study

- Our study seeks to extend previous work by including a number of new studies and by distinguishing Asian population explicitly.
- The number of included studies is not sufficient to provide a robust estimate, so the results should be interpreted in the context of the limitations of the available data.
- Heterogeneous definitions of blood circulating Vitamin D categories were used across studies. The variability in definitions could limit comparability between studies.
- Our study included seven case-control studies; the study design implies that the measurement of blood circulating Vitamin D is measured in individuals already diagnosed with colorectal cancer. Therefore, results from case-control studies need to be interpreted cautiously because of the potential for reverse causation.
- Time of blood sampling in relation to outcome ascertainment also varied among studies. Such cross-sectional measurements may not accurately reflect an individual's Vitamin D status across time.

## INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer and second in terms of mortality, with over 1.8 million new cases and 881,000 deaths worldwide in 2018, accounting for about 1 in 10 cancer cases and deaths.<sup>1</sup> Some Asian countries where the incidence of colorectal cancer was historically low, such as Japan, Israel, Singapore, China, and the Philippines, are experiencing rising incidence rates over the past decades. In 2012, Japan (Miyagi Prefecture Cancer Registry) presented the highest colorectal cancer incidence in the world for men (62 per 100,000 persons) and women (37 per 100,000 persons).<sup>2</sup> Observational studies have identified several risk factors associated with an increased incidence of colorectal cancer including lifestyle factors (e.g., obesity, physical inactivity, smoking, and heavy alcohol use) and non-modifiable factors (e.g., aging, personal and family history of colorectal cancer or adenoma).<sup>3</sup> Other observational studies conducted in Western countries suggested blood circulating 25-hydroxyvitamin D (25(OH)D) (also named as Vitamin D) has a protective role in the development of colorectal cancer.<sup>4-8</sup> Some meta-analyses have consistently reported that there was an inverse association of plasma Vitamin D concentration in the blood with incidence and mortality of colorectal cancer.<sup>9-15</sup>

The prevalence of Vitamin D deficiency has increased in recent decades.<sup>16 17</sup> In a recent population-based study of Asian adults, approximately 75% had suboptimal Vitamin D concentrations.<sup>18</sup> The Endocrine Society Clinical Practice Guideline defines Vitamin D deficiency as 25(OH)D level <20 ng/mL and insufficiency as 21 to 29 ng/mL.<sup>19</sup> Feldman et al.<sup>20</sup> reported anti-neoplastic actions of Vitamin D, particularly in colorectal cancer.<sup>9</sup> Touvier et al.<sup>12</sup> reported that improving Vitamin D levels could be beneficial in reducing colorectal cancer incidence. Data from a cohort of healthy women shows that plasma Vitamin D levels were inversely related to the occurrence and death from colorectal cancer.<sup>21</sup> In the Nurses' Health Study, total circulating Vitamin D was associated with lower risk of colorectal cancer in white women.<sup>22</sup> A recent international study of 17 cohorts in Western population found that Vitamin D deficiency was associated with increased colorectal cancer risk, and Vitamin D above sufficiency levels was associated with 19% to 27% lower risk.<sup>23</sup> Compared with Western countries, there was inconsistent conclusion about the relationship between blood circulating Vitamin D level and colorectal cancer risk in studies of Asian countries,<sup>24-30</sup> given that lifestyle, ethnic and environmental factors are different between Asian and Western countries.

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3 We hypothesized that the association between blood circulating Vitamin D and colorectal  
4 cancer in Asian countries is distinct from Western countries. Thus, this review aimed to  
5 summarize epidemiological evidence regarding blood circulating Vitamin D level and  
6 colorectal cancer risk in Asian countries. This study underlines the public health importance  
7 of reaching and maintaining an optimal vitamin D status in Asian population and may help to  
8 guide clinical and nutritional practice in Asians countries.  
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## 14 15 **METHODS**

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18 We performed the systematic review according to a predetermined protocol and reported in  
19 accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses  
20 Protocols (PRISMA-P) guidelines.<sup>31</sup> Two reviewers (L.Z. and Z.Q.) independently undertook  
21 the literature search, assessment for eligibility, data extraction, and qualitative assessment.  
22 Any inconsistencies between the two reviewers were reviewed by a third reviewer (Y.J.) and  
23 resolved by consensus.  
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### 30 **Eligibility criteria**

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33 *Participants:* Our study draws participants from the Asian population in Asia countries,  
34 according to the list of sovereign states and dependent territories in Asia by the United Nation  
35 (<https://unstats.un.org/unsd/methodology/m49/>), with 48 countries located in five regions  
36 (Central Asia, Eastern Asia, Southern Asia, Southeastern Asia, and Western Asia).  
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40 Population of Asian origin but who live in Western countries were excluded.

41  
42 *Exposure:* The exposure is blood circulating 25-hydroxyvitamin D (25(OH)D) level which is  
43 commonly measured to assess and monitor Vitamin D status in individuals. Most studies only  
44 report the total level and do not distinguish D2 and D3 forms of the vitamin. In our meta-  
45 analysis, we consider the total level of Vitamin D as the exposure.  
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49 *Comparators (controls):* In order to be eligible for inclusion, studies must compare outcomes  
50 in a group of exposed individuals with highest category of blood circulating Vitamin D level  
51 and a group of unexposed individuals with lowest category of blood circulating Vitamin D  
52 level.  
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56 *Outcome:* Studies included in the review have a diagnosis of colorectal cancer or colorectal  
57 adenoma, clinically confirmed by colonoscopy or pathology.  
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3 *Study design:* We target observational studies (case-control, cross-sectional and cohort).  
4 Studies conducted post-1980 and reported in English were considered eligible. Animal  
5 studies were not included.  
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10 Studies were excluded if they: 1) were reviews, editorial, case report, or guideline articles; 2)  
11 did not explicitly state the blood circulating Vitamin D level and its association with  
12 colorectal cancer risk; 3) allowed controls to have a previous disease history of cancer; 4)  
13 focused on Western population or Asian population living in Western countries; or 5)  
14 investigated the blood circulating Vitamin D level and its association with survival of  
15 colorectal cancer. By consensus among all three reviewers (L.Z., Z.Q. and Y.J.), if data  
16 sources were duplicated in more than one study, only the original study was included in the  
17 meta-analysis.  
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### 24 25 **Search strategy**

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28 We conducted a literature search using Medline, Embase, and Web of Science to search all  
29 relevant articles that reported the association plasma or serum Vitamin D level and the risk of  
30 colorectal neoplasia in Asian countries, published from January 1980 to 31 January 2019.  
31 The Medical Subject Heading (MeSH) terms were used in conjunction with the following  
32 keywords to search for the studies: (colorectal neoplasm or colon neoplasm or colorectal  
33 cancer or colon cancer) AND (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-  
34 hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-  
35 hydroxylase or Vitamin D) AND (Asia\* or Afghanistan or Armenia or Azerbaijan or Bahrain  
36 or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or  
37 Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan  
38 or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or  
39 North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi  
40 Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or  
41 Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan  
42 or Vietnam or Yemen). Full search strings are presented in **Table S1**. References from any  
43 relevant articles, editorials, conference abstracts, letters, and reviews were also thoroughly  
44 reviewed to identify any additional relevant studies. Full manuscripts of every article with a  
45 relevant title and abstract were then reviewed for eligibility.  
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## Data extraction and qualitative assessment

Two reviewers (L.Z. and Z.Q.) independently extracted the following study-level characteristics from each eligible study: first author, year of publication, type of study, country where the study was conducted, selection criteria, the numbers of cases and controls (for case-control studies or cross-sectional studies) and the numbers of total participants and incident cases (for cohort studies), population characteristics (sex and age), follow-up period (for cohort studies), sample size, levels of Vitamin D in both case and control group, measures and ranges of Vitamin D, adjusted variables, and risk estimates with corresponding 95% CI for each category. For studies that reported both crude and adjusted estimates of the association between blood circulating Vitamin D and risk of colorectal cancer or adenoma, we used the adjusted estimates for the meta-analysis. For studies that reported several adjusted estimates of association, we used the estimates adjusted for the most variables.

We applied the Newcastle-Ottawa Scale (NOS) quality assessment tool to evaluate the quality of the selected observational studies. This tool was used to measure the key aspects of the methodology in selected studies with regard to design quality and the risk of biased estimates based on three design criteria: 1) selection of study participants; 2) comparability of study groups; and 3) assessment of outcome and exposure with a star system (with a maximum of 9 stars). We judged studies that received a score of 7-9 stars to be at low risk of bias, studies that scored 4-6 stars to be at medium risk, and those that scored 3 or less to be at high risk of bias. A funnel plot was used to assess the publication bias. Any disagreement on the data extraction and quality assessment of the studies were resolved through comprehensive discussion (L.Z., Z.Q. and J.Y).



## Statistical analysis

Study-specific OR estimates were combined using a random-effects model, which considers both within-study and between-study variation's and their corresponding 95% CIs were extracted directly from articles where available, with adjusted ORs extracted preferentially over unadjusted ORs. The dose-response analysis was utilized to assess the relationship between blood circulating Vitamin D level and colorectal cancer risk using the generalized least squares (GLS) method since this method could resolve the inconsistency issue of different Vitamin D levels analyzed in included studies.<sup>32 33</sup> For the dose-response meta-analysis of blood circulating Vitamin D levels, we used the method proposed by previously published studies to compute the trend from the correlated log OR estimates across categories of Vitamin D levels.<sup>34</sup> This analytical method collected the distribution of cases and controls, median values of blood circulating Vitamin D levels, and corresponding OR estimates in each category for each study. The assigned value of the lowest category was designated as a reference level. If the study did not provide median values of blood Vitamin D, the midpoint of the upper and lower boundaries in each category was assigned. For the open-ended exposure categories, the length of the open-ended interval was assumed to be the same as that of the adjacent interval. We examined a potential non-linear dose-response relationship between blood circulating Vitamin D level with colorectal cancer risk by modelling Vitamin D levels using random-effect restricted cubic splines with three knots at percentiles 25%, 50%, and 75% of the distribution (spline model). A *P*-value for nonlinearity was calculated by testing the null hypothesis that the regression coefficient of the second spline was equal to zero by Wald-type test of nonlinear hypotheses.<sup>34</sup> A small *P*-value (<0.05) of Wald-type test indicates departure from linearity. This meta-analysis comparing the appropriate open categories (or highest category) of exposure to the lowest category was performed. The non-linear dose-response relationship was also performed by several representative point values and the risk estimates of a subgroup analysis based on the range of exposure.

The statistical heterogeneity among studies was evaluated using Cochran's Q test and  $I^2$  statistic, with values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively.<sup>35</sup> The criterion for identifying heterogeneity was a *P* value less than 0.05 for the Q test. If substantial heterogeneity was detected, we performed univariate meta-regression analyses to explore the proportion of between-study variance explained by study quality, participant characteristics, and study characteristics. We were unable to

1  
2  
3 perform a multivariate meta-regression analysis as only a small number of included studies  
4 reported information for all study-level factors. We also performed subgroup analyses  
5 comparing pooled association estimates and heterogeneity with stratification by ~~year of~~  
6 ~~publication~~, participants sex, outcome type, the subregion of Asia, blood sample type, and  
7 range of Vitamin D levels (The range is the difference in the midpoint between the highest  
8 and lowest categories of blood circulating Vitamin D in each study). An estimation of  
9 publication bias was evaluated by the Beggs funnel plot, in which the SE of log (OR) of each  
10 study was plotted against its log (OR). An asymmetrical plot suggests possible publication  
11 bias. Egger's linear regression test assessed funnel plot asymmetry, a statistical approach to  
12 identify funnel plot asymmetry on the natural logarithm scale of the ORs. All statistical  
13 analyses were performed using Stata software (version 14.2; StataCorp LP, College Station,  
14 Texas). All of the *P* values were two-sided, and  $P < 0.05$  was considered as statistically  
15 significant.  
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## RESULTS

### Selection of studies

A detailed PRISMA flow diagram<sup>31</sup> of literature search and inclusion is shown in **Figure 1**. A total of 611 studies were initially identified with this literature search (250 from Medline, 272 from Embase and 89 from Web of Science), but 114 studies were excluded due to duplication and 465 were excluded after screening the titles and abstracts. Then 24 studies were excluded after a full-text review (details shown in **Table S2**). Finally, a total of eight studies were identified as eligible for this meta-analysis.

### Study characteristics

The eight studies of blood circulating Vitamin D level were included with a total of 2,916 cases and 6,678 controls (**Tables 1 and 2**). The eight studies included in the meta-analysis were published between 2007 and 2018, with seven studies from Eastern Asia in which four are from Japan,<sup>24 25 27 30</sup> two from Korea,<sup>26 28</sup> and one from China;<sup>36</sup> and one study is from Western Asia.<sup>29</sup> Regarding the study design, seven were case-control,<sup>24-29 36</sup> and one was a nested case-cohort study.<sup>30</sup> Of these eight studies, four studies provided the main endpoint of colorectal cancer<sup>24 29 30 36</sup> and four studies provided the main endpoint of colorectal adenoma.<sup>25-28</sup>

### Meta-analysis and dose-response analysis

The multivariable-adjusted ORs for each study and the combination of all eight studies for the highest versus lowest categories of blood circulating Vitamin D levels are shown in **Figure 2**. The mean blood circulating Vitamin D level of the included study population was 20.21 ng/mL, with an SD of 7.92 ng/mL, the minimal concentration of 3.65 ng/mL, and the maximal concentration of 36.5 ng/mL. Results from the studies on blood circulating Vitamin D levels in relation to colorectal cancer risk were inconsistent, with both inverse and positive associations reported. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D level was 0.75 [95% CI, 0.58-0.97], which indicate a higher blood circulating Vitamin D level had a significant inverse association with risk of colorectal cancer. There was statistically significant heterogeneity among the studies ( $I^2=53.9\%$ ,  $P=0.034$ ).

We evaluated the non-linear dose-response relationship between blood circulating Vitamin D levels and colorectal cancer risk. A 16 ng/mL increment (about 2 SDs=15.84 ng/mL) in blood circulating Vitamin D levels conferred an OR of 0.79 [95% CI, 0.64-0.97], which meant the risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in blood Vitamin D, and the reduction was statistically significant. A moderate heterogeneity existed ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ) in the overall analysis of blood circulating Vitamin D levels, without a significant non-linear dose-response relationship ( $P_{\text{non-linearity}}=0.11$ ), suggesting that the non-linear dose-response relationship does not depart from linearity. Therefore, the linear model is the more fitted model in this dose-response relationship. Similar trends were observed with linear and spline models (**Figure 3**).

### Subgroup analysis

When we stratified the analysis according to blood sample type, the pooled ORs of serum sample and plasma for the highest versus lowest categories of blood circulating Vitamin D levels were 0.52 [95% CI, 0.34-0.80] and 0.77 [95% CI, 0.59-1.00] respectively. The results showed that there was a substantial risk reduction (48%) for blood serum Vitamin D levels associated with risk of colorectal cancer. There was no evidence of significant statistical heterogeneity among studies ( $I^2=22.67\%$ ,  $P=0.21$ ). We then performed subgroup analysis of blood circulating Vitamin D range in each study. The pooled ORs was 0.93 [95% CI, 0.70-1.25] for studies with range  $\leq 15$  ng/mL and 0.62 [95% CI, 0.47-0.83] for studies with range  $> 15$  ng/mL. There was no evidence of statistical heterogeneity among studies ( $I^2=28.48\%$ ,  $P=0.08$ ). When stratifying by outcome, the pooled ORs were 0.67 [95% CI, 0.40-1.14] for studies when the outcome was colorectal adenoma and 0.83 [95% CI, 0.66-1.06] when the outcome was colorectal cancer respectively with no significantly statistical heterogeneity among studies ( $I^2=59.48\%$ ,  $P=0.73$ ). When we stratified the studies by sex, the pooled ORs were 0.59 [95% CI, 0.13-2.74] for studies with estimates for women, and 1.13 [95% CI, 0.81-1.58] for men respectively with no significantly statistical heterogeneity among studies ( $I^2=45.89\%$ ,  $P=0.70$ ). We also conducted analyses that were stratified according to the region of the population. The pooled ORs was 0.75 [95% CI, 0.57-1.00] for Eastern Asia and 0.69 [95% CI, 0.36-1.34] for Western Asia. There was no significantly statistical heterogeneity among studies ( $I^2=59.79\%$ ,  $P=0.87$ ). The above subgroup meta-analyses are shown in **Figure S1** and summarized in **Table 3**.

### Qualitative assessment and publication bias

The NOS tool was used to conduct a qualitative assessment of the selected studies to review the quality of the studies and detect possible bias. Of the eight studies, five studies were at low risk of bias was 8-9 stars.<sup>24-27 30</sup> The three studies at medium risk (5-7 stars) mainly due to the bias from representativeness of cases or controls, control definition, and response rate.<sup>29 36 37</sup> ( shown in **Table S3.**) The Funnel plot and Eggers statistical test indicated no evidence of publication bias in the studies included in the meta-analysis ( $P=0.338$ ) (**Figure S2.**)

## DISCUSSION

Colorectal cancer is one of the cancers with high morbidity and mortality in the world. The one-carbon metabolism pathway requires adequate Vitamin D, and this raises the possibility that Vitamin D may have an essential role in the risk of colorectal cancer. Many epidemiological studies from Europe and the United States believe that increasing the concentration of circulating Vitamin D can reduce the morbidity and mortality of colorectal cancer.<sup>38</sup> However, the association between blood circulating Vitamin D levels and risk of colorectal cancer in the Asian population is still under debate due to a lack of sufficient evidence. This systematic review highlights the inconsistencies among studies addressing the role of blood circulating Vitamin D and colorectal cancer risk in the Asian population. Our systematic review identified eight studies of 2916 cases and 6678 controls that addressed the relationship between blood circulating Vitamin D levels and colorectal cancer risk. This meta-analysis found that 25% reduced risk of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D levels (OR=0.75, 0.58-0.97) up to 36.5 ng/mL, which indicated higher blood circulating Vitamin D level had a significant inverse association with risk of colorectal cancer in Asian population. Our meta-analysis results showed that the negative correlation between Vitamin D and the risk of colorectal cancer is similar to that of European and American population studies<sup>11 12 21 22 39-42</sup> and consistent with the result of a meta-analysis from Ekmekcioglu C et al.<sup>13</sup>, which revealed a pooled relative risk (RR) of 0.62 (0.56–0.70) for colorectal cancer when comparing individuals with the highest category of 25(OH)D with those in the lowest.

Our results found a 16 ng/mL increment in blood circulating Vitamin D levels conferred an OR of 0.79 [95% CI, 0.64-0.97], which meant the risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in blood Vitamin D, and the reduction was statistically significant. Our study also suggested a linear dose-response relationship ( $P_{\text{non-linearity}} = 0.11$ ), which was consistent with several studies conducted in Western population revealing a similar protective dose-response association of blood circulating Vitamin D and colorectal cancer risk. For example, a meta-analysis reported a 26% lower risk of colorectal cancer per 10 ng/mL increment in blood circulating Vitamin D levels.<sup>11</sup> Most experts define Vitamin D deficiency as a Vitamin D level of less than 20 ng/mL.<sup>43 44</sup> Vitamin D concentration of 21 to 29 ng/mL can be considered to indicate a relative insufficiency of Vitamin D, and a level of 30 ng/mL or higher can be considered to indicate sufficient Vitamin D.<sup>45</sup> With the use of such definitions, it has been estimated that many people have Vitamin D deficiency or

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3 insufficiency.<sup>43-44</sup> Previous research has implied the association of Vitamin D deficiency with  
4 an increased incidence of bone fractures.<sup>46</sup> There have also been data showing Vitamin D  
5 deficiency to be associated with cancer,<sup>47-49</sup> diabetes,<sup>50</sup> cognitive impairment,<sup>51</sup> and all-cause  
6 mortality.<sup>52</sup> Among colorectal cancer patients, the prevalence of Vitamin D deficiency was  
7 much higher (nearly 90%) than among patients with other chronic diseases.<sup>53</sup> Humans obtain  
8 Vitamin D from exposure to sunlight, from natural diets, fortified diets, supplementation.<sup>46</sup> It  
9 is possible that the dose-response between Vitamin D concentrations and colorectal cancer  
10 risk is different between Asian and Western population due to ethnic, anthropometric,  
11 dietary, and environmental factors.<sup>12</sup> Lifestyle and diet can promote the development of  
12 early-onset colon lesions by regulating growth factors that interact with inflammatory  
13 pathways.<sup>41</sup> An association between Vitamin D status and reduced risk of colorectal cancer  
14 has been found in ethnically diverse populations.<sup>5</sup> Vitamin D interacts with calcium to  
15 enhance the reduction of colon cancer risk.<sup>54-56</sup> Studies have shown that Vitamin D and  
16 calcium may interact and that both are needed in reducing cancer risk.<sup>57</sup> However, even after  
17 adjusting for calcium intake in some studies,<sup>6,58</sup> Vitamin D was associated with a lower risk.  
18 The independent effects of Vitamin D are supported, but the combined effects of the Vitamin  
19 D and calcium may be greater than the sum of their independent effects.<sup>59</sup> Vitamin A has an  
20 antagonistic effect on Vitamin D<sup>58</sup> and taking both at the same time can lead to decreased  
21 calcium absorption. Still, Vitamin A is often combined with Vitamin D in supplements.  
22 Vitamin D supplementation significantly reduced total cancer mortality but did not reduce  
23 total cancer incidence.<sup>60</sup> Patients with low-risk prostate cancer under active surveillance may  
24 benefit from vitamin D3 supplementation at 4000 IU/d.<sup>61</sup> Among patients with metastatic  
25 colorectal cancer, addition of high-dose Vitamin D3, vs standard-dose Vitamin D3, to  
26 standard chemotherapy resulted in a difference in median progression-free survival that was  
27 not statistically significant, but with a significantly improved supportive effect.<sup>62</sup>  
28 Epidemiological evidence links the incidence of colorectal cancer to lifestyle, smoking,  
29 physical activity, alcohol consumption, and sleep.<sup>63</sup> It has also been linked to reduced fruit  
30 and vegetable consumption and increased consumption of red meat. Dairy products, fish and  
31 other foods and cooking methods also play an essential role.<sup>64</sup> In addition, some drugs such  
32 as non-steroidal anti-inflammatory drugs and cyclooxygenase inhibitors are also involved.<sup>65</sup>  
33 Women on estrogen therapy, for example, did not reduce their risk of colorectal cancer by  
34 taking Vitamin D and calcium supplements.<sup>66</sup> Increased dietary fiber intake reduces the risk  
35 of colorectal cancer, and obesity and low physical activity reduce plasma 25(OH)D  
36 concentration, thereby increasing the risk of colorectal cancer.<sup>42</sup> For every 1 kg/m<sup>2</sup> increase  
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3 in BMI of colorectal cancer patients, serum Vitamin D level decreased significantly (0.46  
4 ng/mL).<sup>67</sup> Most variation in Vitamin D levels usually comes from exposure to the sun, which  
5 is an essential source of Vitamin D for people who get more from fish, even in Japan.<sup>68</sup>  
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9 This meta-analytic comparison revealed a statistically significant beneficial effect of blood  
10 circulating Vitamin D for colorectal cancer. However, the diversity of the studies and the  
11 presence of moderate heterogeneity in our study ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ) in the overall  
12 analysis of blood circulating Vitamin D levels may preclude making meaningful conclusions  
13 from the pooled analysis because the pooled estimate may not reflect the true underlying  
14 effect. Much of the heterogeneity was not explained through subgroup analyses in our study.  
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20 The potential reasons for the heterogeneity in the strength of the association may include  
21 many factors. Firstly, the type of food consumption and vitamin supplements varied  
22 according to the specific dietary habits and lifestyle in each subregion of Asia. A systemic  
23 review studied correlations between various diet types, food or nutrients and colorectal  
24 cancer risk among Asian population, and suggested that red meats, processed meats,  
25 preserved foods, saturated/animal fats, cholesterol, high sugar foods, spicy foods, tubers or  
26 refined carbohydrates have been found to have a positive association with colorectal cancer  
27 risk.<sup>69</sup> Besides diet, other personal and lifestyle factors (e.g., exposure to sunlight, obesity,  
28 smoking and drinking habit) may alter the strength of the association and contribute to the  
29 heterogeneity of the association in Asian countries. In our meta-analysis, we had seven  
30 studies<sup>24-28 30 36</sup> from Eastern Asia and one study from Western Asia,<sup>29</sup> however the number  
31 of studies from these regions were imbalanced, yet we still present it in the subgroup  
32 analysis; Besides, with systematic search, we did not have available studies from Central  
33 Asia, Southern Asia, Southeastern Asia included in this meta-analysis, therefore we were  
34 cautious to interpret the subgroup analysis. Our study revealed no evidence of publication  
35 bias, and most of the studies included in our meta-analysis verified the diagnoses of  
36 colorectal cancer for cases. Histologic confirmation of cancer diagnoses for cases was an  
37 optimal validation for the case-control design in our meta-analysis.  
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52 Possible confounders for the association between colorectal cancer and blood circulating  
53 Vitamin D levels include sex, age, family history of colorectal cancer, smoking, alcohol  
54 drinking, body mass index, and diabetes. Most studies in our meta-analysis provided risk  
55 estimates that were adjusted for age,<sup>24 26-30 36</sup> sex,<sup>24 26 27 29 30 36</sup> body mass index,<sup>24-30 36</sup>  
56 smoking,<sup>24-30 36</sup> drinking,<sup>24-26 29 36</sup> physical activity,<sup>30</sup> alcohol consumption,<sup>24-30</sup> and family  
57 history of colorectal cancer;<sup>24 25 29</sup> fewer were adjusted for folate,<sup>24 37</sup> energy intake,<sup>24 27</sup>  
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3 hypertension,<sup>36</sup> vitamin D binding protein,<sup>36</sup> blood collection,<sup>24 27</sup> and adjusted for vitamin  
4 supplement use.<sup>24</sup> Therefore, for these studies, the observed reduced risk of colorectal cancer  
5 associated with a Vitamin D level is less likely to be confounded by these known  
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7 confounders.  
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10 Our analysis had the following limitations. First, the number of included studies is not  
11 sufficient to provide a robust estimate of association of blood circulating Vitamin D levels  
12 and colorectal cancer risk, so the analysis and results should be interpreted in the context of  
13 the limitations of the available data. Second, heterogeneous definitions of blood circulating  
14 Vitamin D categories were used across studies. The variability in definitions could limit  
15 comparability between studies. Third, our study included seven case-control studies; the  
16 study design implies that the measurement of blood circulating Vitamin D is measured in  
17 individuals already diagnosed with colorectal cancer. Therefore, results from this study  
18 design need to be interpreted cautiously because of the potential for reverse causation. Time  
19 of blood sampling in relation to outcome ascertainment also varied among studies. Such  
20 cross-sectional measurements may not accurately reflect an individual's Vitamin D status  
21 across time. Forth, some studies included in our meta-analysis did not adjust for some  
22 potentially relevant confounders which may have led to residual confounding and may  
23 explain some of the observed heterogeneity. Fifth, difference in the method used for  
24 measuring blood circulating Vitamin D levels may also be a source of heterogeneity between  
25 the included studies. Sixth, in the dose-response analysis, the literature selected listed the  
26 median Vitamin D value, instead of the original Vitamin D value, which could also lead to  
27 inaccurate results. Seventh, Although we conducted the investigation of quality levels of the  
28 observational studies in conducting this meta-analysis by the NOS quality assessment tool,  
29 Bae<sup>70</sup> suggested that it is more reasonable to control for quality level by performing  
30 subgroup analysis according to study design rather than by using high-quality based on the  
31 NOS tool. In this context, however, we did not perform a subgroup analysis according to  
32 study design since the included 8 studies were case-control and nested case-cohort studies.  
33 Finally, a recent meta-analysis investigated the association between blood circulating  
34 Vitamin D levels and survival of colorectal cancer and found that the pooled hazard ratios  
35 (95% confidence intervals) were 0.68 [0.55–0.85] and 0.67 [0.57–0.78] for overall and  
36 colorectal cancer-specific survival comparing highest versus lowest categories of blood  
37 Vitamin D, respectively.<sup>15</sup> However, our study did not explore the association of the blood  
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3 circulating Vitamin D levels and colorectal cancer mortality in Asian countries. In future  
4 studies, we would like to explore this association in Asian countries.  
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## 7 **CONCLUSION**

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10 The blood circulating Vitamin D is inversely associated with colorectal cancer prevalence in  
11 the Asian population. Our findings on the inverse association between blood circulating  
12 Vitamin D and the risk of colorectal cancer in Asian population suggest that Asian should  
13 improve their nutritional status and maintain a higher blood circulating Vitamin D level. This  
14 meta-analysis also provides valuable information for future research of association between  
15 blood circulating Vitamin D and colorectal cancer risk in Asian population. Further studies  
16 may focus on evaluating more detailed Vitamin D levels with colorectal cancer. A  
17 multinational, population-based study in Asian countries may resolve the issue of  
18 heterogeneity to generate detailed information on blood circulating Vitamin D levels and risk  
19 of colorectal cancer. In considering Vitamin D supplementation also associate with cancer  
20 incidence and mortality,<sup>60</sup> we may investigate these associations in the future studies.  
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## 31 **Authors Contributions**

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34 LZ: Study concept and design; acquisition of data; statistical analysis; interpretation of data;  
35 drafting and critical review of the manuscript for important intellectual content; approval of  
36 the final version of the manuscript.  
37

38 HZ: Interpretation of data; drafting and critical review of the manuscript for important  
39 intellectual content; approval of the final version of the manuscript.  
40

41 YZ: Interpretation of data; drafting and critical review of the manuscript for important  
42 intellectual content; approval of the final version of the manuscript.  
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45 CH: Drafting and critical review of the manuscript for important intellectual content;  
46 approval of the final version of the manuscript.  
47

48 AA: Drafting and critical review of the manuscript for important intellectual content;  
49 approval of the final version of the manuscript.  
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51

52 XQ: Interpretation of data; drafting and critical review of the manuscript for important  
53 intellectual content; approval of the final version of the manuscript.  
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56 PJ: Interpretation of data; drafting and critical review of the manuscript for important  
57 intellectual content; approval of the final version of the manuscript.  
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3 YJ: Study concept and design; acquisition of data interpretation of data; drafting and critical  
4 review of the manuscript for important intellectual content; approval of the final version of  
5 the manuscript.  
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8 ZQ: Study concept and design; acquisition of data; interpretation of data; drafting and critical  
9 review of the manuscript for important intellectual content; approval of the final version of  
10 the manuscript.  
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For peer review only

## Figure legends/captions

**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

**Figure 2.** Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D. F, female; M, male;

**Figure 3.** Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modeled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line,  $P_{\text{non-linearity}}=0.11$ ). Lines with short dashes represent the linear trend.

**Table 1.** Summary of characteristic for 8 studies included in the meta-analysis

Author(year)	Study design	Country	Study period	Outcome	Outcome ascertainment	Sex	Sample size (n); characteristics				Blood sample type	Vitamin D testing method	
							Case control	or	No.	Male (%)	Mean (SD)/Median age(range)		
Budhathoki et al. (2018) <sup>30</sup>	Nested case-cohort	Japan	1990-2009	colorectal cancer	NA	All	case control		637 4044	52 34	56.2(7.5) 53.7(7.9)	plasma	chemiluminescent enzyme immunoassay
Choi et al. (2015) <sup>37</sup>	case-control	Korea	2011-2012	colorectal adenoma	colonoscopy	All	case control		112 112	51 51	60.3 (5.3) 59.5 (5.4)	serum	chemiluminescent enzyme immunoassay
						Men	case control		57 57	100 100	NA NA	serum	
						Women	case control		55 55	0 0	NA NA	serum	
							case control		55 55	0 0	NA NA		
Hong et al. (2012) <sup>26</sup>	case-control	Korea	2009-2010	colorectal adenoma	colonoscopy	All	case control		143 143	68 68	58.7 (6.0) 58.7 (6.0)	serum	direct competitive electrochemiluminescence immunoassay
Otani et al. (2007) <sup>24</sup>	case-control	Japan	1990-2003	colorectal cancer	pathologically confirmed	All	case control		323 621	52 52	NA NA	plasma	competitive protein-binding assay
						Men	case control		163 324	100 100	56.9 56.9		
						Women	case control		160 297	0 0	56.5 56.4		
							case control		297 297	0 0	56.4 56.4		
Takahashi et al. (2010) <sup>25</sup>	case-control	Japan	1997-2004	colorectal adenoma	colonoscopy	Men	case control		656 648	100 100	52.0 (1.4) 51.8 (1.5)	plasma or serum	radioimmunoassay method
Yamaji et al. (2012) <sup>27</sup>	case-control	Japan	2004-2005	colorectal adenoma	colonoscopy	All	case control		737 703	67 65	NA NA	plasma	radioimmunoassay method
Ying et al. (2015) <sup>36</sup>	case-control	China	2010-2014	colorectal cancer	colonoscopy	All	case control		212 212	NA NA	63(56-73) 63(57-73)	plasma	direct competitive enzyme-linked immunosorbent assay
						cases control		96 195	67 54	57.8(10.0) 51.2(12.9)	serum	NA	

**Abbreviations:** SD, Standard deviation; NA, Not available; All\*: all participants, including men and women

**Table 2.** Summary of risk estimate for 8 studies included in the meta-analysis

Author(year)	sex	Vitamin D level cut points (ng/ml)	Vitamin D medium level (ng/ml)	Number of cases	Number of controls	N	OR	LCI	UCI	Adjusted variables
Budhathoki et al. (2018) <sup>30</sup>	All	1st Q, 12.5-16.5 ng/ml	14.7	134	1004	1138	1			age, sex, BMI, smoking, alcohol use, physical activity, family history of cancer, and history of diabetes
		2nd Q, 17.6-21.6 ng/ml	19.9	165	1000	1165	1.08	0.84	1.39	
		3rd Q, 21.2-25.6 ng/ml	21.3	160	1016	1176	0.96	0.74	1.26	
		4th Q, 25.8-33.0 ng/ml	26.5	178	1024	1202	0.95	0.73	1.23	
Choi et al. (2015) <sup>37</sup>	All	1st Q, 10.3 ng/ml	10.3	37	28	65	1			age, sex, BMI, alcohol drinking, smoking status, folate intake; women: additional menopausal status and hormone replacement use
		2nd Q, 14.3 ng/ml	14.3	26	28	54	0.72	0.31	1.66	
		3rd Q, 17.9 ng/ml	17.9	28	28	56	0.73	0.29	1.82	
		4th Q, 24.2 ng/ml	24.2	21	28	49	0.49	0.19	1.27	
	Men	1st Q, 11.1 ng/ml	11.1	15	14	29	1			
		2nd Q, 14.6 ng/ml	14.6	13	14	27	1.31	0.36	4.82	
		3rd Q, 17.7 ng/ml	17.7	17	15	32	1.26	0.4	8.12	
		4th Q, 22.7 ng/ml	22.7	12	14	26	1.8	0.19	8.12	
	Women	1st Q, 9.5 ng/ml	9.5	16	14	30	1			
		2nd Q, 13.5 ng/ml	13.5	22	14	36	0.89	0.26	3.1	
		3rd Q, 18.4 ng/ml	18.4	8	14	22	0.54	0.13	2.16	
		4th Q, 25.4 ng/ml	25.4	9	13	22	0.22	0.04	1.15	
Hong et al. (2012) <sup>26</sup>	All	1st Q, <14.3 ng/ml	10.1	31	42	73	1			age, sex, BMI, smoking, alcohol drinking, physical activity, corrected calcium level
		2nd Q, 14.3-18.5 ng/ml	16.4	29	42	71	0.87	0.43	1.74	
		3rd Q, 18.6-23.8 ng/ml	21.2	41	31	72	0.4	0.2	0.82	
		4th Q, >=23.9 ng/ml	29.1	42	28	70	0.38	0.18	0.8	
Otani et al. (2007) <sup>24</sup>	Men	1st Q, <22.9 ng/ml	18.3	43	74	117	1			age, sex, BMI, smoking, alcohol consumption, physical exercise, Vitamin supplement use, family history of colorectal cancer, total energy intake, dietary fiber intake, folate intake, calcium intake, Vitamin D intake, n-3 fatty acid intake, red meat intake, fish intake
		2nd Q, 22.9-27.5 ng/ml	25.2	40	85	125	0.76	0.42	1.4	
		3rd Q, 27.6-32.0 ng/ml	29.8	36	85	121	0.76	0.39	1.5	
		4th Q, >32.1 ng/ml	36.5	44	80	124	0.73	0.35	1.5	
	Women	1st Q, <18.7 ng/ml	15.2	41	77	118	1			
		2nd Q, 18.7-22.2 ng/ml	20.5	34	73	107	1	0.55	1.9	
		3rd Q, 22.3-26.9 ng/ml	24.6	44	71	115	1.2	0.65	2.3	
		4th Q, >27.0 ng/ml	31.6	41	76	117	1.1	0.5	2.3	
Takahashi et al. (2010) <sup>25</sup>	Men	1st Q, <22 ng/ml	20	128	142	270	1			BMI, hospital, rank in the Self Defense Forces, smoking, alcohol drinking, parental history of colorectal cancer, physical activity, type of blood sample, the month of blood drawing
		2nd Q, 23-25 ng/ml	24	162	156	318	1.21	0.86	1.7	
		3rd Q, 26-29 ng/ml	27.5	223	208	431	1.21	0.87	1.69	
		4th Q, >30 ng/ml	33	143	142	285	1.25	0.85	1.84	
Yamaji et al. (2012) <sup>27</sup>	All	1st Q, 14-19 ng/ml	16.5	145	129	274	1			age, sex, BMI, screening periods, the season of blood collection, smoking, alcohol drinking, family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, height, daily energy intake
		2nd Q, 20-23 ng/ml	21.5	132	128	260	0.86	0.6	1.24	
		3rd Q, 24-26 ng/ml	25	157	145	302	0.91	0.64	1.29	
		4th Q, 27-31 ng/ml	29	175	144	319	1.03	0.73	1.46	
		5th Q, 31-34 ng/ml	32.5	128	157	285	0.64	0.45	0.92	
Ying et al. (2015) <sup>36</sup>	All	1st Q, <7.29 ng/ml	3.65	80	53	133	1			age, sex, BMI, smoking, drinking, history of diabetes, hypertension, vitamin D binding protein
		2nd Q, 7.29-14.61 ng/ml	10.95	49	53	102	0.62	0.35	1.12	
		3rd Q, 14.61-28.84 ng/ml	21.73	46	53	99	0.67	0.38	1.20	
		4th Q, >=28.84 ng/ml	35.96	37	53	90	0.53	0.29	0.98	
Yurekli et al.(2015) <sup>29</sup>	All	1st Q, <8.6 ng/ml	7.1	24	48	72	1			age, sex, BMI, smoking, alcohol intake
		2nd Q, 8.6-10.1 ng/ml	9.35	20	53	73	0.48	0.24	0.98	
		3rd Q, 10.1-14.5 ng/ml	12.3	25	48	73	0.51	0.25	1.03	
		4th Q, >=14.5 ng/ml	18.9	27	46	73	0.69	0.36	1.36	

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**Abbreviations:** NA, Not available; OR: odds ratio; LCI: 95% CI's lower confidence interval; UCI: 95% CI's upper confidence interval; BMI: body mass index  
All\*: all participants, including men and women

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**Table 3.** Summary of subgroup analysis for the associations of blood circulating Vitamin D and the risk colorectal cancer and adenoma by year of publication, outcome, participants sex, the subregion of Asia, and blood sample type.

Subgroup analysis	Number of estimates	Random effect The summary OR (95% CI)	Ratio of ORs (95% CI)	$I^2$ (%)	$P$ value
<u>Outcome</u>					
Colorectal adenoma	4	0.67 (0.40-1.14)			
Colorectal cancer	4	0.83 (0.66-1.06)	1.11(0.54-2.28)	59.48	0.73
<u>Sex</u>					
Women	2	0.59 (0.13-2.74)			
Men	3	1.13(0.81-1.58)	1.46 (0.03-63.5)	45.89	0.70
<u>Subregion</u>					
Eastern Asia	7	0.75 (0.57-1.00)			
Western Asia	1	0.69 (0.36-1.34)	0.92 (0.28-2.99)	59.79	0.87
<u>Blood sample type</u>					
Serum	3	0.52 (0.34-0.80)			
Plasma	4	0.77 (0.59-1.00)	1.49 (0.73-3.03)	22.67	0.21
<u>Range</u>					
≤15 ng/mL	4	0.93 (0.70-1.25)			
>15 ng/mL	4	0.62 (0.47-0.83)	0.65(0.39-1.07)	28.48	0.08

Abbreviations: OR, Odds ratio;  $I^2$ , Percent residual variation due to heterogeneity; CI, Confidence interval.  
\* $I^2$  and P value related to subgroup differences.



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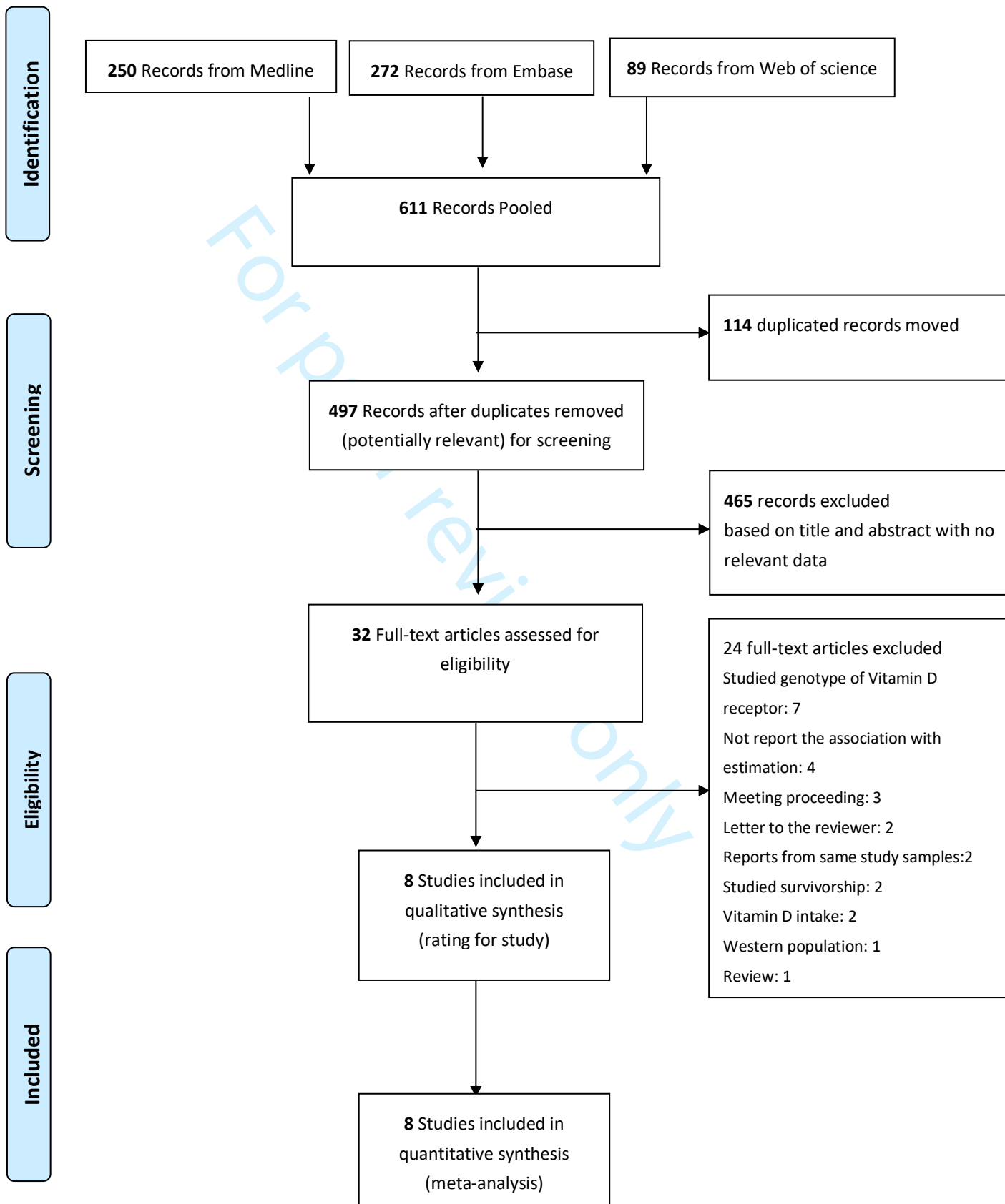
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**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

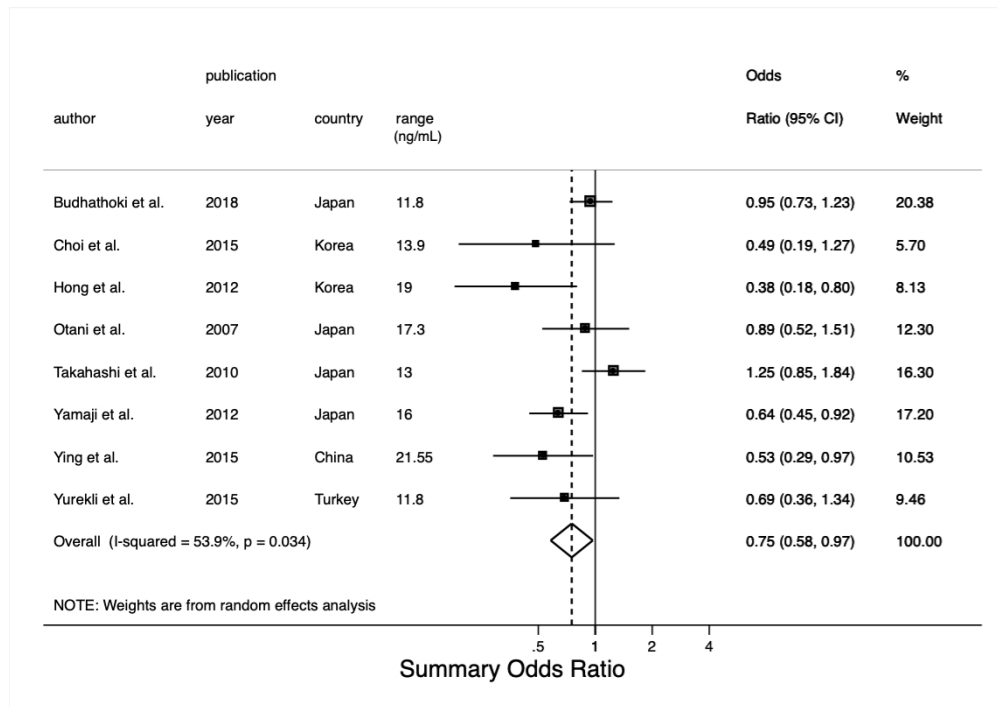


Figure 2. Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D.



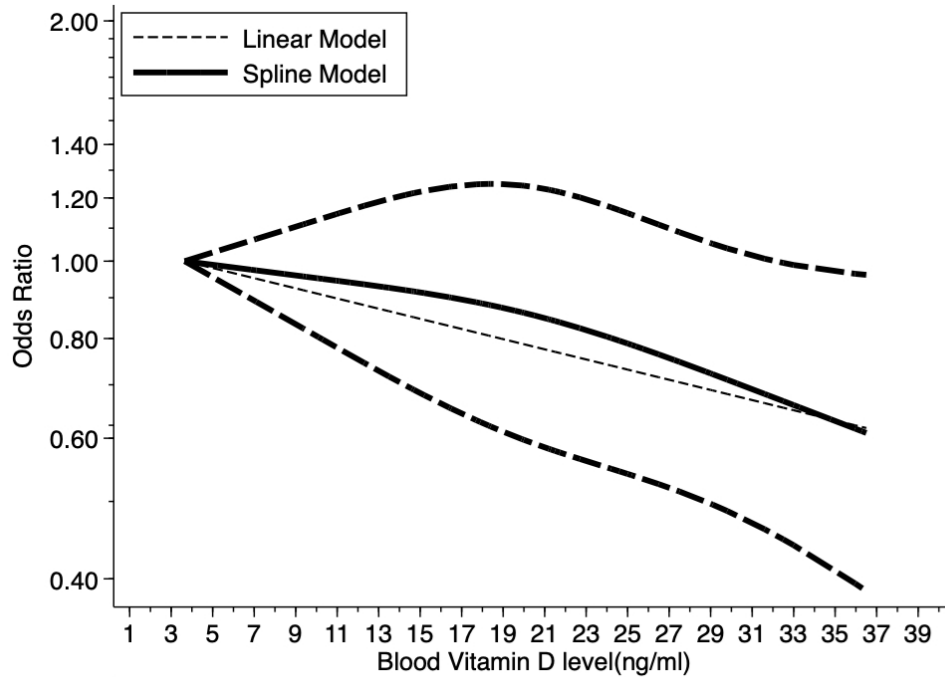


Figure 3. Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modeled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line,  $P_{\text{non-linearity}}=0.11$ ). Lines with short dashes represent the linear trend.

## Supplementary Material

**Table S1.** Literature search results from Medline, Embase, and Web of Science.

	Medline	Results
1	exp colorectal neoplasms/	187315
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	2688871
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	80293
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1074938
5	1 or 2	2690742
6	3 and 4 and 5	258
7	limit 6 to English language	250

	Embase	Results
1	exp colorectal neoplasms/	26250
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1075151
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	137427
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or	1562807

	Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	
5	1 or 2	1075151
6	3 and 4 and 5	279
7	limit 6 to English language	272

	<b>Web of science</b>	<b>Results</b>
1	TS= (colorectal neoplasm or colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*)	460,541
2	TS= (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D)	99,758
3	TS=(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen)	2,184,572
4	#1 AND #2 AND #3	106
5	(#4) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)	89

**Table S2.** List and details of the excluded studies from the meta-analysis

Reason for exclusion	Author	Year	Study title	Journal
Studied genotype of Vitamin D receptor(n=7)	Wong HL, et al.	2003	Vitamin D receptor start codon polymorphism and colorectal cancer risk: Effect modification by dietary calcium and fat in Singapore Chinese	Carcinogenesis
	Li C, et al.	2009	Vitamin D receptor gene polymorphisms and the risk of colorectal cancer in a Chinese population	Digestive Diseases and Sciences
	Kang JW et al.	2015	Role of vitamin D and local vitamin D receptor expression in colon carcinogenesis	Journal of Gastroenterology and Hepatology
	Takehige N, et al.	2015	Associations between vitamin D receptor (VDR) gene polymorphisms and colorectal cancer risk and effect modifications of dietary calcium and vitamin D in a Japanese population	Asian Pac J Cancer Prev
	Aumpansub P, et al.	2016	Strong association of single nucleotide polymorphisms of vitamin D receptor gene, BSML, and colorectal cancer in Asian population	Gastroenterology
	Budhathoki S, et al.	2016	Vitamin D receptor gene polymorphism and the risk of colorectal cancer: A nested case-control study	PLoS ONE
	Gong C, et al.	2017	Dietary factors and polymorphisms in vitamin D metabolism genes: the risk and prognosis of colorectal cancer in northeast China	Scientific Reports
Not report the association with estimation(n=4)	Mizoue T, et al.	2005	Dietary patterns and colorectal adenomas in Japanese men - The self-defense forces health study.	American Journal of Epidemiology
	Atoum MF, et al.	2014	Association between circulating vitamin D, the TaqI vitamin D receptor gene polymorphism and colorectal cancer risk among Jordanians. Asian Pacific journal of cancer prevention	Asian Pacific Journal of Cancer Prevention
	Kumagai Y, et al.	2014	Dietary patterns and colorectal cancer risk in Japan: the Ohsaki Cohort Study.	Cancer Causes & Control
	Hessami Arani S, et al.	2017	Rising rates of colorectal cancer among younger iranians: Is diet to blame?	Current Oncology
Meeting proceeding (n=3)	Grant WB, et al.	2011	Ecological study findings regarding vitamin D and cancer.	Anticancer Research
	Li K, et al.	2014	The association between serum vitamin D concentrations and colorectal cancer.	Clinical Chemistry and Laboratory Medicine
	Ozer C, et al.	2015	The relationship between serum 25-hydroxy vitamin D levels and insulin resistance in breast and colon cancer.	Clinical Chemistry and Laboratory Medicine
Letter to the reviewer (n=2)	Dunnigan MG, et al.	1990	Serum 25-hydroxyvitamin D and colon cancer.	Lancet
	Qu B, et al.	2017	Role of Circulating and Supplemental Calcium and Vitamin D in the Occurrence and Development of Colorectal Adenoma or Colorectal Cancer.	J Clin Gastroenterol
Reports from same study sample (n=2)	Byeon JS, et al.	2007	Colorectal neoplasm in asymptomatic Asians: a prospective multinational multicenter colonoscopy survey.	Gastrointestinal Endoscopy
	Shin A, et al.	2011	Site-specific risk factors for colorectal cancer in a korean population.	PLoS ONE
Studied survivorship (n=2)	Wesa KM, et al.	2015	Serum 25-hydroxy vitamin D and survival in advanced colorectal cancer: a retrospective analysis.	Nutr Cancer
	Woo KW, et al.	2015	Vitamin D deficiency in Hong Kong advanced cancer patients: Result of first 50 patients.	Annals of Oncology
Vitamin D intake (n=2)	Mizoue T, et al.	2008	Calcium, dairy foods, vitamin D, and colorectal cancer risk: The Fukuoka colorectal cancer study.	Cancer Epidemiology Biomarkers and Prevention
	Ishihara J, et al.	2008	Dietary calcium, vitamin D, and the risk of colorectal cancer.	American Journal of Clinical Nutrition
Western population (n=1)	Sy AM, et al.	2013	Association between serum vitamin D levels and colonic carcinomatous polyps.	Journal of Gastrointestinal Cancer
Review (n=1)	Grant WB, et al.	2009	Ecological Studies Of Ultraviolet B, Vitamin D And Cancer Since 2000.	Annals of Epidemiology

**Table S3.** Newcastle-Ottawa Scale for assessing the quality of studies in the systematic review

Author (Year)	Country	Region	Selection				Comparability		Exposure			Total Score
			S1	S2	S3	S4	C1	C2	E1	E2	E3	
Budhathoki et al. (2018)[1]	Japan	Eastern Asian	★	★	★	★	★	★	★	★		8
Choi et al. (2015)[2]	Korea	Eastern Asian	★				★	★	★	★		5
Hong et al. (2012)[3]	Korea	Eastern Asian	★	★	★	★	★	★	★	★		8
Otani et al. (2007)[4]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Takahashi et al. (2010)[5]	Japan	Eastern Asian	★	★	★	★		★	★	★	★	8
Yamaji et al. (2012)[6]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Ying et al. (2015)[7]	China	Eastern Asian	★			★	★	★	★	★	★	7
Yurekli et al.(2015)[8]	Turkey	Western Asian	★				★	★	★	★		5

**Guidelines for review***Selection:*

S1, Case definition adequacy: ★a) requires independent validation (>1 person/record/time/process to extract information or reference to primary record sources such as colonoscopy or medical/hospital records); b) record linkage or self-report with no reference to primary record; c) no description

S2, Representativeness of the cases: ★a) consecutive or representative series of cases; b) potential for selection biases or not stated

S3, Selection of controls: ★a) community controls; b) hospital controls, within same community as cases; c) no description

S4, Definition of controls: ★a) no history of colorectal cancer or adenoma; b) no description of the source

*Comparability:*

C1, ★ Study adjusted for confounder, such as age and sex (the most important factors);

C2, ★ Study adjusted for any additional confounders (1> additional factors, e.g., BMI, drinking or smoking)

*Exposure:*

E1, Ascertainment of exposure: ★a) secure record (e.g., medical records); ★b) structured interview were blind to case/control status; c) interview not blinded to case/control status; d) written self-report or medical record only; e) no description

E2, Same method of ascertainment for cases and controls: ★a) yes; b) no

E3, Non-response rate: ★a) the same rate for both groups; b) non-respondents described; c) rate different and no designation

**Figure S1.** Subgroup meta-analysis, including stratified by outcome (colorectal cancer or colorectal adenoma, **S1A**), sex (women, men or both, **S1B**), sample type (serum or plasma, **S1C**), range ( $\leq 15$  or  $>15$  ng/mL **S1D**), subregion (Eastern Asia or Western Asia, **S1E**).

**Figure S1A**

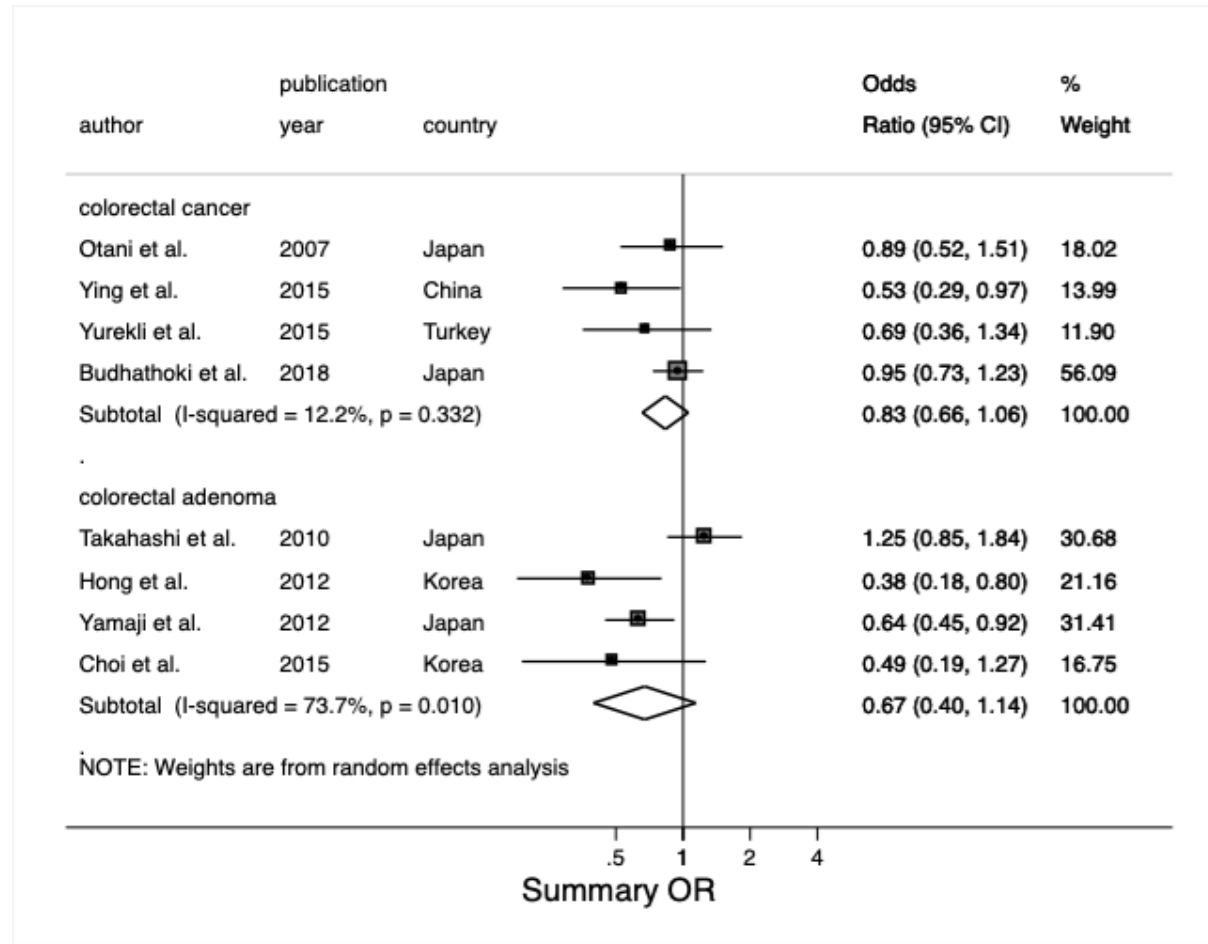


Figure S1B

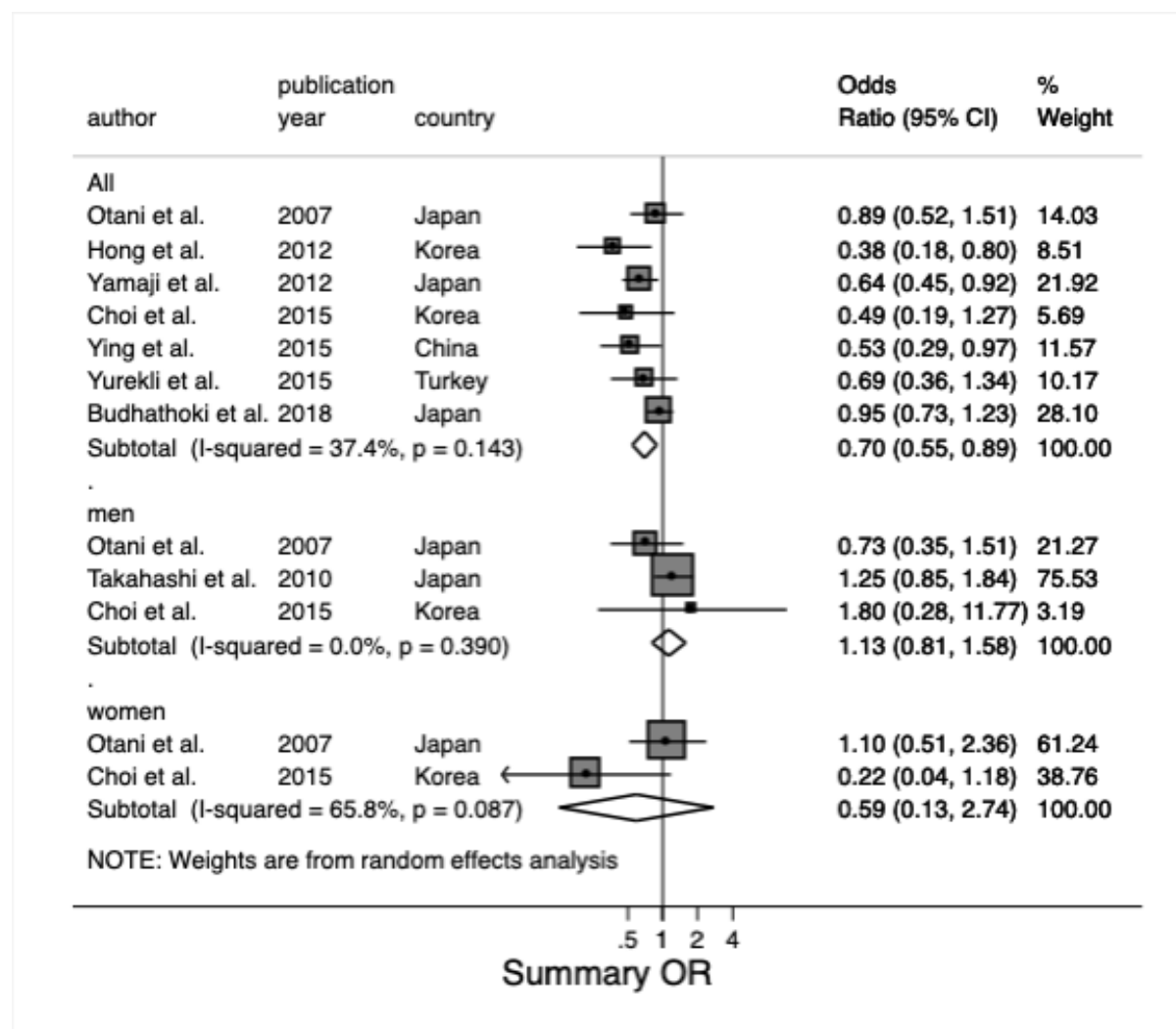
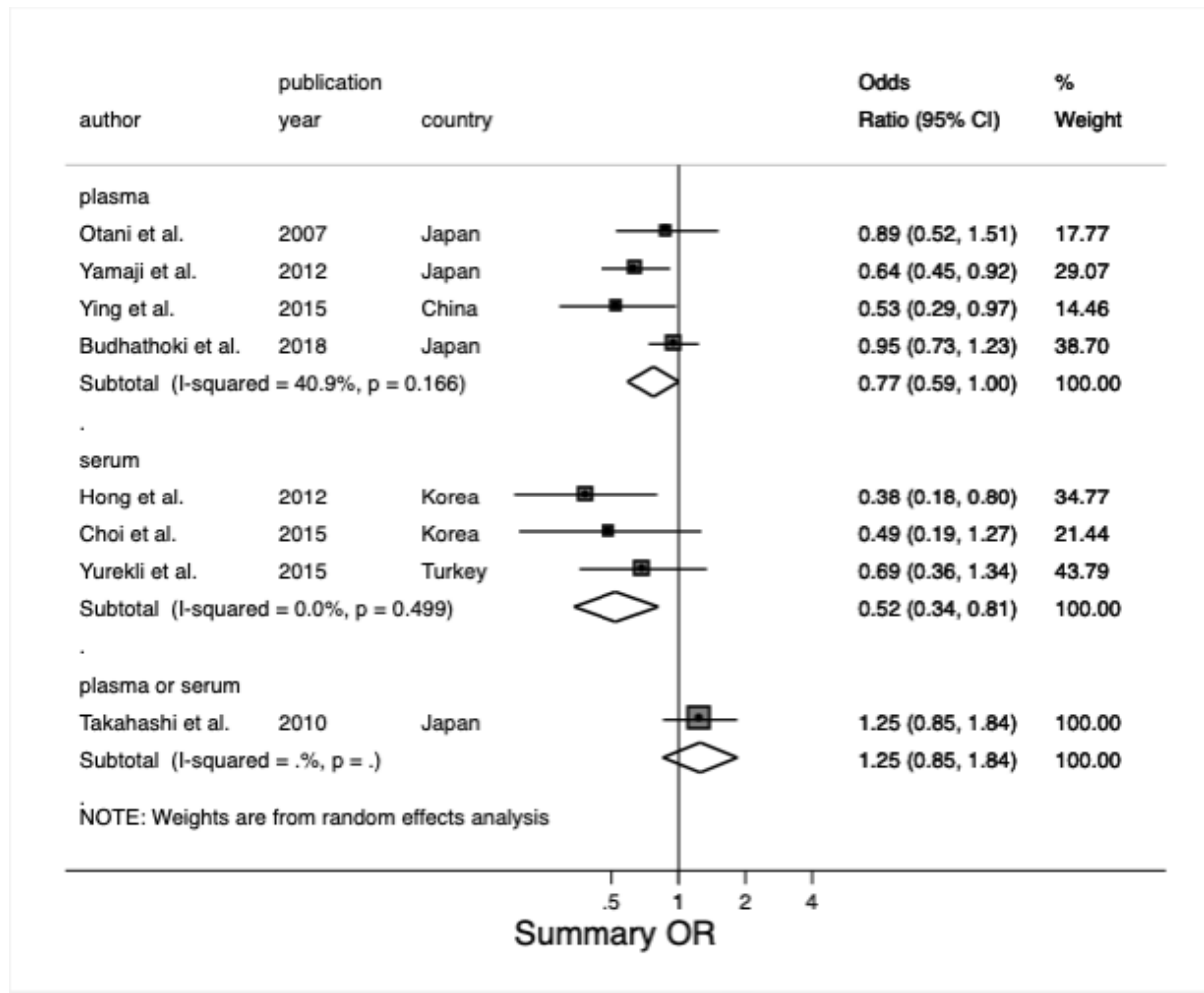


Figure S1C



Only



Figure S1D

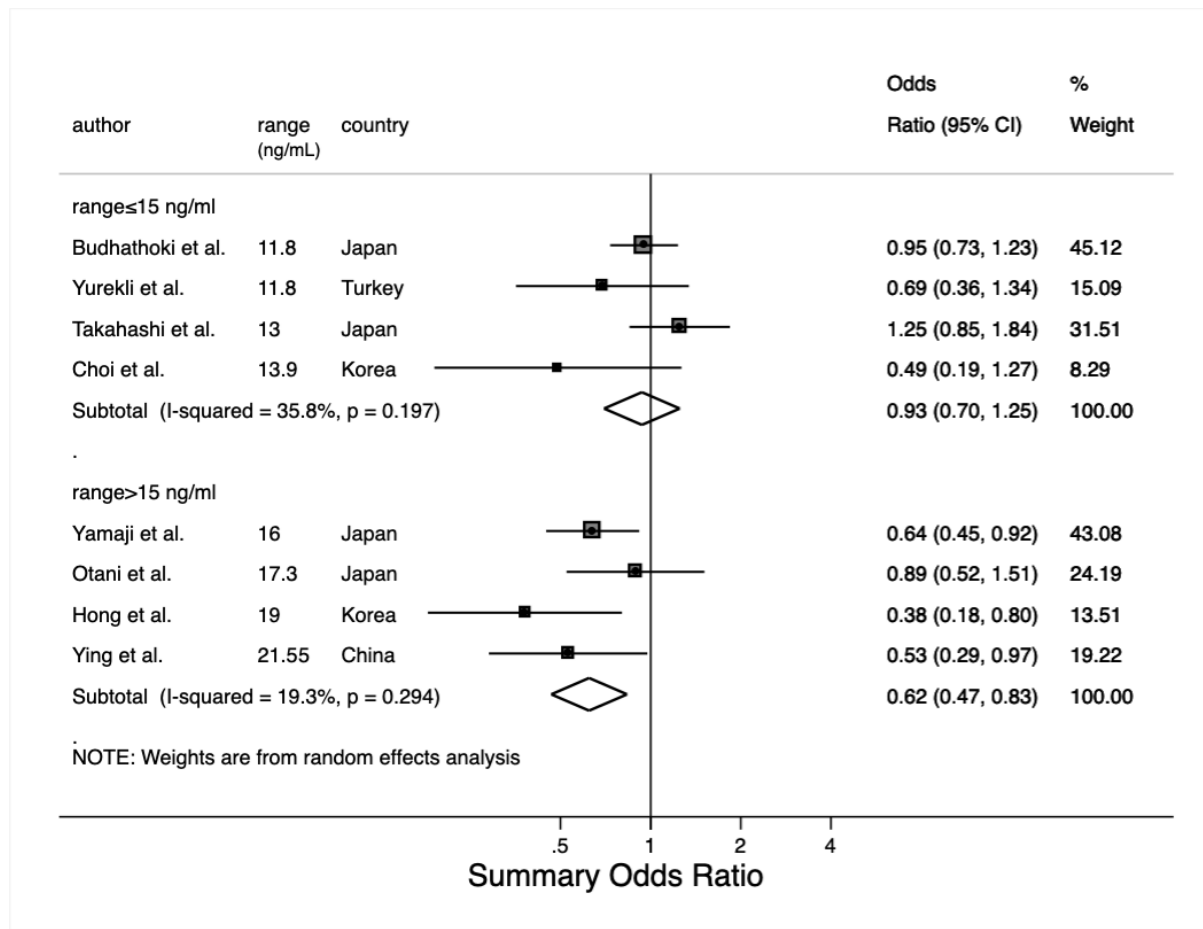
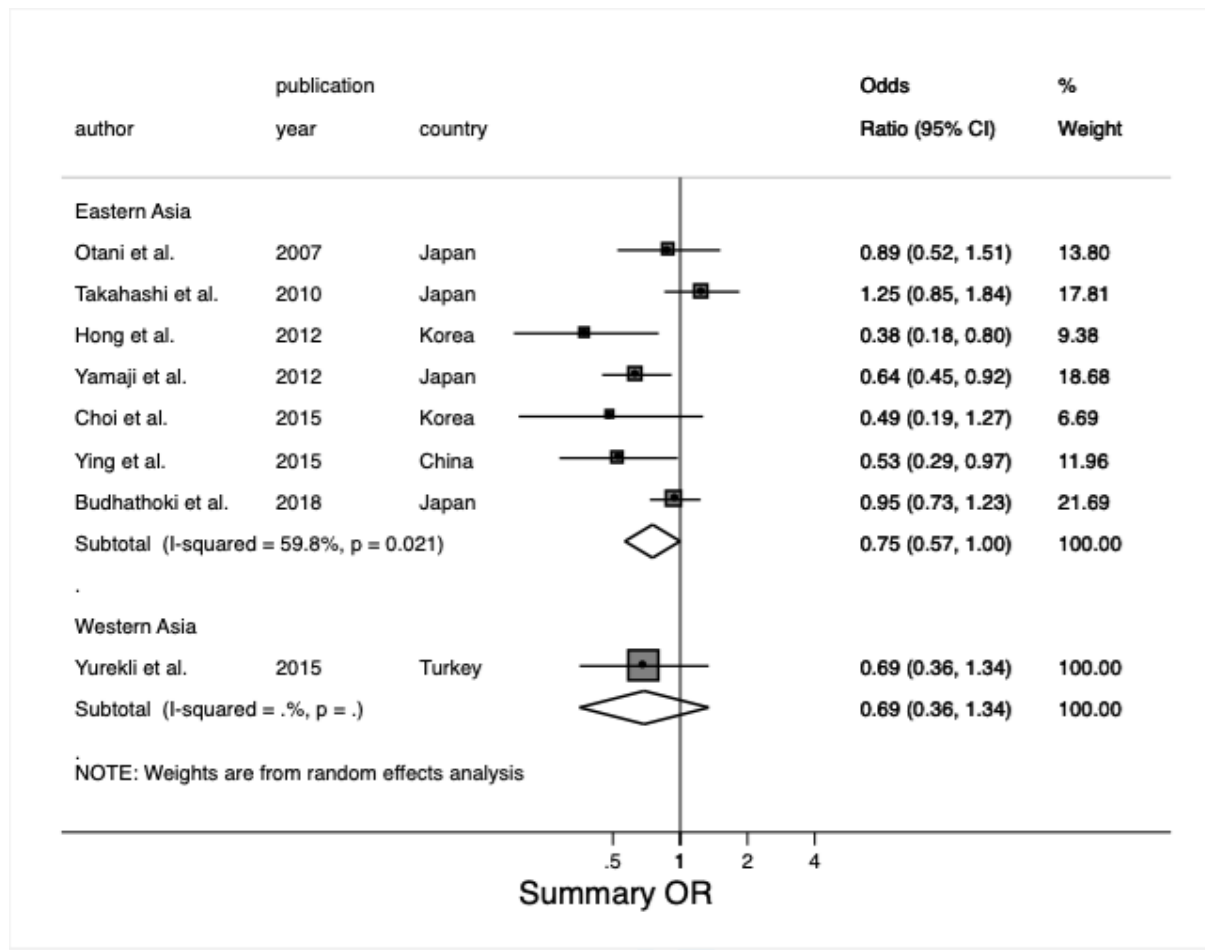
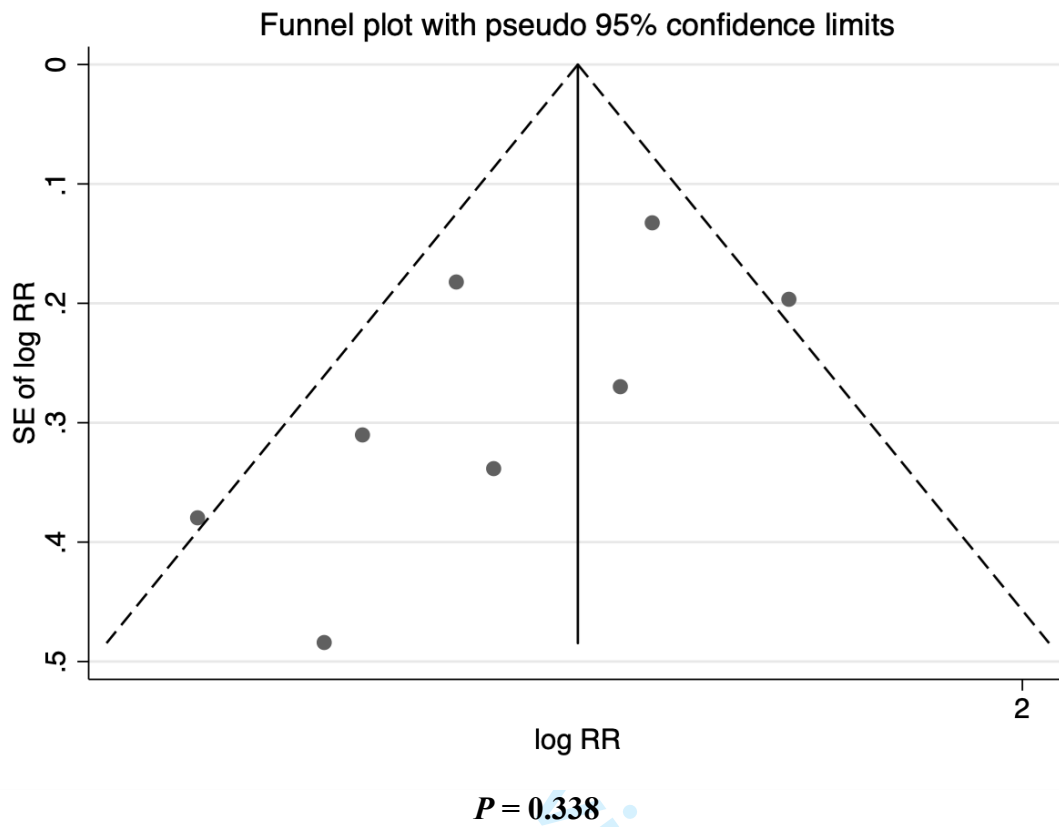


Figure S1E



**Figure S2.** Funnel plot for all studies included in the meta-analysis and between blood circulating Vitamin D and the colorectal cancer risk in Asian countries





# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 6
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Page 5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Page 7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I <sup>2</sup> ) for each meta-analysis.	Page 9



# PRISMA 2009 Checklist

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 10
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 11 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 11, Table 1 and Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 11 and Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 11 and 12
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 12 and 13
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 14 to Page 16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 18
<b>FUNDING</b>			
For peer review only - <a href="http://bmjopen.bmj.com/site/about/guidelines.xhtml">http://bmjopen.bmj.com/site/about/guidelines.xhtml</a>			



# PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Page 2
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From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).

Page 2 of 2

For peer review only

# BMJ Open

## Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-030513.R2
Article Type:	Original research
Date Submitted by the Author:	08-Oct-2019
Complete List of Authors:	Zhang, Lin; Peking Union Medical College Hospital, Clinical Laboratory; University of Melbourne School of Population and Global Health, Centre of Epidemiology and Biostatistics Zou, Huachun; Sun Yat-sen University, School of Public Health Zhao, Yang; The University of Melbourne, Melbourne School of Population and Global Health; WHO Collaborating Centre on Implementation Research for Prevention & Control of NCDs, Hu, Chunlei; Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Department of Gastrointestinal Surgery, Peking University Shenzhen Hospital Atanda, Adejare; Morgan State University, School of Community Health and Policy Qin, Xuzhen; Peking Union Medical College Hospital, Clinical Laboratory Jia, Peng; University of Twente, Faculty of Geo-Information Science and Earth Observation (ITC); International Initiative on Spatial Lifecourse Epidemiology (ISLE) Jiang, Yu; Chinese Academy of Medical Sciences & Peking Union Medical College, School of public health Qi, Zhihong; Peking Union Medical College Hospital, Clinical Laboratory
<b>Primary Subject Heading</b>:	Epidemiology
Secondary Subject Heading:	Oncology, Public health
Keywords:	Colorectal cancer, Colorectal adenoma, Vitamin D, 25-hydroxyvitamin D, Asia, Meta-analysis

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# Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

Lin Zhang,<sup>1,2,3,4,12</sup> Huachun Zou,<sup>5,6,7</sup> Yang Zhao,<sup>8</sup> Chunlei Hu,<sup>9</sup> Adejare Atanda,<sup>10</sup> Xuzhen Qin,<sup>1</sup> Peng Jia,<sup>11,12</sup> Yu Jiang,<sup>2</sup> Zhihong Qi<sup>1</sup>

<sup>1</sup> Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>2</sup> Peking Union Medical College School of Public Health, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>3</sup> Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, Victoria 3100, Australia

<sup>4</sup> The University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Parkville, Victoria 3100, Australia

<sup>5</sup> School of Public Health (Shenzhen), Sun Yat-sen University, Shenzhen 518107, Guangdong, China

<sup>6</sup> School of Public Health, Sun Yat-sen University, Guangzhou 510085, Guangdong, China

<sup>7</sup> Kirby Institute, University of New South Wales, Sydney 2052, NSW, Australia

<sup>8</sup> School of Population and Global Health, The University of Melbourne & WHO Collaborating Centre on Implementation Research for Prevention & Control of NCDs, Parkville, Victoria 3100, Australia

<sup>9</sup> Department of Gastrointestinal Surgery, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, China

<sup>10</sup> School of Community Health and Policy, Morgan State University, Baltimore, Maryland, USA

<sup>11</sup> Faculty of Geo-Information Science and Earth Observation (ITC), University of Twente, Enschede 7500, The Netherlands

<sup>12</sup> International Initiative on Spatial Lifecourse Epidemiology (ISLE)

## \*Correspondence to:

Prof. Zhihong Qi

Clinical Laboratory, Peking Union Medical College Hospital  
Chinese Academy of Medical Sciences & Peking Union Medical College  
Shuai Fu Yuan 1#, DongCheng District, Beijing 100730, China

Tel.: + 86 10 69159740

Email: cizeng1010@sina.com

Prof. Yu Jiang

Chinese Academy of Medical Sciences & Peking Union Medical College,  
DongDan SanTiao 9, DongCheng District, Beijing 100730, China

Tel.: + 86 10 65105916

Email: wingedsky@gmail.com



**Transparency declaration**

The Corresponding Authors affirm that this manuscript is an accurate account of the study proposed with no important aspects omitted, and that any discrepancies from the study as planned and registered will be explained upon the completion of the proposed study.

**Ethical approval**

Ethics approval is not required for this study because it is a systematic review.

**Details of funding**

This study was supported by National Key R&D Program of China (No. 2018YFC1315000/2018YFC1315003).

**Details of the role of the study sponsor**

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**Conflict of interest**

The authors have declared that no competing interests exist.

**Statement of independence of researchers form funders**

Not applicable.

**Patient involvement**

No patients were involved in the design or analysis of this study.

**Data sharing statement**

All data relevant to the study are included in the article or uploaded as supplementary information

## ABSTRACT

### Objectives

We conducted this dose-response meta-analysis to assess the association between blood circulating Vitamin D levels and colorectal cancer risk in Asian population.

### Design

This study was a systematic review and dose-response meta-analysis for observational studies which investigated the relationship between blood circulating Vitamin D levels and colorectal cancer risk in Asian population.

### Data Sources

Relevant studies were identified by searching literature in MEDLINE, EMBASE, and Web of Science until 31 January 2019. Eligibility criteria: original studies published in peer-reviewed journals investigating association between blood circulating Vitamin D levels and the risk of colorectal cancer and/or adenoma in Asian countries.

### Data extraction and synthesis:

Two authors independently extracted data and assessed the quality of included studies. Study-specific ORs were pooled using a random-effects model. A dose-response meta-analysis was performed with generalized least squares regression. We applied the Newcastle-Ottawa Scale (NOS) quality assessment tool to evaluate the quality of the selected observational studies.

### Results

The eight included studies encompassed a total of 2,916 cases and 6,678 controls. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D levels was 0.75 [95% CI, 0.58-0.97] up to 36.5 ng/mL in Asian population. There was heterogeneity among the studies ( $I^2=53.9%$ ,  $P_{\text{heterogeneity}}=0.034$ ). The dose-response meta-analysis indicated a significant linear relationship ( $P_{\text{non-linearity}}=0.11$ ). An increment of 16 ng/mL in blood circulating Vitamin D level corresponded to an OR of 0.79 [95% CI, 0.64-0.97].

### Conclusions

The results of this meta-analysis indicated that blood circulating Vitamin D level is associated with decreased risk of colorectal cancer in Asian countries. This dose-response meta-analysis further shows that the strength of this association among Asian population is similar to that among Western population. Our study suggested that Asian population should improve nutritional status and maintain a higher level of blood circulating Vitamin D.

### Strengths and limitations of this study

- Our study seeks to extend previous work by including a number of new studies and by distinguishing Asian population explicitly.
- The number of included studies is not sufficient to provide a robust estimate, so the results should be interpreted in the context of the limitations of the available data.
- Heterogeneous definitions of blood circulating Vitamin D categories were used across studies. The variability in definitions could limit comparability between studies.
- Our study included seven case-control studies; the study design implies that the measurement of blood circulating Vitamin D is measured in individuals already diagnosed with colorectal cancer. Therefore, results from case-control studies need to be interpreted cautiously because of the potential for reverse causation.
- Time of blood sampling in relation to outcome ascertainment also varied among studies. Such cross-sectional measurements may not accurately reflect an individual's Vitamin D status across time.

## INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer and second in terms of mortality, with over 1.8 million new cases and 881,000 deaths worldwide in 2018, accounting for about 1 in 10 cancer cases and deaths.<sup>1</sup> Some Asian countries where the incidence of colorectal cancer was historically low, such as Japan, Israel, Singapore, China, and the Philippines, are experiencing rising incidence rates over the past decades. In 2012, Japan (Miyagi Prefecture Cancer Registry) presented the highest colorectal cancer incidence in the world for men (62 per 100,000 persons) and women (37 per 100,000 persons).<sup>2</sup> Observational studies have identified several risk factors associated with an increased incidence of colorectal cancer including lifestyle factors (e.g., obesity, physical inactivity, smoking, and heavy alcohol use) and non-modifiable factors (e.g., aging, personal and family history of colorectal cancer or adenoma).<sup>3</sup> Other observational studies conducted in Western countries suggested blood circulating 25-hydroxyvitamin D (25(OH)D) (also named as Vitamin D) has a protective role in the development of colorectal cancer.<sup>4-8</sup> Some meta-analyses have consistently reported that there was an inverse association of plasma Vitamin D concentration in the blood with incidence and mortality of colorectal cancer.<sup>9-15</sup>

The prevalence of Vitamin D deficiency has increased in recent decades.<sup>16 17</sup> In a recent population-based study of Asian adults, approximately 75% had suboptimal Vitamin D concentrations.<sup>18</sup> The Endocrine Society Clinical Practice Guideline defines Vitamin D deficiency as 25(OH)D level <20 ng/mL and insufficiency as 21 to 29 ng/mL.<sup>19</sup> Feldman et al.<sup>20</sup> reported anti-neoplastic actions of Vitamin D, particularly in colorectal cancer.<sup>9</sup> Touvier et al.<sup>12</sup> reported that improving Vitamin D levels could be beneficial in reducing colorectal cancer incidence. Data from a cohort of healthy women shows that plasma Vitamin D levels were inversely related to the occurrence and death from colorectal cancer.<sup>21</sup> In the Nurses' Health Study, total circulating Vitamin D was associated with lower risk of colorectal cancer in white women.<sup>22</sup> A recent international study of 17 cohorts in Western population found that Vitamin D deficiency was associated with increased colorectal cancer risk, and Vitamin D above sufficiency levels was associated with 19% to 27% lower risk.<sup>23</sup> Compared with Western countries, there was inconsistent conclusion about the relationship between blood circulating Vitamin D level and colorectal cancer risk in studies of Asian countries,<sup>24-30</sup> given that lifestyle, ethnic and environmental factors are different between Asian and Western countries.

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3 We hypothesized that the association between blood circulating Vitamin D and colorectal  
4 cancer in Asian countries is distinct from Western countries. Thus, this review aimed to  
5 summarize epidemiological evidence regarding blood circulating Vitamin D level and  
6 colorectal cancer risk in Asian countries. This study underlines the public health importance  
7 of reaching and maintaining an optimal vitamin D status in Asian population and may help to  
8 guide clinical and nutritional practice in Asians countries.  
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## 14 15 **METHODS**

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18 We performed the systematic review according to a predetermined protocol and reported in  
19 accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses  
20 Protocols (PRISMA-P) guidelines.<sup>31</sup> Two reviewers (L.Z. and Z.Q.) independently undertook  
21 the literature search, assessment for eligibility, data extraction, and qualitative assessment.  
22 Any inconsistencies between the two reviewers were reviewed by a third reviewer (Y.J.) and  
23 resolved by consensus.  
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### 30 **Eligibility criteria**

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33 *Participants:* Our study draws participants from the Asian population in Asia countries,  
34 according to the list of sovereign states and dependent territories in Asia by the United Nation  
35 (<https://unstats.un.org/unsd/methodology/m49/>), with 48 countries located in five regions  
36 (Central Asia, Eastern Asia, Southern Asia, Southeastern Asia, and Western Asia).  
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40 Population of Asian origin but who live in Western countries were excluded.

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42 *Exposure:* The exposure is blood circulating 25-hydroxyvitamin D (25(OH)D) level which is  
43 commonly measured to assess and monitor Vitamin D status in individuals. Most studies only  
44 report the total level and do not distinguish D2 and D3 forms of the vitamin. In our meta-  
45 analysis, we consider the total level of Vitamin D as the exposure.  
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49 *Comparators (controls):* In order to be eligible for inclusion, studies must compare outcomes  
50 in a group of exposed individuals with highest category of blood circulating Vitamin D level  
51 and a group of unexposed individuals with lowest category of blood circulating Vitamin D  
52 level.  
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56 *Outcome:* Studies included in the review have a diagnosis of colorectal cancer or colorectal  
57 adenoma, clinically confirmed by colonoscopy or pathology.  
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3 *Study design:* We target observational studies (case-control, cross-sectional and cohort).  
4 Studies conducted post-1980 and reported in English were considered eligible. Animal  
5 studies were not included.  
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10 Studies were excluded if they: 1) were reviews, editorial, case report, or guideline articles; 2)  
11 did not explicitly state the blood circulating Vitamin D level and its association with  
12 colorectal cancer risk; 3) allowed controls to have a previous disease history of cancer; 4)  
13 focused on Western population or Asian population living in Western countries; or 5)  
14 investigated the blood circulating Vitamin D level and its association with survival of  
15 colorectal cancer. By consensus among all three reviewers (L.Z., Z.Q. and Y.J.), if data  
16 sources were duplicated in more than one study, only the original study was included in the  
17 meta-analysis.  
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### 24 25 **Search strategy**

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28 We conducted a literature search using Medline, Embase, and Web of Science to search all  
29 relevant articles that reported the association plasma or serum Vitamin D level and the risk of  
30 colorectal neoplasia in Asian countries, published from January 1980 to 31 January 2019.  
31 The Medical Subject Heading (MeSH) terms were used in conjunction with the following  
32 keywords to search for the studies: (colorectal neoplasm or colon neoplasm or colorectal  
33 cancer or colon cancer) AND (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-  
34 hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-  
35 hydroxylase or Vitamin D) AND (Asia\* or Afghanistan or Armenia or Azerbaijan or Bahrain  
36 or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or  
37 Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan  
38 or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or  
39 North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi  
40 Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or  
41 Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan  
42 or Vietnam or Yemen). Full search strings are presented in **Table S1**. References from any  
43 relevant articles, editorials, conference abstracts, letters, and reviews were also thoroughly  
44 reviewed to identify any additional relevant studies. Full manuscripts of every article with a  
45 relevant title and abstract were then reviewed for eligibility.  
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## Data extraction and qualitative assessment

Two reviewers (L.Z. and Z.Q.) independently extracted the following study-level characteristics from each eligible study: first author, year of publication, type of study, country where the study was conducted, selection criteria, the numbers of cases and controls (for case-control studies or cross-sectional studies) and the numbers of total participants and incident cases (for cohort studies), population characteristics (sex and age), follow-up period (for cohort studies), sample size, levels of Vitamin D in both case and control group, measures and ranges of Vitamin D, adjusted variables, and risk estimates with corresponding 95% CI for each category. For studies that reported both crude and adjusted estimates of the association between blood circulating Vitamin D and risk of colorectal cancer or adenoma, we used the adjusted estimates for the meta-analysis. For studies that reported several adjusted estimates of association, we used the estimates adjusted for the most variables.

We applied the Newcastle-Ottawa Scale (NOS) quality assessment tool to evaluate the quality of the selected observational studies. This tool was used to measure the key aspects of the methodology in selected studies with regard to design quality and the risk of biased estimates based on three design criteria: 1) selection of study participants; 2) comparability of study groups; and 3) assessment of outcome and exposure with a star system (with a maximum of 9 stars). We judged studies that received a score of 7-9 stars to be at low risk of bias, studies that scored 4-6 stars to be at medium risk, and those that scored 3 or less to be at high risk of bias. A funnel plot was used to assess the publication bias. Any disagreement on the data extraction and quality assessment of the studies were resolved through comprehensive discussion (L.Z., Z.Q. and J.Y).

## Statistical analysis

Study-specific OR estimates were combined using a random-effects model, which considers both within-study and between-study variation's and their corresponding 95% CIs were extracted directly from articles where available, with adjusted ORs extracted preferentially over unadjusted ORs. The dose-response analysis was utilized to assess the relationship between blood circulating Vitamin D level and colorectal cancer risk using the generalized least squares (GLS) method since this method could resolve the inconsistency issue of different Vitamin D levels analyzed in included studies.<sup>32 33</sup> For the dose-response meta-analysis of blood circulating Vitamin D levels, we used the method proposed by previously published studies to compute the trend from the correlated log OR estimates across categories of Vitamin D levels.<sup>34</sup> This analytical method collected the distribution of cases and controls, median values of blood circulating Vitamin D levels, and corresponding OR estimates in each category for each study. The assigned value of the lowest category was designated as a reference level. If the study did not provide median values of blood Vitamin D, the midpoint of the upper and lower boundaries in each category was assigned. For the open-ended exposure categories, the length of the open-ended interval was assumed to be the same as that of the adjacent interval. We examined a potential non-linear dose-response relationship between blood circulating Vitamin D level with colorectal cancer risk by modelling Vitamin D levels using random-effect restricted cubic splines with three knots at percentiles 25%, 50%, and 75% of the distribution (spline model). A *P*-value for nonlinearity was calculated by testing the null hypothesis that the regression coefficient of the second spline was equal to zero by Wald-type test of nonlinear hypotheses.<sup>34</sup> A small *P*-value (<0.05) of Wald-type test indicates departure from linearity. This meta-analysis comparing the appropriate open categories (or highest category) of exposure to the lowest category was performed. The non-linear dose-response relationship was also performed by several representative point values and the risk estimates of a subgroup analysis based on the range of exposure.

The statistical heterogeneity among studies was evaluated using Cochran's Q test and  $I^2$  statistic, with values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively.<sup>35</sup> The criterion for identifying heterogeneity was a *P* value less than 0.05 for the Q test. If substantial heterogeneity was detected, we performed univariate meta-regression analyses to explore the proportion of between-study variance explained by study quality, participant characteristics, and study characteristics. We were unable to



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3 perform a multivariate meta-regression analysis as only a small number of included studies  
4 reported information for all study-level factors. We also performed subgroup analyses  
5 comparing pooled association estimates and heterogeneity with stratification by ~~year of~~  
6 ~~publication~~, participants sex, outcome type, the subregion of Asia, blood sample type, and  
7 range of Vitamin D levels (The range is the difference in the midpoint between the highest  
8 and lowest categories of blood circulating Vitamin D in each study).An estimation of  
9 publication bias was evaluated by the Beggs funnel plot, in which the SE of log (OR) of each  
10 study was plotted against its log (OR). An asymmetrical plot suggests possible publication  
11 bias. Egger's linear regression test assessed funnel plot asymmetry, a statistical approach to  
12 identify funnel plot asymmetry on the natural logarithm scale of the ORs. All statistical  
13 analyses were performed using Stata software (version 14.2; StataCorp LP, College Station,  
14 Texas). All of the *P* values were two-sided, and  $P < 0.05$  was considered as statistically  
15 significant.  
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## RESULTS

### Selection of studies

A detailed PRISMA flow diagram<sup>31</sup> of literature search and inclusion is shown in **Figure 1**. A total of 611 studies were initially identified with this literature search (250 from Medline, 272 from Embase and 89 from Web of Science), but 114 studies were excluded due to duplication and 465 were excluded after screening the titles and abstracts. Then 24 studies were excluded after a full-text review (details shown in **Table S2**). Finally, a total of eight studies were identified as eligible for this meta-analysis.

### Study characteristics

The eight studies of blood circulating Vitamin D level were included with a total of 2,916 cases and 6,678 controls (**Tables 1 and 2**). The eight studies included in the meta-analysis were published between 2007 and 2018, with seven studies from Eastern Asia in which four are from Japan,<sup>24 25 27 30</sup> two from Korea,<sup>26 28</sup> and one from China;<sup>36</sup> and one study is from Western Asia.<sup>29</sup> Regarding the study design, seven were case-control,<sup>24-29 36</sup> and one was a nested case-cohort study.<sup>30</sup> Of these eight studies, four studies provided the main endpoint of colorectal cancer<sup>24 29 30 36</sup> and four studies provided the main endpoint of colorectal adenoma.<sup>25-28</sup>

### Meta-analysis and dose-response analysis

The multivariable-adjusted ORs for each study and the combination of all eight studies for the highest versus lowest categories of blood circulating Vitamin D levels are shown in **Figure 2**. The mean blood circulating Vitamin D level of the included study population was 20.21 ng/mL, with an SD of 7.92 ng/mL, the minimal concentration of 3.65 ng/mL, and the maximal concentration of 36.5 ng/mL. Results from the studies on blood circulating Vitamin D levels in relation to colorectal cancer risk were inconsistent, with both inverse and positive associations reported. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D level was 0.75 [95% CI, 0.58-0.97], which indicate a higher blood circulating Vitamin D level had a significant inverse association with risk of colorectal cancer. There was statistically significant heterogeneity among the studies ( $I^2=53.9\%$ ,  $P=0.034$ ).

We evaluated the non-linear dose-response relationship between blood circulating Vitamin D levels and colorectal cancer risk. A 16 ng/mL increment (about 2 SDs=15.84 ng/mL) in blood circulating Vitamin D levels conferred an OR of 0.79 [95% CI, 0.64-0.97], which meant the risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in blood Vitamin D, and the reduction was statistically significant. A moderate heterogeneity existed ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ) in the overall analysis of blood circulating Vitamin D levels, without a significant non-linear dose-response relationship ( $P_{\text{non-linearity}}=0.11$ ), suggesting that the non-linear dose-response relationship does not depart from linearity. Therefore, the linear model is the more fitted model in this dose-response relationship. Similar trends were observed with linear and spline models (**Figure 3**).

### Subgroup analysis

When we stratified the analysis according to blood sample type, the pooled ORs of serum sample and plasma for the highest versus lowest categories of blood circulating Vitamin D levels were 0.52 [95% CI, 0.34-0.80] and 0.77 [95% CI, 0.59-1.00] respectively. The results showed that there was a substantial risk reduction (48%) for blood serum Vitamin D levels associated with risk of colorectal cancer. There was no evidence of significant statistical heterogeneity among studies ( $I^2=22.67\%$ ,  $P=0.21$ ). We then performed subgroup analysis of blood circulating Vitamin D range in each study. The pooled ORs was 0.93 [95% CI, 0.70-1.25] for studies with range  $\leq 15$  ng/mL and 0.62 [95% CI, 0.47-0.83] for studies with range  $> 15$  ng/mL. There was no evidence of statistical heterogeneity among studies ( $I^2=28.48\%$ ,  $P=0.08$ ). When stratifying by outcome, the pooled ORs were 0.67 [95% CI, 0.40-1.14] for studies when the outcome was colorectal adenoma and 0.83 [95% CI, 0.66-1.06] when the outcome was colorectal cancer respectively with no significantly statistical heterogeneity among studies ( $I^2=59.48\%$ ,  $P=0.73$ ). When we stratified the studies by sex, the pooled ORs were 0.59 [95% CI, 0.13-2.74] for studies with estimates for women, and 1.13 [95% CI, 0.81-1.58] for men respectively with no significantly statistical heterogeneity among studies ( $I^2=45.89\%$ ,  $P=0.70$ ). We also conducted analyses that were stratified according to the region of the population. The pooled ORs was 0.75 [95% CI, 0.57-1.00] for Eastern Asia and 0.69 [95% CI, 0.36-1.34] for Western Asia. There was no significantly statistical heterogeneity among studies ( $I^2=59.79\%$ ,  $P=0.87$ ). The above subgroup meta-analyses are shown in **Figure S1** and summarized in **Table 3**.

### Qualitative assessment and publication bias

The NOS tool was used to conduct a qualitative assessment of the selected studies to review the quality of the studies and detect possible bias. Of the eight studies, five studies were at low risk of bias was 8-9 stars.<sup>24-27 30</sup> The three studies at medium risk (5-7 stars) mainly due to the bias from representativeness of cases or controls, control definition, and response rate.<sup>29 36 37</sup>( shown in **Table S3.**) The Funnel plot and Eggers statistical test indicated no evidence of publication bias in the studies included in the meta-analysis ( $P=0.338$ ) (**Figure S2.**)

## DISCUSSION

Colorectal cancer is one of the cancers with high morbidity and mortality in the world. The one-carbon metabolism pathway requires adequate Vitamin D, and this raises the possibility that Vitamin D may have an essential role in the risk of colorectal cancer. Many epidemiological studies from Europe and the United States believe that increasing the concentration of circulating Vitamin D can reduce the morbidity and mortality of colorectal cancer.<sup>38</sup> However, the association between blood circulating Vitamin D levels and risk of colorectal cancer in the Asian population is still under debate due to a lack of sufficient evidence. This systematic review highlights the inconsistencies among studies addressing the role of blood circulating Vitamin D and colorectal cancer risk in the Asian population. Our systematic review identified eight studies of 2916 cases and 6678 controls that addressed the relationship between blood circulating Vitamin D levels and colorectal cancer risk. This meta-analysis found that 25% reduced risk of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D levels (OR=0.75, 0.58-0.97) up to 36.5 ng/mL, which indicated higher blood circulating Vitamin D level had a significant inverse association with risk of colorectal cancer in Asian population. Our meta-analysis results showed that the negative correlation between Vitamin D and the risk of colorectal cancer is similar to that of European and American population studies<sup>11 12 21 22 39-42</sup> and consistent with the result of a meta-analysis from Ekmekcioglu C et al.<sup>13</sup>, which revealed a pooled relative risk (RR) of 0.62 (0.56–0.70) for colorectal cancer when comparing individuals with the highest category of 25(OH)D with those in the lowest.

Our results found a 16 ng/mL increment in blood circulating Vitamin D levels conferred an OR of 0.79 [95% CI, 0.64-0.97], which meant the risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in blood Vitamin D, and the reduction was statistically significant. Our study also suggested a linear dose-response relationship ( $P_{\text{non-linearity}} = 0.11$ ), which was consistent with several studies conducted in Western population revealing a similar protective dose-response association of blood circulating Vitamin D and colorectal cancer risk. For example, a meta-analysis reported a 26% lower risk of colorectal cancer per 10 ng/mL increment in blood circulating Vitamin D levels.<sup>11</sup> Most experts define Vitamin D deficiency as a Vitamin D level of less than 20 ng/mL.<sup>43 44</sup> Vitamin D concentration of 21 to 29 ng/mL can be considered to indicate a relative insufficiency of Vitamin D, and a level of 30 ng/mL or higher can be considered to indicate sufficient Vitamin D.<sup>45</sup> With the use of such definitions, it has been estimated that many people have Vitamin D deficiency or

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3 insufficiency.<sup>43 44</sup> Previous research has implied the association of Vitamin D deficiency with  
4 an increased incidence of bone fractures.<sup>46</sup> There have also been data showing Vitamin D  
5 deficiency to be associated with cancer,<sup>47-49</sup> diabetes,<sup>50</sup> cognitive impairment,<sup>51</sup> and all-cause  
6 mortality.<sup>52</sup> Among colorectal cancer patients, the prevalence of Vitamin D deficiency was  
7 much higher (nearly 90%) than among patients with other chronic diseases.<sup>53</sup> Humans obtain  
8 Vitamin D from exposure to sunlight, from natural diets, fortified diets, supplementation.<sup>46</sup> It  
9 is possible that the dose-response between Vitamin D concentrations and colorectal cancer  
10 risk is different between Asian and Western population due to ethnic, anthropometric,  
11 dietary, and environmental factors.<sup>12</sup> Lifestyle and diet can promote the development of  
12 early-onset colon lesions by regulating growth factors that interact with inflammatory  
13 pathways.<sup>41</sup> An association between Vitamin D status and reduced risk of colorectal cancer  
14 has been found in ethnically diverse populations.<sup>5</sup> Vitamin D interacts with calcium to  
15 enhance the reduction of colon cancer risk.<sup>54-56</sup> Studies have shown that Vitamin D and  
16 calcium may interact and that both are needed in reducing cancer risk.<sup>57</sup> However, even after  
17 adjusting for calcium intake in some studies,<sup>6 58</sup> Vitamin D was associated with a lower risk.  
18 The independent effects of Vitamin D are supported, but the combined effects of the Vitamin  
19 D and calcium may be greater than the sum of their independent effects.<sup>59</sup> Vitamin A has an  
20 antagonistic effect on Vitamin D<sup>58</sup> and taking both at the same time can lead to decreased  
21 calcium absorption. Still, Vitamin A is often combined with Vitamin D in supplements.  
22 Vitamin D supplementation significantly reduced total cancer mortality but did not reduce  
23 total cancer incidence.<sup>60</sup> Patients with low-risk prostate cancer under active surveillance may  
24 benefit from vitamin D3 supplementation at 4000 IU/d.<sup>61</sup> Among patients with metastatic  
25 colorectal cancer, addition of high-dose Vitamin D3, vs standard-dose Vitamin D3, to  
26 standard chemotherapy resulted in a difference in median progression-free survival that was  
27 not statistically significant, but with a significantly improved supportive effect.<sup>62</sup>  
28 Epidemiological evidence links the incidence of colorectal cancer to lifestyle, smoking,  
29 physical activity, alcohol consumption, and sleep.<sup>63</sup> It has also been linked to reduced fruit  
30 and vegetable consumption and increased consumption of red meat. Dairy products, fish and  
31 other foods and cooking methods also play an essential role.<sup>64</sup> In addition, some drugs such  
32 as non-steroidal anti-inflammatory drugs and cyclooxygenase inhibitors are also involved.<sup>65</sup>  
33 Women on estrogen therapy, for example, did not reduce their risk of colorectal cancer by  
34 taking Vitamin D and calcium supplements.<sup>66</sup> Increased dietary fiber intake reduces the risk  
35 of colorectal cancer, and obesity and low physical activity reduce plasma 25(OH)D  
36 concentration, thereby increasing the risk of colorectal cancer.<sup>42</sup> For every 1 kg/m<sup>2</sup> increase  
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3 in BMI of colorectal cancer patients, serum Vitamin D level decreased significantly (0.46  
4 ng/mL).<sup>67</sup> Most variation in Vitamin D levels usually comes from exposure to the sun, which  
5 is an essential source of Vitamin D for people who get more from fish, even in Japan.<sup>68</sup>  
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9 This meta-analytic comparison revealed a statistically significant beneficial effect of blood  
10 circulating Vitamin D for colorectal cancer. However, the diversity of the studies and the  
11 presence of moderate heterogeneity in our study ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ) in the overall  
12 analysis of blood circulating Vitamin D levels may preclude making meaningful conclusions  
13 from the pooled analysis because the pooled estimate may not reflect the true underlying  
14 effect. Much of the heterogeneity was not explained through subgroup analyses in our study.  
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20 The potential reasons for the heterogeneity in the strength of the association may include  
21 many factors. Firstly, the type of food consumption and vitamin supplements varied  
22 according to the specific dietary habits and lifestyle in each subregion of Asia. A systemic  
23 review studied correlations between various diet types, food or nutrients and colorectal  
24 cancer risk among Asian population, and suggested that red meats, processed meats,  
25 preserved foods, saturated/animal fats, cholesterol, high sugar foods, spicy foods, tubers or  
26 refined carbohydrates have been found to have a positive association with colorectal cancer  
27 risk.<sup>69</sup> Besides diet, other personal and lifestyle factors (e.g., exposure to sunlight, obesity,  
28 smoking and drinking habit) may alter the strength of the association and contribute to the  
29 heterogeneity of the association in Asian countries. In our meta-analysis, we had seven  
30 studies<sup>24-28 30 36</sup> from Eastern Asia and one study from Western Asia,<sup>29</sup> however the number  
31 of studies from these regions were imbalanced, yet we still present it in the subgroup  
32 analysis; Besides, with systematic search, we did not have available studies from Central  
33 Asia, Southern Asia, Southeastern Asia included in this meta-analysis, therefore we were  
34 cautious to interpret the subgroup analysis. Our study revealed no evidence of publication  
35 bias, and most of the studies included in our meta-analysis verified the diagnoses of  
36 colorectal cancer for cases. Histologic confirmation of cancer diagnoses for cases was an  
37 optimal validation for the case-control design in our meta-analysis.  
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52 Possible confounders for the association between colorectal cancer and blood circulating  
53 Vitamin D levels include sex, age, family history of colorectal cancer, smoking, alcohol  
54 drinking, body mass index, and diabetes. Most studies in our meta-analysis provided risk  
55 estimates that were adjusted for age,<sup>24 26-30 36</sup> sex,<sup>24 26 27 29 30 36</sup> body mass index,<sup>24-30 36</sup>  
56 smoking,<sup>24-30 36</sup> drinking,<sup>24-26 29 36</sup> physical activity,<sup>30</sup> alcohol consumption,<sup>24-30</sup> and family  
57 history of colorectal cancer;<sup>24 25 29</sup> fewer were adjusted for folate,<sup>24 37</sup> energy intake,<sup>24 27</sup>  
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3 hypertension,<sup>36</sup> vitamin D binding protein,<sup>36</sup> blood collection,<sup>24 27</sup> and adjusted for vitamin  
4 supplement use.<sup>24</sup> Therefore, for these studies, the observed reduced risk of colorectal cancer  
5 associated with a Vitamin D level is less likely to be confounded by these known  
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7 confounders.  
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10 Our analysis had the following limitations. First, the number of included studies is not  
11 sufficient to provide a robust estimate of association of blood circulating Vitamin D levels  
12 and colorectal cancer risk, so the analysis and results should be interpreted in the context of  
13 the limitations of the available data. Second, heterogeneous definitions of blood circulating  
14 Vitamin D categories were used across studies. The variability in definitions could limit  
15 comparability between studies. Third, our study included seven case-control studies; the  
16 study design implies that the measurement of blood circulating Vitamin D is measured in  
17 individuals already diagnosed with colorectal cancer. Therefore, results from this study  
18 design need to be interpreted cautiously because of the potential for reverse causation. Time  
19 of blood sampling in relation to outcome ascertainment also varied among studies. Such  
20 cross-sectional measurements may not accurately reflect an individual's Vitamin D status  
21 across time. Forth, some studies included in our meta-analysis did not adjust for some  
22 potentially relevant confounders which may have led to residual confounding and may  
23 explain some of the observed heterogeneity. Fifth, difference in the method used for  
24 measuring blood circulating Vitamin D levels may also be a source of heterogeneity between  
25 the included studies. Sixth, in the dose-response analysis, the literature selected listed the  
26 median Vitamin D value, instead of the original Vitamin D value, which could also lead to  
27 inaccurate results. Seventh, Although we conducted the investigation of quality levels of the  
28 observational studies in conducting this meta-analysis by the NOS quality assessment tool,  
29 Bae<sup>70</sup> suggested that it is more reasonable to control for quality level by performing  
30 subgroup analysis according to study design rather than by using high-quality based on the  
31 NOS tool. In this context, however, we did not perform a subgroup analysis according to  
32 study design since the included 8 studies were case-control and nested case-cohort studies.  
33 Finally, a recent meta-analysis investigated the association between blood circulating  
34 Vitamin D levels and survival of colorectal cancer and found that the pooled hazard ratios  
35 (95% confidence intervals) were 0.68 [0.55–0.85] and 0.67 [0.57–0.78] for overall and  
36 colorectal cancer-specific survival comparing highest versus lowest categories of blood  
37 Vitamin D, respectively.<sup>15</sup> However, our study did not explore the association of the blood  
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3 circulating Vitamin D levels and colorectal cancer mortality in Asian countries. In future  
4 studies, we would like to explore this association in Asian countries.  
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## 7 **CONCLUSION**

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10 The blood circulating Vitamin D is inversely associated with colorectal cancer prevalence in  
11 the Asian population. Our findings on the inverse association between blood circulating  
12 Vitamin D and the risk of colorectal cancer in Asian population suggest that Asian should  
13 improve their nutritional status and maintain a higher blood circulating Vitamin D level. This  
14 meta-analysis also provides valuable information for future research of association between  
15 blood circulating Vitamin D and colorectal cancer risk in Asian population. Further studies  
16 may focus on evaluating more detailed Vitamin D levels with colorectal cancer. A  
17 multinational, population-based study in Asian countries may resolve the issue of  
18 heterogeneity to generate detailed information on blood circulating Vitamin D levels and risk  
19 of colorectal cancer. In considering Vitamin D supplementation also associate with cancer  
20 incidence and mortality,<sup>60</sup> we may investigate these associations in the future studies.  
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## 31 **Authors Contributions**

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34 LZ: Study concept and design; acquisition of data; statistical analysis; interpretation of data;  
35 drafting and critical review of the manuscript for important intellectual content; approval of  
36 the final version of the manuscript.  
37

38 HZ: Interpretation of data; drafting and critical review of the manuscript for important  
39 intellectual content; approval of the final version of the manuscript.  
40

41 YZ: Interpretation of data; drafting and critical review of the manuscript for important  
42 intellectual content; approval of the final version of the manuscript.  
43  
44

45 CH: Drafting and critical review of the manuscript for important intellectual content;  
46 approval of the final version of the manuscript.  
47

48 AA: Drafting and critical review of the manuscript for important intellectual content;  
49 approval of the final version of the manuscript.  
50  
51

52 XQ: Interpretation of data; drafting and critical review of the manuscript for important  
53 intellectual content; approval of the final version of the manuscript.  
54  
55

56 PJ: Interpretation of data; drafting and critical review of the manuscript for important  
57 intellectual content; approval of the final version of the manuscript.  
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3 YJ: Study concept and design; acquisition of data interpretation of data; drafting and critical  
4 review of the manuscript for important intellectual content; approval of the final version of  
5 the manuscript.  
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8 ZQ: Study concept and design; acquisition of data; interpretation of data; drafting and critical  
9 review of the manuscript for important intellectual content; approval of the final version of  
10 the manuscript.  
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## Figure legends/captions

**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

**Figure 2.** Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D. F, female; M, male;

**Figure 3.** Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modeled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line,  $P_{\text{non-linearity}}=0.11$ ). Lines with short dashes represent the linear trend.

**Table 1.** Summary of characteristic for 8 studies included in the meta-analysis

Author(year)	Study design	Country	Study period	Outcome	Outcome ascertainment	Sex	Sample size (n); characteristics				Blood sample type	Vitamin D testing method	
							Case control	or	No.	Male (%)	Mean (SD)/Median age(range)		
Budhathoki et al. (2018) <sup>30</sup>	Nested case-cohort	Japan	1990-2009	colorectal cancer	NA	All	case control		637 4044	52 34	56.2(7.5) 53.7(7.9)	plasma	chemiluminescent enzyme immunoassay
Choi et al. (2015) <sup>37</sup>	case-control	Korea	2011-2012	colorectal adenoma	colonoscopy	All	case control		112 112	51 51	60.3 (5.3) 59.5 (5.4)	serum	chemiluminescent enzyme immunoassay
						Men	case control		57 57	100 100	NA NA	serum	
						Women	case control		55 55	0 0	NA NA	serum	
							case control		55 55	0 0	NA NA		
Hong et al. (2012) <sup>26</sup>	case-control	Korea	2009-2010	colorectal adenoma	colonoscopy	All	case control		143 143	68 68	58.7 (6.0) 58.7 (6.0)	serum	direct competitive electrochemiluminescence immunoassay
Otani et al. (2007) <sup>24</sup>	case-control	Japan	1990-2003	colorectal cancer	pathologically confirmed	All	case control		323 621	52 52	NA NA	plasma	competitive protein-binding assay
						Men	case control		163 324	100 100	56.9 56.9		
						Women	case control		160 297	0 0	56.5 56.4		
							case control		297 297	0 0	56.4 56.4		
Takahashi et al. (2010) <sup>25</sup>	case-control	Japan	1997-2004	colorectal adenoma	colonoscopy	Men	case control		656 648	100 100	52.0 (1.4) 51.8 (1.5)	plasma or serum	radioimmunoassay method
Yamaji et al. (2012) <sup>27</sup>	case-control	Japan	2004-2005	colorectal adenoma	colonoscopy	All	case control		737 703	67 65	NA NA	plasma	radioimmunoassay method
Ying et al. (2015) <sup>36</sup>	case-control	China	2010-2014	colorectal cancer	colonoscopy	All	case control		212 212	NA NA	63(56-73) 63(57-73)	plasma	direct competitive enzyme-linked immunosorbent assay
						cases control		96 195	67 54	57.8(10.0) 51.2(12.9)	serum	NA	

**Abbreviations:** SD, Standard deviation; NA, Not available; All\*: all participants, including men and women

**Table 2.** Summary of risk estimate for 8 studies included in the meta-analysis

Author(year)	sex	Vitamin D level cut points (ng/ml)	Vitamin D medium level (ng/ml)	Number of cases	Number of controls	N	OR	LCI	UCI	Adjusted variables
Budhathoki et al. (2018) <sup>30</sup>	All	1st Q, 12.5-16.5 ng/ml	14.7	134	1004	1138	1			age, sex, BMI, smoking, alcohol use, physical activity, family history of cancer, and history of diabetes
		2nd Q, 17.6-21.6 ng/ml	19.9	165	1000	1165	1.08	0.84	1.39	
		3rd Q, 21.2-25.6 ng/ml	21.3	160	1016	1176	0.96	0.74	1.26	
		4th Q, 25.8-33.0 ng/ml	26.5	178	1024	1202	0.95	0.73	1.23	
Choi et al. (2015) <sup>37</sup>	All	1st Q, 10.3 ng/ml	10.3	37	28	65	1			age, sex, BMI, alcohol drinking, smoking status, folate intake; women: additional menopausal status and hormone replacement use
		2nd Q, 14.3 ng/ml	14.3	26	28	54	0.72	0.31	1.66	
		3rd Q, 17.9 ng/ml	17.9	28	28	56	0.73	0.29	1.82	
		4th Q, 24.2 ng/ml	24.2	21	28	49	0.49	0.19	1.27	
	Men	1st Q, 11.1 ng/ml	11.1	15	14	29	1			
		2nd Q, 14.6 ng/ml	14.6	13	14	27	1.31	0.36	4.82	
		3rd Q, 17.7 ng/ml	17.7	17	15	32	1.26	0.4	8.12	
		4th Q, 22.7 ng/ml	22.7	12	14	26	1.8	0.19	8.12	
	Women	1st Q, 9.5 ng/ml	9.5	16	14	30	1			
		2nd Q, 13.5 ng/ml	13.5	22	14	36	0.89	0.26	3.1	
		3rd Q, 18.4 ng/ml	18.4	8	14	22	0.54	0.13	2.16	
		4th Q, 25.4 ng/ml	25.4	9	13	22	0.22	0.04	1.15	
Hong et al. (2012) <sup>26</sup>	All	1st Q, <14.3 ng/ml	10.1	31	42	73	1			age, sex, BMI, smoking, alcohol drinking, physical activity, corrected calcium level
		2nd Q, 14.3-18.5 ng/ml	16.4	29	42	71	0.87	0.43	1.74	
		3rd Q, 18.6-23.8 ng/ml	21.2	41	31	72	0.4	0.2	0.82	
		4th Q, >=23.9 ng/ml	29.1	42	28	70	0.38	0.18	0.8	
Otani et al. (2007) <sup>24</sup>	Men	1st Q, <22.9 ng/ml	18.3	43	74	117	1			age, sex, BMI, smoking, alcohol consumption, physical exercise, Vitamin supplement use, family history of colorectal cancer, total energy intake, dietary fiber intake, folate intake, calcium intake, Vitamin D intake, n-3 fatty acid intake, red meat intake, fish intake
		2nd Q, 22.9-27.5 ng/ml	25.2	40	85	125	0.76	0.42	1.4	
		3rd Q, 27.6-32.0 ng/ml	29.8	36	85	121	0.76	0.39	1.5	
		4th Q, >32.1 ng/ml	36.5	44	80	124	0.73	0.35	1.5	
	Women	1st Q, <18.7 ng/ml	15.2	41	77	118	1			
		2nd Q, 18.7-22.2 ng/ml	20.5	34	73	107	1	0.55	1.9	
		3rd Q, 22.3-26.9 ng/ml	24.6	44	71	115	1.2	0.65	2.3	
		4th Q, >27.0 ng/ml	31.6	41	76	117	1.1	0.5	2.3	
Takahashi et al. (2010) <sup>25</sup>	Men	1st Q, <22 ng/ml	20	128	142	270	1			BMI, hospital, rank in the Self Defense Forces, smoking, alcohol drinking, parental history of colorectal cancer, physical activity, type of blood sample, the month of blood drawing
		2nd Q, 23-25 ng/ml	24	162	156	318	1.21	0.86	1.7	
		3rd Q, 26-29 ng/ml	27.5	223	208	431	1.21	0.87	1.69	
		4th Q, >30 ng/ml	33	143	142	285	1.25	0.85	1.84	
Yamaji et al. (2012) <sup>27</sup>	All	1st Q, 14-19 ng/ml	16.5	145	129	274	1			age, sex, BMI, screening periods, the season of blood collection, smoking, alcohol drinking, family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, height, daily energy intake
		2nd Q, 20-23 ng/ml	21.5	132	128	260	0.86	0.6	1.24	
		3rd Q, 24-26 ng/ml	25	157	145	302	0.91	0.64	1.29	
		4th Q, 27-31 ng/ml	29	175	144	319	1.03	0.73	1.46	
		5th Q, 31-34 ng/ml	32.5	128	157	285	0.64	0.45	0.92	
Ying et al. (2015) <sup>36</sup>	All	1st Q, <7.29 ng/ml	3.65	80	53	133	1			age, sex, BMI, smoking, drinking, history of diabetes, hypertension, vitamin D binding protein
		2nd Q, 7.29-14.61 ng/ml	10.95	49	53	102	0.62	0.35	1.12	
		3rd Q, 14.61-28.84 ng/ml	21.73	46	53	99	0.67	0.38	1.20	
		4th Q, >=28.84 ng/ml	35.96	37	53	90	0.53	0.29	0.98	
Yurekli et al.(2015) <sup>29</sup>	All	1st Q, <8.6 ng/ml	7.1	24	48	72	1			age, sex, BMI, smoking, alcohol intake
		2nd Q, 8.6-10.1 ng/ml	9.35	20	53	73	0.48	0.24	0.98	
		3rd Q, 10.1-14.5 ng/ml	12.3	25	48	73	0.51	0.25	1.03	
		4th Q, >=14.5 ng/ml	18.9	27	46	73	0.69	0.36	1.36	

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3 **Abbreviations:** NA, Not available; OR: odds ratio; LCI: 95% CI's lower confidence interval; UCI: 95% CI's upper confidence interval; BMI: body mass  
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5 All\*: all participants, including men and women  
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**Table 3.** Summary of subgroup analysis for the associations of blood circulating Vitamin D and the risk colorectal cancer and adenoma by year of publication, outcome, participants sex, the subregion of Asia, and blood sample type.

Subgroup analysis	Number of estimates	Random effect The summary OR (95% CI)	Ratio of ORs (95% CI)	$I^2$ (%)	$P$ value
<u>Outcome</u>					
Colorectal adenoma	4	0.67 (0.40-1.14)			
Colorectal cancer	4	0.83 (0.66-1.06)	1.11(0.54-2.28)	59.48	0.73
<u>Sex</u>					
Women	2	0.59 (0.13-2.74)			
Men	3	1.13(0.81-1.58)	1.46 (0.03-63.5)	45.89	0.70
<u>Subregion</u>					
Eastern Asia	7	0.75 (0.57-1.00)			
Western Asia	1	0.69 (0.36-1.34)	0.92 (0.28-2.99)	59.79	0.87
<u>Blood sample type</u>					
Serum	3	0.52 (0.34-0.80)			
Plasma	4	0.77 (0.59-1.00)	1.49 (0.73-3.03)	22.67	0.21
<u>Range</u>					
≤15 ng/mL	4	0.93 (0.70-1.25)			
>15 ng/mL	4	0.62 (0.47-0.83)	0.65(0.39-1.07)	28.48	0.08

Abbreviations: OR, Odds ratio;  $I^2$ , Percent residual variation due to heterogeneity; CI, Confidence interval.  
\* $I^2$  and  $P$  value related to subgroup differences.

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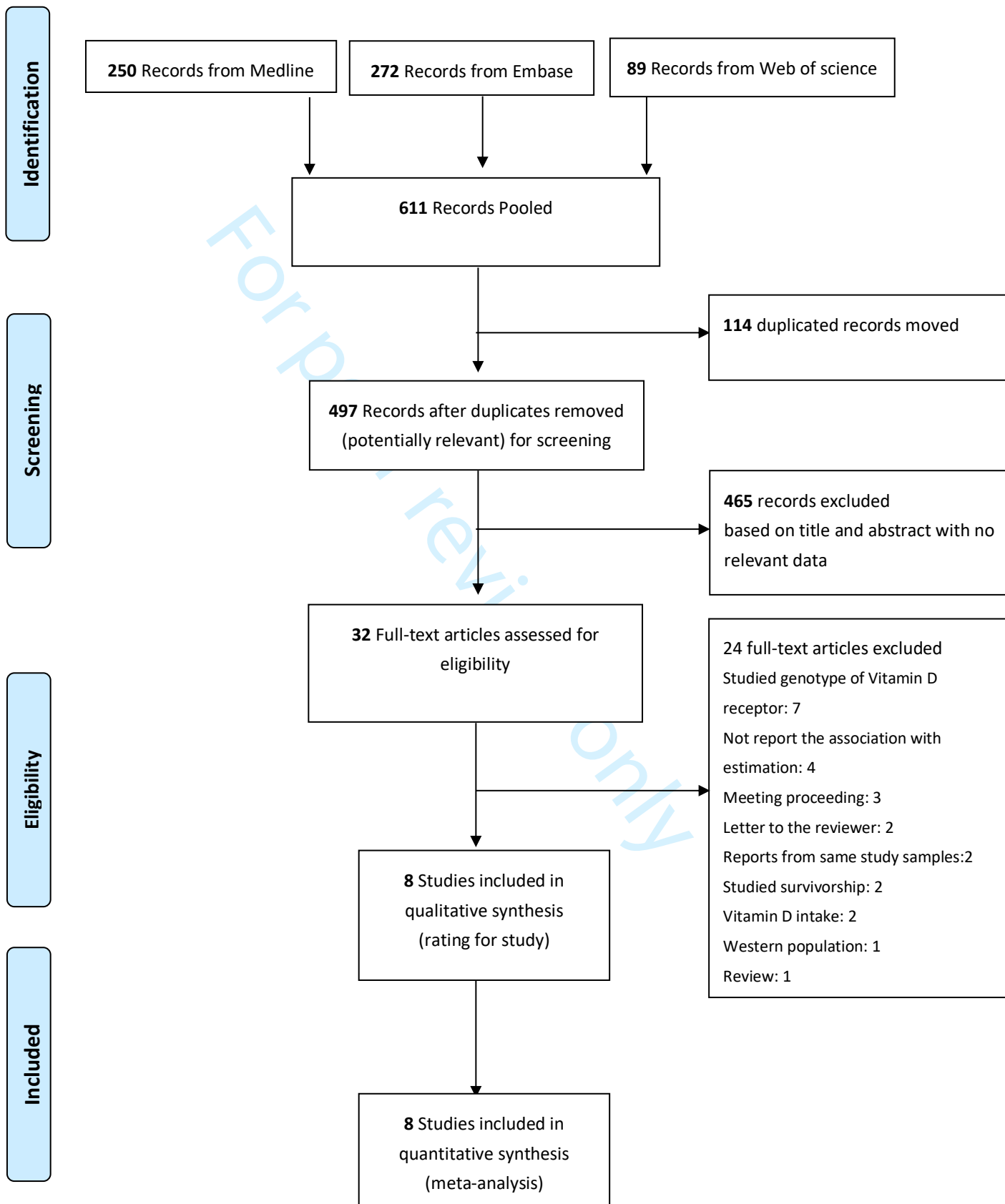
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**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

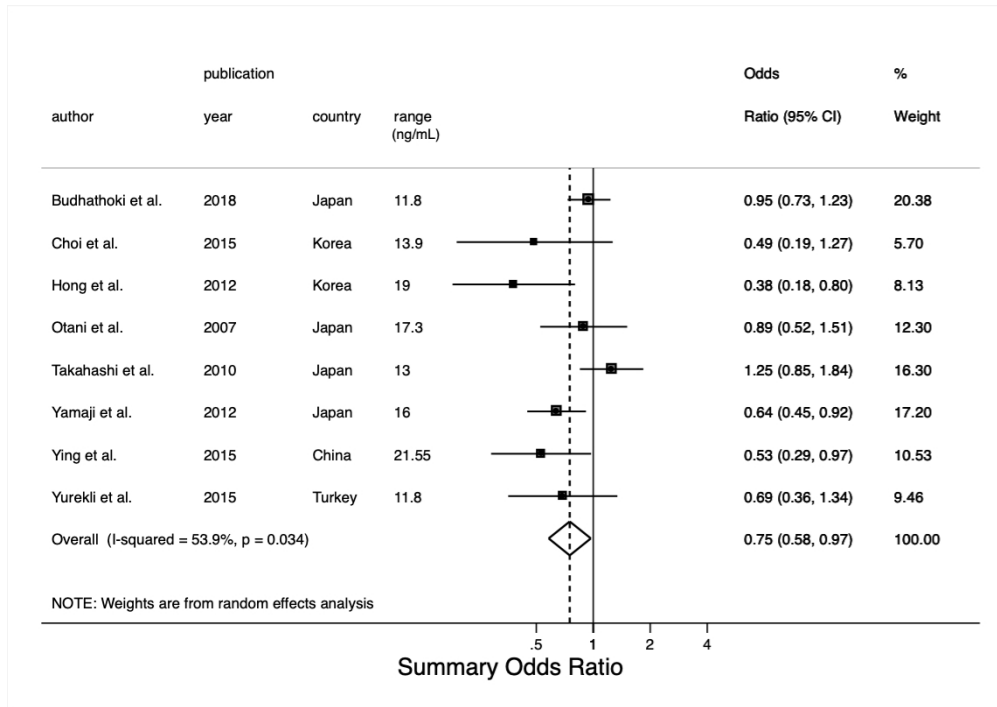


Figure 2. Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D.

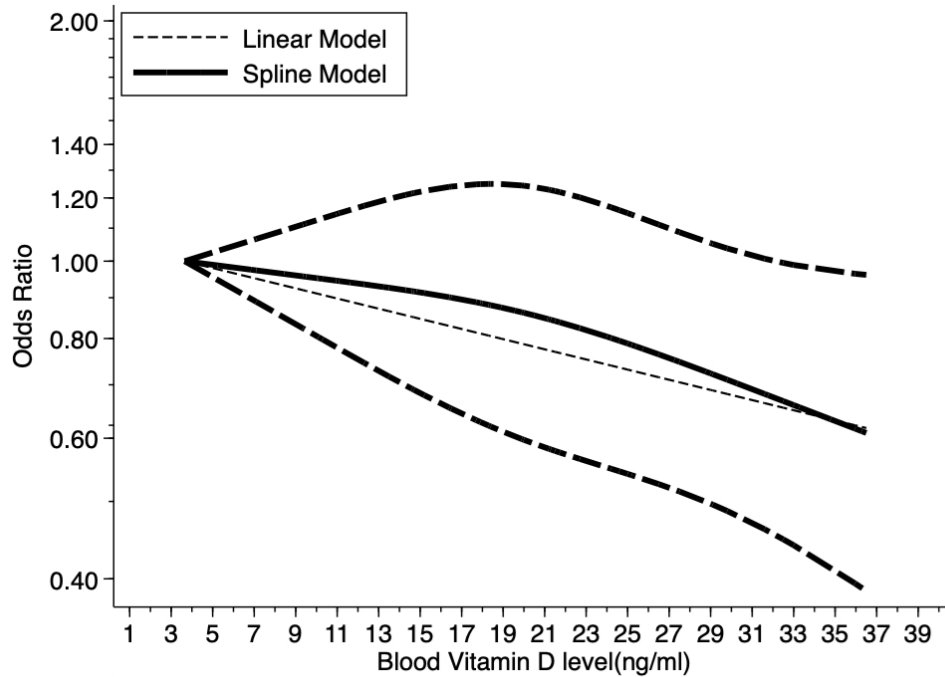


Figure 3. Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modeled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line,  $P_{\text{non-linearity}}=0.11$ ). Lines with short dashes represent the linear trend.



## Supplementary Material

**Table S1.** Literature search results from Medline, Embase, and Web of Science.

	Medline	Results
1	exp colorectal neoplasms/	187315
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	2688871
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	80293
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1074938
5	1 or 2	2690742
6	3 and 4 and 5	258
7	limit 6 to English language	250

	Embase	Results
1	exp colorectal neoplasms/	26250
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1075151
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	137427
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or	1562807

	Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	
5	1 or 2	1075151
6	3 and 4 and 5	279
7	limit 6 to English language	272

	Web of science	Results
1	TS= (colorectal neoplasm or colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*)	460,541
2	TS= (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D)	99,758
3	TS=(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen)	2,184,572
4	#1 AND #2 AND #3	106
5	(#4) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)	89

**Table S2.** List and details of the excluded studies from the meta-analysis

Reason for exclusion	Author	Year	Study title	Journal
Studied genotype of Vitamin D receptor(n=7)	Wong HL, et al.	2003	Vitamin D receptor start codon polymorphism and colorectal cancer risk: Effect modification by dietary calcium and fat in Singapore Chinese	Carcinogenesis
	Li C, et al.	2009	Vitamin D receptor gene polymorphisms and the risk of colorectal cancer in a Chinese population	Digestive Diseases and Sciences
	Kang JW et al.	2015	Role of vitamin D and local vitamin D receptor expression in colon carcinogenesis	Journal of Gastroenterology and Hepatology
	Takehige N, et al.	2015	Associations between vitamin D receptor (VDR) gene polymorphisms and colorectal cancer risk and effect modifications of dietary calcium and vitamin D in a Japanese population	Asian Pac J Cancer Prev
	Aumpansub P, et al.	2016	Strong association of single nucleotide polymorphisms of vitamin D receptor gene, BSML, and colorectal cancer in Asian population	Gastroenterology
	Budhathoki S, et al.	2016	Vitamin D receptor gene polymorphism and the risk of colorectal cancer: A nested case-control study	PLoS ONE
	Gong C, et al.	2017	Dietary factors and polymorphisms in vitamin D metabolism genes: the risk and prognosis of colorectal cancer in northeast China	Scientific Reports
Not report the association with estimation(n=4)	Mizoue T, et al.	2005	Dietary patterns and colorectal adenomas in Japanese men - The self-defense forces health study.	American Journal of Epidemiology
	Atoum MF, et al.	2014	Association between circulating vitamin D, the TaqI vitamin D receptor gene polymorphism and colorectal cancer risk among Jordanians. Asian Pacific journal of cancer prevention	Asian Pacific Journal of Cancer Prevention
	Kumagai Y, et al.	2014	Dietary patterns and colorectal cancer risk in Japan: the Ohsaki Cohort Study.	Cancer Causes & Control
	Hessami Arani S, et al.	2017	Rising rates of colorectal cancer among younger iranians: Is diet to blame?	Current Oncology
Meeting proceeding (n=3)	Grant WB, et al.	2011	Ecological study findings regarding vitamin D and cancer.	Anticancer Research
	Li K, et al.	2014	The association between serum vitamin D concentrations and colorectal cancer.	Clinical Chemistry and Laboratory Medicine
	Ozer C, et al.	2015	The relationship between serum 25-hydroxy vitamin D levels and insulin resistance in breast and colon cancer.	Clinical Chemistry and Laboratory Medicine
Letter to the reviewer (n=2)	Dunnigan MG, et al.	1990	Serum 25-hydroxyvitamin D and colon cancer.	Lancet
	Qu B, et al.	2017	Role of Circulating and Supplemental Calcium and Vitamin D in the Occurrence and Development of Colorectal Adenoma or Colorectal Cancer.	J Clin Gastroenterol
Reports from same study sample (n=2)	Byeon JS, et al.	2007	Colorectal neoplasm in asymptomatic Asians: a prospective multinational multicenter colonoscopy survey.	Gastrointestinal Endoscopy
	Shin A, et al.	2011	Site-specific risk factors for colorectal cancer in a korean population.	PLoS ONE
Studied survivorship (n=2)	Wesa KM, et al.	2015	Serum 25-hydroxy vitamin D and survival in advanced colorectal cancer: a retrospective analysis.	Nutr Cancer
	Woo KW, et al.	2015	Vitamin D deficiency in Hong Kong advanced cancer patients: Result of first 50 patients.	Annals of Oncology
Vitamin D intake (n=2)	Mizoue T, et al.	2008	Calcium, dairy foods, vitamin D, and colorectal cancer risk: The Fukuoka colorectal cancer study.	Cancer Epidemiology Biomarkers and Prevention
	Ishihara J, et al.	2008	Dietary calcium, vitamin D, and the risk of colorectal cancer.	American Journal of Clinical Nutrition
Western population (n=1)	Sy AM, et al.	2013	Association between serum vitamin D levels and colonic carcinomatous polyps.	Journal of Gastrointestinal Cancer
Review (n=1)	Grant WB, et al.	2009	Ecological Studies Of Ultraviolet B, Vitamin D And Cancer Since 2000.	Annals of Epidemiology

**Table S3.** Newcastle-Ottawa Scale for assessing the quality of studies in the systematic review

Author (Year)	Country	Region	Selection				Comparability		Exposure			Total Score
			S1	S2	S3	S4	C1	C2	E1	E2	E3	
Budhathoki et al. (2018)[1]	Japan	Eastern Asian	★	★	★	★	★	★	★	★		8
Choi et al. (2015)[2]	Korea	Eastern Asian	★				★	★	★	★		5
Hong et al. (2012)[3]	Korea	Eastern Asian	★	★	★	★	★	★	★	★		8
Otani et al. (2007)[4]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Takahashi et al. (2010)[5]	Japan	Eastern Asian	★	★	★	★		★	★	★	★	8
Yamaji et al. (2012)[6]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Ying et al. (2015)[7]	China	Eastern Asian	★			★	★	★	★	★	★	7
Yurekli et al.(2015)[8]	Turkey	Western Asian	★				★	★	★	★		5

**Guidelines for review***Selection:*

S1, Case definition adequacy: ★a) requires independent validation (>1 person/record/time/process to extract information or reference to primary record sources such as colonoscopy or medical/hospital records); b) record linkage or self-report with no reference to primary record; c) no description

S2, Representativeness of the cases: ★a) consecutive or representative series of cases; b) potential for selection biases or not stated

S3, Selection of controls: ★a) community controls; b) hospital controls, within same community as cases; c) no description

S4, Definition of controls: ★a) no history of colorectal cancer or adenoma; b) no description of the source

*Comparability:*

C1, ★ Study adjusted for confounder, such as age and sex (the most important factors);

C2, ★ Study adjusted for any additional confounders (1> additional factors, e.g., BMI, drinking or smoking)

*Exposure:*

E1, Ascertainment of exposure: ★a) secure record (e.g., medical records); ★b) structured interview were blind to case/control status; c) interview not blinded to case/control status; d) written self-report or medical record only; e) no description

E2, Same method of ascertainment for cases and controls: ★a) yes; b) no

E3, Non-response rate: ★a) the same rate for both groups; b) non-respondents described; c) rate different and no designation

**Figure S1.** Subgroup meta-analysis, including stratified by outcome (colorectal cancer or colorectal adenoma, **S1A**), sex (women, men or both, **S1B**), sample type (serum or plasma, **S1C**), range ( $\leq 15$  or  $>15$  ng/mL **S1D**), subregion (Eastern Asia or Western Asia, **S1E**).

**Figure S1A**

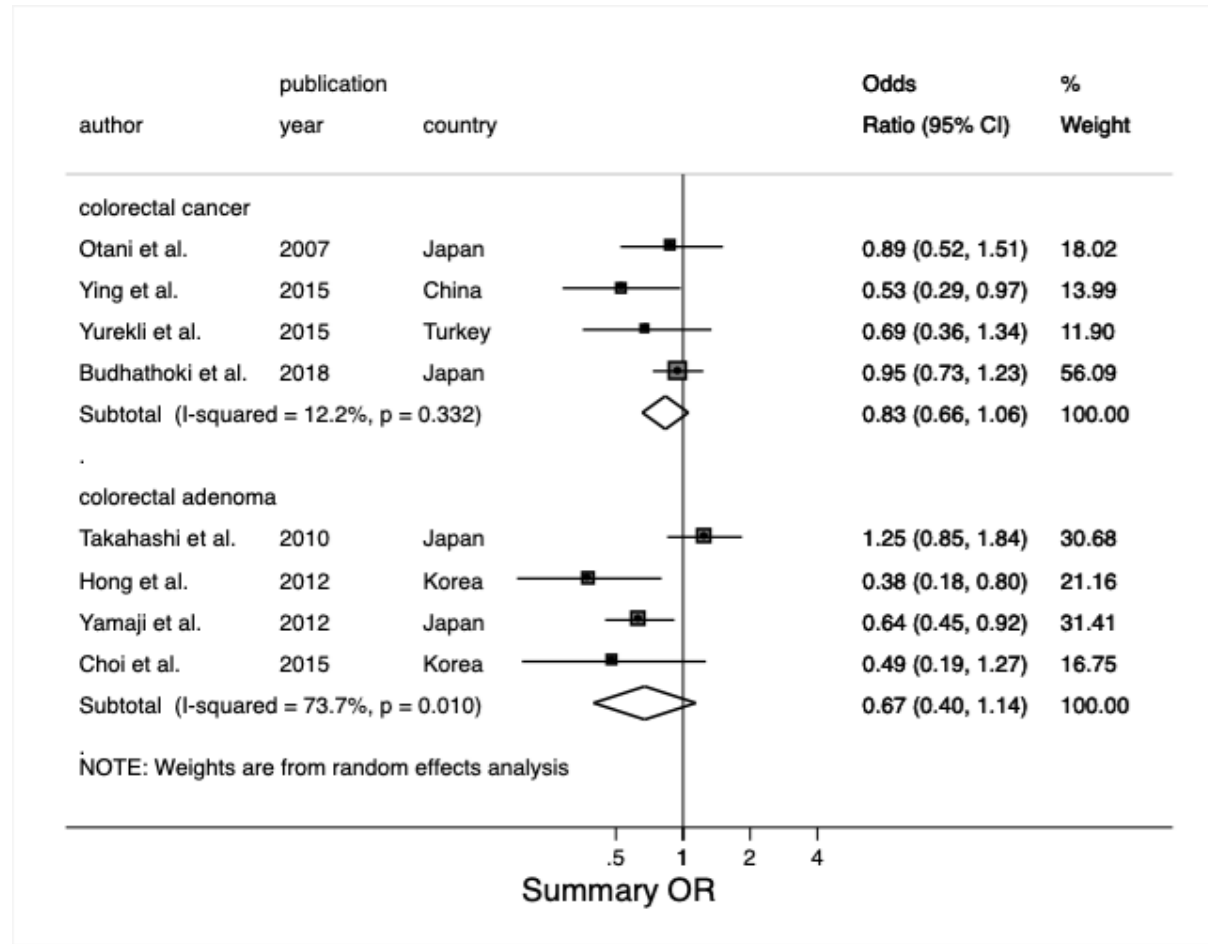


Figure S1B

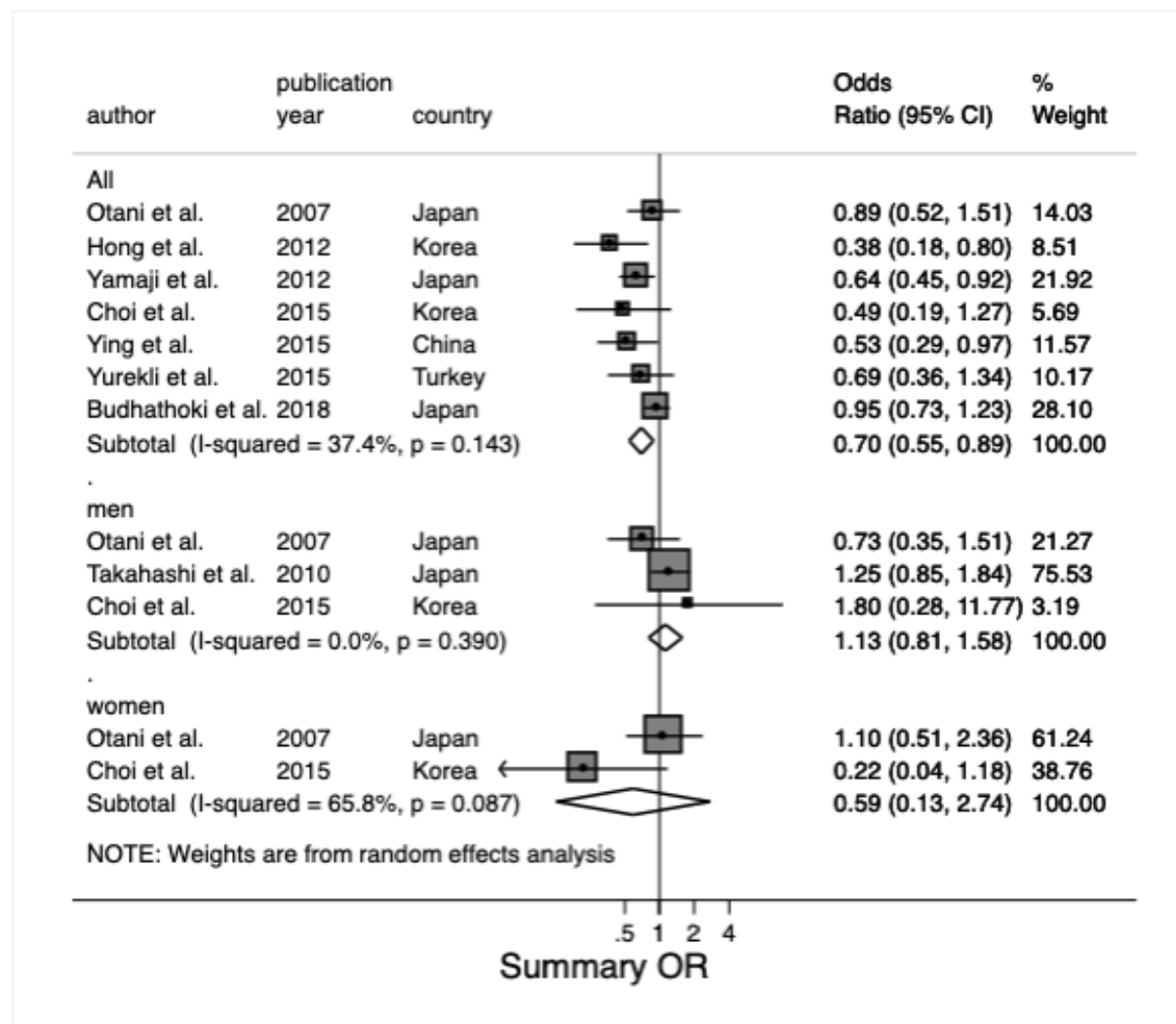
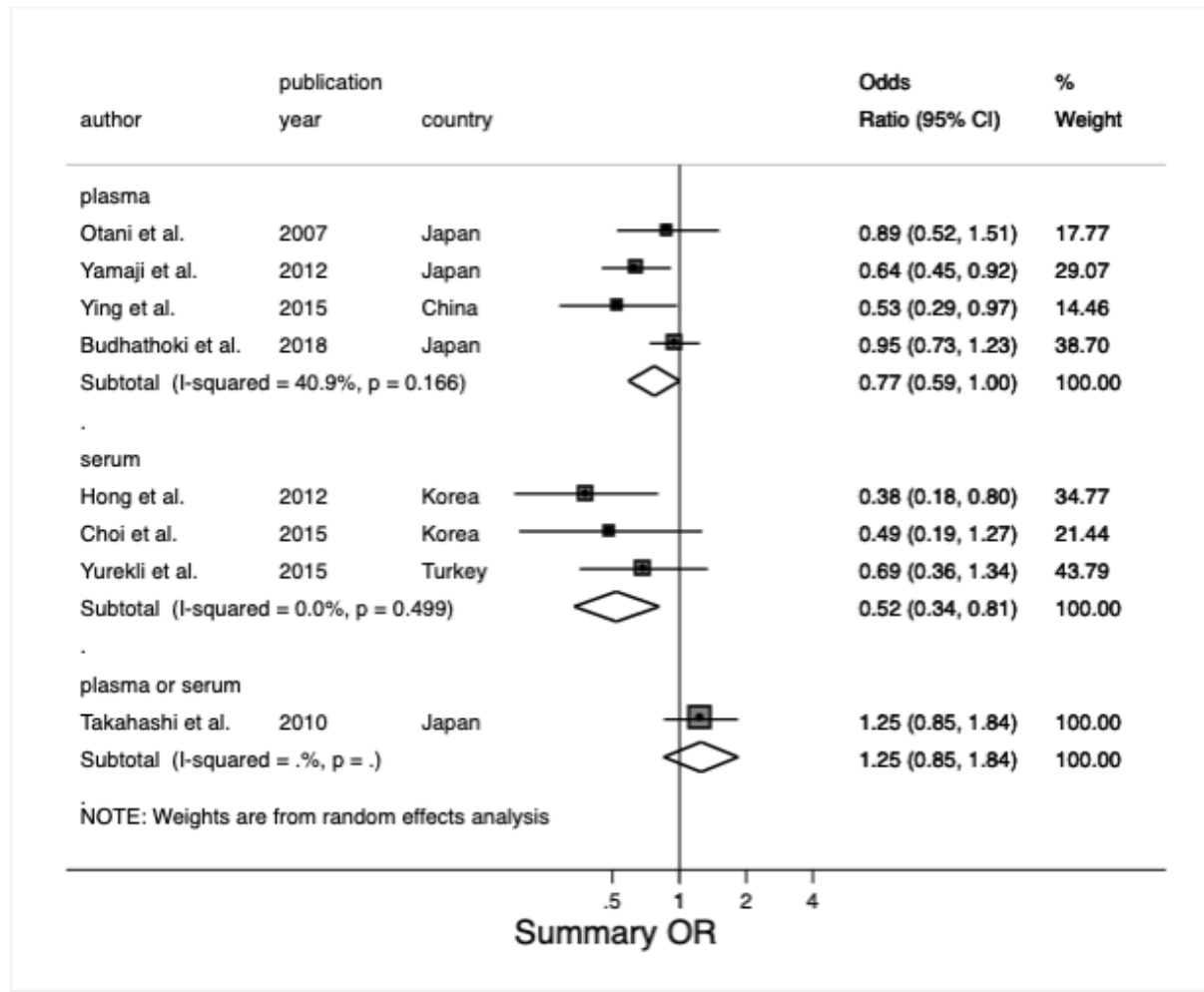


Figure S1C



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Figure S1D

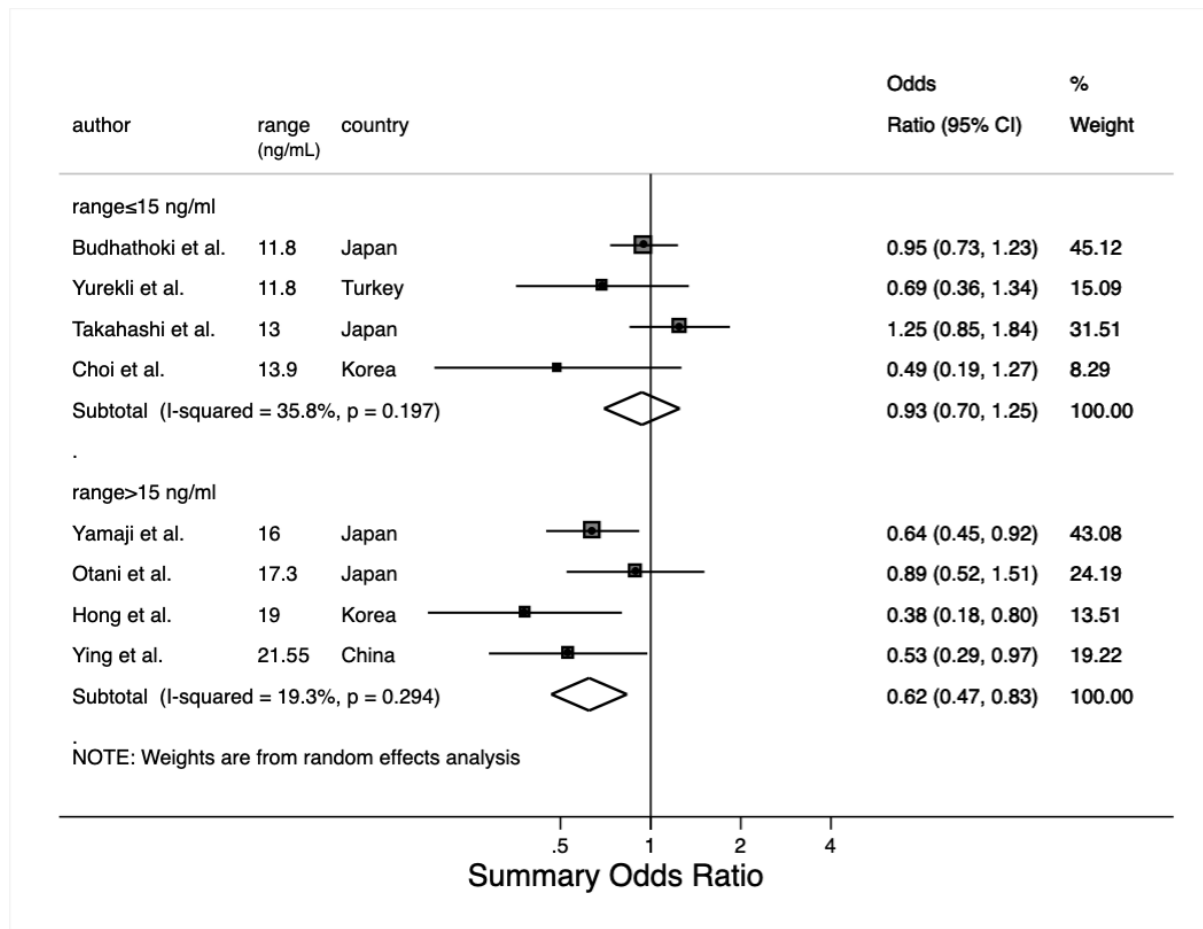
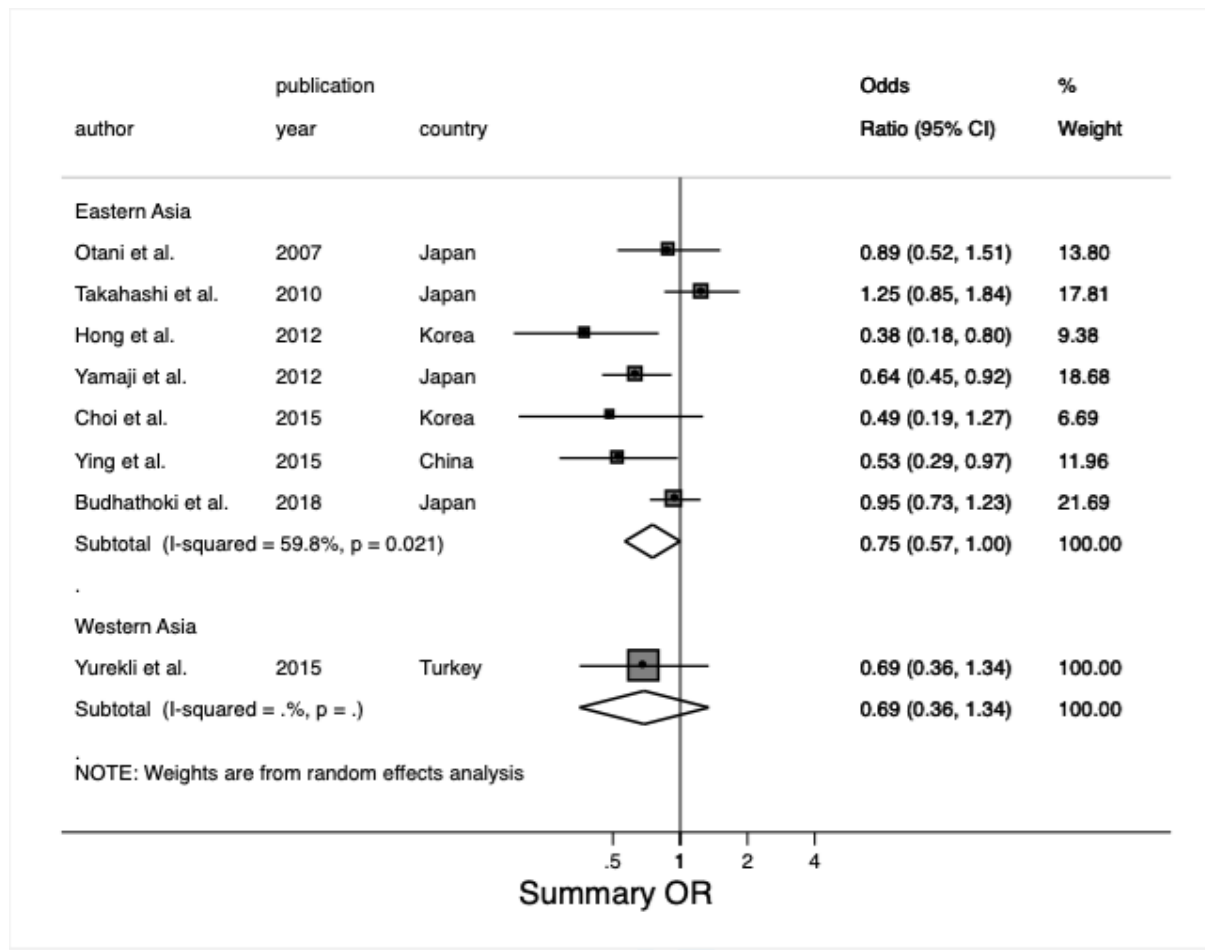
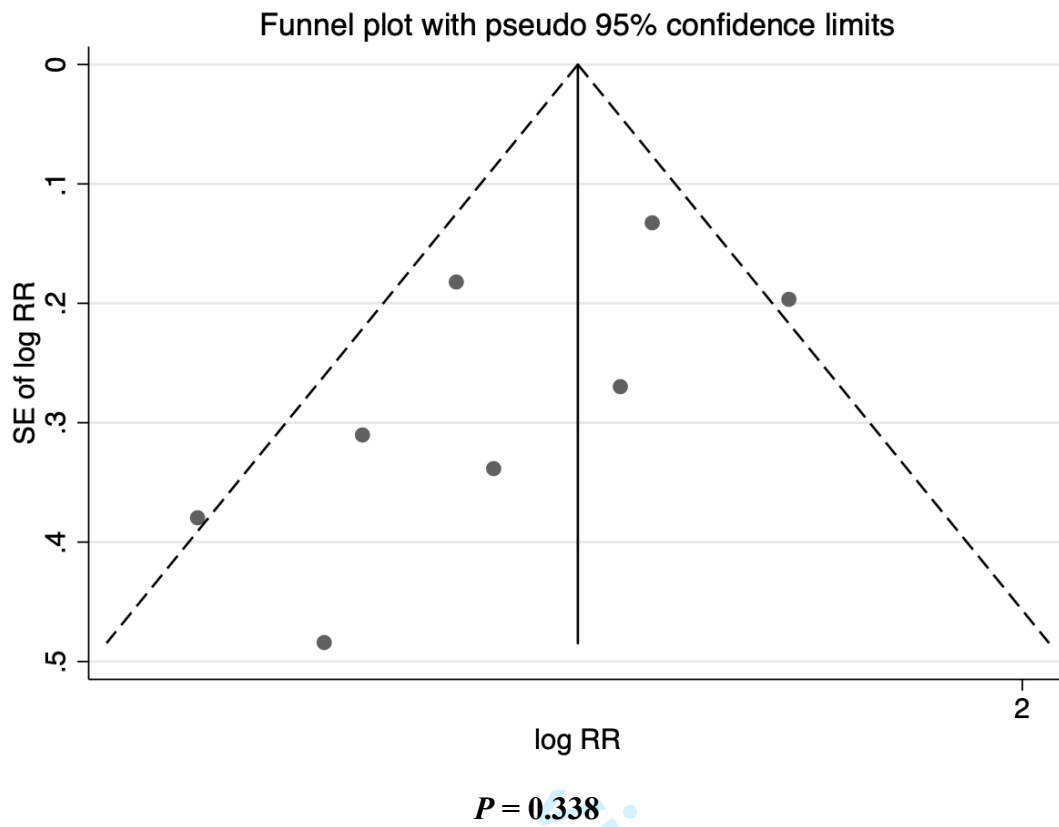




Figure S1E



**Figure S2.** Funnel plot for all studies included in the meta-analysis and between blood circulating Vitamin D and the colorectal cancer risk in Asian countries





# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 6
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Page 5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Page 7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I <sup>2</sup> ) for each meta-analysis.	Page 9



# PRISMA 2009 Checklist

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 10
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 11 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 11, Table 1 and Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 11 and Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 11 and 12
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 12 and 13
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 14 to Page 16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 18
<b>FUNDING</b>			
For peer review only - <a href="http://bmjopen.bmj.com/site/about/guidelines.xhtml">http://bmjopen.bmj.com/site/about/guidelines.xhtml</a>			



# PRISMA 2009 Checklist

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# BMJ Open

## Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

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# Association Between Blood Circulating Vitamin D and Colorectal Cancer Risk in Asian Countries: A Systematic Review and Dose-Response Meta-analysis

Lin Zhang,<sup>1,2,3,4,12</sup> Huachun Zou,<sup>5,6,7</sup> Yang Zhao,<sup>8</sup> Chunlei Hu,<sup>9</sup> Adejare (Jay) Atanda,<sup>10</sup> Xuzhen Qin,<sup>1</sup> Peng Jia,<sup>11,12</sup> Yu Jiang,<sup>2</sup> Zhihong Qi<sup>1</sup>

<sup>1</sup> Clinical Laboratory, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>2</sup> Peking Union Medical College School of Public Health, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China.

<sup>3</sup> Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, Victoria 3100, Australia

<sup>4</sup> The University of Melbourne Centre for Cancer Research, Victorian Comprehensive Cancer Centre, Parkville, Victoria 3100, Australia

<sup>5</sup> School of Public Health (Shenzhen), Sun Yat-sen University, Shenzhen 518107, Guangdong, China

<sup>6</sup> School of Public Health, Sun Yat-sen University, Guangzhou 510085, Guangdong, China

<sup>7</sup> Kirby Institute, University of New South Wales, Sydney 2052, NSW, Australia

<sup>8</sup> School of Population and Global Health, The University of Melbourne & WHO Collaborating Centre on Implementation Research for Prevention & Control of NCDs, Parkville, Victoria 3100, Australia

<sup>9</sup> Department of Gastrointestinal Surgery, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, China

<sup>10</sup> School of Community Health and Policy, Morgan State University, Baltimore, Maryland, USA

<sup>11</sup> Faculty of Geo-Information Science and Earth Observation (ITC), University of Twente, Enschede 7500, The Netherlands

<sup>12</sup> International Initiative on Spatial Lifecourse Epidemiology (ISLE)

## \*Correspondence to:

Prof. Zhihong Qi

Clinical Laboratory, Peking Union Medical College Hospital  
Chinese Academy of Medical Sciences & Peking Union Medical College  
Shuai Fu Yuan 1#, DongCheng District, Beijing 100730, China

Tel.: + 86 10 69159740

Email: cizeng1010@sina.com

Prof. Yu Jiang

Chinese Academy of Medical Sciences & Peking Union Medical College,  
DongDan SanTiao 9, DongCheng District, Beijing 100730, China

Tel.: + 86 10 65105916

Email: wingedsky@gmail.com

**Transparency declaration**

The Corresponding Authors affirm that this manuscript is an accurate account of the study proposed with no important aspects omitted, and that any discrepancies from the study as planned and registered will be explained upon the completion of the proposed study.

**Ethical approval**

Ethics approval is not required for this study because it is a systematic review.

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**Conflict of interest**

The authors have declared that no competing interests exist.

**Statement of independence of researchers form funders**

Not applicable.

**Patient involvement**

No patients were involved in the design or analysis of this study.

**Data sharing statement**

All data relevant to the study are included in the article or uploaded as supplementary information.



## ABSTRACT

### Objectives

To assess the association between blood circulating Vitamin D levels and colorectal cancer risk in the Asian population.

### Design

This is a systematic review and dose-response meta-analysis of observational studies that investigated the relationship between blood circulating Vitamin D levels and colorectal cancer risk in the Asian population.

### Data Sources

Relevant studies were identified through a literature search in MEDLINE, EMBASE, and Web of Science from January 1980 to 31 January 2019. Eligibility criteria: original studies published in peer-reviewed journals investigating the association between blood circulating Vitamin D levels and the risk of colorectal cancer and/or adenoma in Asian countries.

### Data extraction and synthesis:

Two authors independently extracted data and assessed the quality of included studies. Study-specific ORs were pooled using a random-effects model. A dose-response meta-analysis was performed with generalized least squares regression. We applied the Newcastle-Ottawa Scale quality assessment to evaluate the quality of the selected studies.

### Results

The eight included studies encompassed a total of 2,916 cases and 6,678 controls. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D levels was 0.75 [95% CI, 0.58-0.97] up to 36.5 ng/mL in the Asian population. There was heterogeneity among the studies ( $I^2=53.9%$ ,  $P_{\text{heterogeneity}}=0.034$ ). The dose-response meta-analysis indicated a significant linear relationship ( $P_{\text{non-linearity}}=0.11$ ). An increment of 16 ng/mL in blood circulating Vitamin D level corresponded to an OR of 0.79 [95% CI, 0.64-0.97].

### Conclusions

The results of this meta-analysis indicate that blood circulating Vitamin D level is associated with decreased risk of colorectal cancer in Asian countries. The dose-response meta-analysis shows that the strength of this association among the Asian population is similar to that among the Western population. Our study suggests that the Asian population should improve nutritional status and maintain a higher level of blood circulating Vitamin D.

#### Strengths and limitations of this study

- Our study seeks to extend previous work by including a number of new studies and by distinguishing the Asian population explicitly.
- The number of included studies is not sufficient to provide a robust estimate, so the results should be interpreted in the context of the limitations of the available data.
- Heterogeneous definitions of blood circulating Vitamin D categories were used across studies. The variability in definitions could limit comparability between studies.
- Our study included seven case-control studies; the study design implies that the measurement of blood circulating Vitamin D is measured in individuals already diagnosed with colorectal cancer. Results from case-control studies need to be interpreted cautiously because of the potential for reverse causation.
- Time of blood sampling in relation to outcome ascertainment also varied among studies. Such cross-sectional measurements may not accurately reflect an individual's Vitamin D status across time.

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## INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer and second in terms of mortality. With over 1.8 million new cases, and 881,000 deaths worldwide in 2018, it accounts for about 1 in 10 cancer cases and deaths.<sup>1</sup> Some Asian countries where the incidence of colorectal cancer was historically low, such as Japan, Israel, Singapore, China, and the Philippines, have experienced rising incidence rates over the past decades. In 2012, Japan (Miyagi Prefecture Cancer Registry) presented the highest colorectal cancer incidence in the world for men (62 per 100,000 persons) and women (37 per 100,000 persons).<sup>2</sup> Observational studies have identified several risk factors associated with an increased incidence of colorectal cancer including lifestyle factors (e.g., obesity, physical inactivity, smoking, and heavy alcohol use) and non-modifiable factors (e.g., aging, personal and family history of colorectal cancer or adenoma).<sup>3</sup> Other observational studies conducted in Western countries suggest blood circulating 25-hydroxyvitamin D (25(OH)D) (Vitamin D) has a protective role in the development of colorectal cancer.<sup>4-8</sup> Some meta-analyses have consistently reported that there was an inverse association between plasma Vitamin D concentration in the blood and incidence of, and mortality from colorectal cancer.<sup>9-15</sup>

The prevalence of Vitamin D deficiency has increased in recent decades.<sup>16 17</sup> In a recent population-based study of Asian adults, approximately 75% had suboptimal Vitamin D concentrations.<sup>18</sup> The Endocrine Society Clinical Practice Guideline defines Vitamin D deficiency as 25(OH)D level <20 ng/mL and insufficiency as 21 to 29 ng/mL.<sup>19</sup> Feldman et al.<sup>20</sup> reported anti-neoplastic actions of Vitamin D, particularly in colorectal cancer.<sup>9</sup> Touvier et al.<sup>12</sup> reported that improving Vitamin D levels could be beneficial in reducing colorectal cancer incidence. Data from a cohort of healthy women showed that plasma Vitamin D levels was inversely related to the occurrence and death from colorectal cancer.<sup>21</sup> In the Nurses' Health Study, total circulating Vitamin D was associated with a lower risk of colorectal cancer in white women.<sup>22</sup> A recent international study of 17 cohorts in Western population found that Vitamin D deficiency was associated with increased colorectal cancer risk, and Vitamin D above sufficiency levels was associated with 19% to 27% lower risk.<sup>23</sup> Compared with Western countries, there was an inconsistent conclusion about the relationship between blood circulating Vitamin D level and colorectal cancer risk in studies of Asian countries,<sup>24-30</sup> given that lifestyle, ethnic and environmental factors are different between Asian and Western countries.

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3 We hypothesized that the association between blood circulating Vitamin D and  
4 colorectal cancer in Asian countries is distinct from Western countries. Thus, this review  
5 aimed to summarize epidemiological evidence regarding blood circulating Vitamin D level  
6 and colorectal cancer risk in Asian countries. This study underlines the public health  
7 importance of attaining and maintaining an optimal vitamin D status in the Asian population  
8 and may help to guide clinical and nutritional practice in Asians countries.  
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## 14 **METHODS**

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18 We performed the systematic review according to a predetermined protocol and reported in  
19 accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses  
20 Protocols (PRISMA-P) guidelines.<sup>31</sup> Two reviewers (L.Z. and Z.Q.) independently undertook  
21 the literature search, assessment for eligibility, data extraction, and qualitative assessment.  
22 Any inconsistencies between the two reviewers were reviewed by a third reviewer (Y.J.) and  
23 resolved by consensus.  
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### 30 **Eligibility criteria**

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33 *Participants:* Our study uses the list of sovereign states and dependent territories in Asia by  
34 the United Nation (<https://unstats.un.org/unsd/methodology/m49/>) to draw participants from  
35 48 countries located in five regions (Central Asia, Eastern Asia, Southern Asia, Southeastern  
36 Asia, and Western Asia). Asians, and people of Asian origin who live in Western countries  
37 were excluded.  
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42 *Exposure:* The exposure is blood circulating 25-hydroxyvitamin D (25(OH)D) level which is  
43 commonly measured to assess and monitor Vitamin D status in individuals. Most studies only  
44 report the total level and do not distinguish D2 and D3 forms of the vitamin. In our meta-  
45 analysis, we consider the total level of Vitamin D as the exposure.  
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49 *Comparators (controls):* In order to be eligible for inclusion, studies must compare outcomes  
50 in a group of exposed individuals with the highest category of blood circulating Vitamin D  
51 level and a group of unexposed individuals with the lowest category of blood circulating  
52 Vitamin D level.  
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56 *Outcome:* Studies included in the review have a diagnosis of colorectal cancer or colorectal  
57 adenoma, clinically confirmed by colonoscopy or pathology.  
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3 *Study design:* We target observational studies (case-control, cross-sectional and cohort).  
4 English language studies conducted post-1980 were considered eligible. Animal studies were  
5 excluded.  
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10 Studies were excluded if they: 1) were reviews, editorial, case report, or guideline  
11 articles; 2) did not explicitly state the blood circulating Vitamin D level and its association  
12 with colorectal cancer risk; 3) allowed controls to have a previous disease history of cancer; 4)  
13 focused on Western population or Asian population living in Western countries; or 5)  
14 investigated the blood circulating Vitamin D level and its association with survival of  
15 colorectal cancer. By consensus among all three reviewers (L.Z., Z.Q. and Y.J.), if data  
16 sources were duplicated in more than one study, only the original study was included in the  
17 meta-analysis.  
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### 25 **Search strategy**

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27 We conducted a literature search using Medline, Embase, and Web of Science, and retrieved  
28 all relevant articles that reported the association plasma or serum Vitamin D level and the risk  
29 of colorectal neoplasia in Asian countries, published from January 1980 to 31 January 2019.  
30 The Medical Subject Heading (MeSH) terms were used in conjunction with the following  
31 keywords for our search: (colorectal neoplasm or colon neoplasm or colorectal cancer or  
32 colon cancer) AND (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-  
33 hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-  
34 hydroxylase or Vitamin D) AND (Asia\* or Afghanistan or Armenia or Azerbaijan or Bahrain  
35 or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or  
36 Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan  
37 or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or  
38 North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi  
39 Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or  
40 Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan  
41 or Vietnam or Yemen). Full search strings are presented in **Table S1**. References from  
42 relevant articles, editorials, conference abstracts, letters, and reviews were thoroughly  
43 reviewed to identify additional studies. Full manuscripts of every article with a relevant title  
44 and abstract were then reviewed for eligibility.  
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## Data extraction and qualitative assessment

Two reviewers (L.Z. and Z.H.Q) independently extracted the following study-level characteristics from each eligible study: first author, year of publication, type of study, country where the study was conducted, selection criteria, the numbers of cases and controls (for case-control studies or cross-sectional studies) and the numbers of total participants and incident cases (for cohort studies), population characteristics (sex and age), follow-up period (for cohort studies), sample size, levels of Vitamin D in both case and control group, measures and ranges of Vitamin D, adjusted variables, and risk estimates with corresponding 95% CI for each category. For studies that reported both crude and adjusted estimates of the association between blood circulating Vitamin D and risk of colorectal cancer or adenoma, we used the adjusted estimates for the meta-analysis. For studies that reported several adjusted estimates of association, we used the estimates adjusted for the most variables.

We applied the Newcastle-Ottawa Scale (NOS) quality assessment tool to evaluate the quality of the selected observational studies. This tool was used to measure the key aspects of the methodology in selected studies with regard to design quality and the risk of biased estimates based on three design criteria: 1) selection of study participants; 2) comparability of study groups; and 3) assessment of outcome and exposure with a star system (with a maximum of 9 stars). We judged studies that received a score of 7-9 stars to be at low risk of bias, studies that scored 4-6 stars to be at medium risk, and those that scored 3 or less to be at high risk of bias. A funnel plot was used to assess the publication bias. Any disagreement on the data extraction and quality assessment of the studies were resolved through comprehensive discussion (L.Z., Z.Q, and Y.J.).

## Statistical analysis

Study-specific OR estimates were combined using a random-effects model, that considers within-study and between-study variations. Corresponding 95% CIs were extracted directly from articles where available, with adjusted ORs extracted preferentially over unadjusted ORs. The dose-response analysis was utilized to assess the relationship between blood circulating Vitamin D level and colorectal cancer risk using the generalized least squares (GLS) method to resolve the inconsistency issue of different Vitamin D levels in included studies.<sup>32 33</sup> For the dose-response meta-analysis of blood circulating Vitamin D levels, we used a method proposed by previous studies to compute the trend from the correlated log OR estimates across categories of Vitamin D levels.<sup>34</sup> This analytical method collected the distribution of cases and controls, median values of blood circulating Vitamin D levels, and corresponding OR estimates in each category for each study. The assigned value of the lowest category was designated as a reference level. If the study did not provide median values of blood Vitamin D, the midpoint of the upper and lower boundaries in each category was assigned. For the open-ended exposure categories, the length of the open-ended interval was assumed to be the same as that of the adjacent interval. We examined a potential non-linear dose-response relationship between blood circulating Vitamin D level with colorectal cancer risk by modelling Vitamin D levels using random-effect restricted cubic splines with three knots at percentiles 25%, 50%, and 75% of the distribution (spline model). A *P*-value for nonlinearity was calculated by testing the null hypothesis that the regression coefficient of the second spline was equal to zero by Wald-type test of nonlinear hypotheses.<sup>34</sup> A small *P*-value (<0.05) of the Wald-type test indicates departure from linearity. The non-linear dose-response relationship was confirmed by several representative point values and the risk estimates of a subgroup analysis based on the range of exposure.

The statistical heterogeneity among studies was evaluated using Cochran's *Q* test and *I*<sup>2</sup> statistic, with values of 25%, 50%, and 75% representing low, moderate, and high heterogeneity, respectively.<sup>35</sup> The criterion for identifying heterogeneity was a *P* value less than 0.05 for the *Q* test. If substantial heterogeneity was detected, we performed univariate meta-regression analyses to explore the proportion of between-study variance explained by study quality, participant characteristics, and study characteristics. We were unable to perform a multivariate meta-regression analysis as only a small number of included studies reported information for all study-level factors. We performed subgroup analyses comparing

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3 pooled association estimates and heterogeneity with stratification by, participants sex,  
4 outcome type, subregion of Asia, blood sample type, and range of Vitamin D levels (The  
5 range is the difference in the midpoint between the highest and lowest categories of blood  
6 circulating Vitamin D in each study). An estimation of publication bias was evaluated by the  
7 Beggs funnel plot, in which the SE of log (OR) of each study was plotted against its log  
8 (OR). An asymmetrical plot suggests possible publication bias. Egger's linear regression test  
9 assessed funnel plot asymmetry, a statistical approach to identify funnel plot asymmetry on  
10 the natural logarithm scale of the ORs. All statistical analyses were performed using Stata  
11 (version 14.2; StataCorp LP, College Station, Texas). All *P* values were two-sided, and *P*  
12 <0.05 was considered as statistically significant.  
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## RESULTS

### Selection of studies

A detailed PRISMA flow diagram<sup>31</sup> of literature search and inclusion criteria is shown in **Figure 1**. A total of 611 studies were initially identified with this literature search (250 from Medline, 272 from Embase and 89 from Web of Science), but 114 studies were excluded due to duplication and 465 were excluded after screening the titles and abstracts. Twenty-four other studies were excluded after full-text review (details shown in **Table S2**). Finally, a total of eight studies were identified as eligible for meta-analysis.

### Study characteristics

The eight studies included had a total of 2,916 cases and 6,678 controls (**Tables 1 and 2**). These studies were published between 2007 and 2018 - seven from Eastern Asia (four are from Japan,<sup>24 25 27 30</sup> two from Korea,<sup>26 28</sup> and one from China;<sup>36</sup>) and one is from Western Asia.<sup>29</sup> Regarding study design, seven were case-control,<sup>24-29 36</sup> and one was a nested case-cohort study.<sup>30</sup> Of the eight studies, four provided the main endpoint of colorectal cancer<sup>24 29 30 36</sup> and the remaining four provided the main endpoint of colorectal adenoma.<sup>25-28</sup>

### Meta-analysis and dose-response analysis

The multivariable-adjusted ORs for each study and the combination of all eight studies for the highest versus lowest categories of blood circulating Vitamin D levels are shown in **Figure 2**. The mean blood circulating Vitamin D level of the included study population was 20.21 ng/mL, with an SD of 7.92 ng/mL, the minimal concentration of 3.65 ng/mL, and the maximal concentration of 36.5 ng/mL. Results from the studies on blood circulating Vitamin D levels in relation to colorectal cancer risk were inconsistent, with both inverse and positive associations reported. The pooled ORs of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D level was 0.75 [95% CI, 0.58-0.97], which indicate higher blood circulating Vitamin D level had a significant inverse association with risk of colorectal cancer. There was statistically significant heterogeneity among the studies ( $I^2=53.9\%$ ,  $P=0.034$ ).

We evaluated the non-linear dose-response relationship between blood circulating Vitamin D levels and colorectal cancer risk. A 16 ng/mL increment (about 2 SDs=15.84

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3 ng/mL) in blood circulating Vitamin D levels conferred an OR of 0.79 [95% CI, 0.64-0.97],  
4 which meant the risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in  
5 blood Vitamin D, and the reduction was statistically significant. A moderate heterogeneity  
6 existed ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ) in the overall analysis of blood circulating Vitamin D  
7 levels, without a significant non-linear dose-response relationship ( $P_{\text{non-linearity}}=0.11$ ),  
8 suggesting that the non-linear dose-response relationship does not depart from linearity.  
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10 Similar trends were observed with linear and spline models (**Figure 3**).  
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### 17 **Subgroup analysis**

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20 When we stratified the analysis according to blood sample type, the pooled ORs of serum  
21 sample and plasma for the highest versus lowest categories of blood circulating Vitamin D  
22 levels were 0.52 [95% CI, 0.34-0.80] and 0.77 [95% CI, 0.59-1.00] respectively. The results  
23 showed that there was a substantial risk reduction (48%) for blood serum Vitamin D levels  
24 associated with the risk of colorectal cancer. There was no evidence of significant statistical  
25 heterogeneity among studies ( $I^2=22.67\%$ ,  $P=0.21$ ). We then performed a subgroup analysis  
26 of blood circulating Vitamin D range in each study. The pooled ORs was 0.93 [95% CI, 0.70-  
27 1.25] for studies with range  $\leq 15$  ng/mL and 0.62 [95% CI, 0.47-0.83] for studies with range  
28  $>15$  ng/mL. There was no evidence of statistical heterogeneity among studies ( $I^2=28.48\%$ ,  
29  $P=0.08$ ). When stratified by outcome, the pooled ORs were 0.67 [95% CI, 0.40-1.14] for  
30 studies when the outcome was colorectal adenoma and 0.83 [95% CI, 0.66-1.06] when the  
31 outcome was colorectal cancer respectively with no significantly statistical heterogeneity  
32 among studies ( $I^2=59.48\%$ ,  $P=0.73$ ). When we stratified the studies by sex, the pooled ORs  
33 were 0.59 [95% CI, 0.13-2.74] for studies with estimates for women, and 1.13 [95% CI, 0.81-  
34 1.58] for men respectively with no significantly statistical heterogeneity among studies  
35 ( $I^2=45.89\%$ ,  $P=0.70$ ). We also stratified according to the geographical region of the  
36 population. The pooled ORs was 0.75 [95% CI, 0.57-1.00] for Eastern Asia and 0.69 [95%  
37 CI, 0.36-1.34] for Western Asia. There was no significantly statistical heterogeneity among  
38 studies ( $I^2=59.79\%$ ,  $P=0.87$ ). The subgroup meta-analyses are shown in **Figure S1** and  
39 summarized in **Table 3**.  
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### 56 **Qualitative assessment and publication bias**

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3 The NOS tool was used to conduct a qualitative assessment of the selected studies to review  
4 the quality of the studies and detect possible bias. Of the eight studies, five were at low risk  
5 of bias (8-9) stars.<sup>24-27 30</sup> Three studies were at medium risk (5-7 stars) mainly due to bias  
6 from representativeness of cases or controls, control definition, and response rate.<sup>29 36</sup>  
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<sup>37</sup>( shown in **Table S3.**) The funnel plot and Eggers statistical test indicated no evidence of publication bias in the studies included in the meta-analysis ( $P=0.338$ ) (**Figure S2.**)

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## DISCUSSION

Colorectal cancer is one of the cancers with high morbidity and mortality in the world. The one-carbon metabolism pathway requires adequate Vitamin D, and this raises the possibility that Vitamin D may have an essential role in the risk of colorectal cancer. Many epidemiological studies from Europe and the United States believe that increasing the concentration of circulating Vitamin D can reduce the morbidity and mortality of colorectal cancer.<sup>38</sup> However, the association between blood circulating Vitamin D levels and the risk of colorectal cancer in the Asian population is still under debate due to a lack of sufficient evidence. This systematic review highlights the inconsistencies among studies addressing the role of blood circulating Vitamin D and colorectal cancer risk in the Asian population. Our systematic review identified eight studies consisting of 2916 cases and 6678 controls that addressed the relationship between blood circulating Vitamin D levels and colorectal cancer risk. Our meta-analysis found 25% reduced risk of colorectal cancer for the highest versus lowest categories of blood circulating Vitamin D levels (OR=0.75, 0.58-0.97) up to 36.5 ng/mL, that indicated higher blood circulating Vitamin D level has a significant inverse association with risk of colorectal cancer in Asian population. Our meta-analysis results showed that the negative correlation between Vitamin D and the risk of colorectal cancer is similar to that of European and American population studies<sup>11 12 21 22 39-42</sup> and consistent with the result of a meta-analysis by Ekmekcioglu C et al.<sup>13</sup>, that found a pooled relative risk (RR) of 0.62 (0.56–0.70) for colorectal cancer when comparing individuals with the highest category of 25(OH)D with those in the lowest.

Our results found a 16 ng/mL increment in blood circulating Vitamin D levels with an OR of 0.79 [95% CI, 0.64-0.97], which meant the risk of colorectal cancer decreased by 21% for every 16 ng/mL increment in blood Vitamin D, and the reduction was statistically significant. Our study also suggested a linear dose-response relationship ( $P_{\text{non-linearity}} = 0.11$ ), that was consistent with several studies conducted in Western populations revealing a similar protective dose-response association of the blood circulating Vitamin D and colorectal cancer risk. For example, a meta-analysis reported a 26% lower risk of colorectal cancer per 10 ng/mL increment in blood circulating Vitamin D levels.<sup>11</sup> Most experts define Vitamin D deficiency as a Vitamin D level of less than 20 ng/mL.<sup>43 44</sup> Vitamin D concentration of 21 to 29 ng/mL can be considered to indicate a relative insufficiency of Vitamin D, and a level of 30 ng/mL or higher can be considered to indicate sufficient Vitamin D.<sup>45</sup> With the use of such definitions, it has been estimated that many people have Vitamin D deficiency or

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3 insufficiency.<sup>43-44</sup> Previous research has implied an association between Vitamin D deficiency  
4 and an increased incidence of bone fractures.<sup>46</sup> There is also data showing Vitamin D  
5 deficiency to be associated with cancer,<sup>47-49</sup> diabetes,<sup>50</sup> cognitive impairment,<sup>51</sup> and all-cause  
6 mortality.<sup>52</sup> Among colorectal cancer patients, the prevalence of Vitamin D deficiency was  
7 much higher (nearly 90%) than among patients with other chronic diseases.<sup>53</sup> Humans obtain  
8 Vitamin D from exposure to sunlight, from natural diets, fortified diets, supplementation  
9 etc.<sup>46</sup> Dose-response between Vitamin D concentrations and colorectal cancer risk may be  
10 different between Asian and Western populations due to ethnic, anthropometric, dietary, and  
11 environmental factors.<sup>12</sup> Lifestyle and diet can promote the development of early-onset colon  
12 lesions by regulating growth factors that interact with inflammatory pathways.<sup>41</sup> An  
13 association between Vitamin D status and reduced risk of colorectal cancer has been found in  
14 ethnically diverse populations.<sup>5</sup> Vitamin D interacts with calcium to enhance the reduction of  
15 colon cancer risk.<sup>54-56</sup> Studies have shown that Vitamin D and calcium may interact and that  
16 both are needed in reducing cancer risk.<sup>57</sup> However, even after adjusting for calcium intake in  
17 some studies,<sup>6-58</sup> Vitamin D was associated with a lower risk. The independent effects of  
18 Vitamin D are supported, but the combined effects of Vitamin D and calcium may be greater  
19 than the sum of their independent effects.<sup>59</sup> Vitamin A has an antagonistic effect on Vitamin  
20 D<sup>58</sup> and taking both at the same time can lead to decreased calcium absorption. Still, Vitamin  
21 A is often combined with Vitamin D in supplements.

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Vitamin D supplementation significantly reduced total cancer mortality but did not  
reduce total cancer incidence.<sup>60</sup> Patients with low-risk prostate cancer under active  
surveillance may benefit from vitamin D3 supplementation at 4000 IU/d.<sup>61</sup> Among patients  
with metastatic colorectal cancer, the addition of high-dose Vitamin D3, vs standard-dose  
Vitamin D3, to standard chemotherapy resulted in a difference in median progression-free  
survival that was not statistically significant, but with a significantly improved supportive  
effect.<sup>62</sup> Epidemiological evidence links the incidence of colorectal cancer to lifestyle,  
smoking, physical activity, alcohol consumption, and sleep.<sup>63</sup> It has also been linked to  
reduced fruit and vegetable consumption and increased consumption of red meat. Dairy  
products, fish and other foods and cooking methods also play an essential role.<sup>64</sup> In addition,  
some drugs such as non-steroidal anti-inflammatory drugs and cyclooxygenase inhibitors are  
also involved.<sup>65</sup> Women on estrogen therapy, for example, did not reduce their risk of  
colorectal cancer by taking Vitamin D and calcium supplements.<sup>66</sup> Increased dietary fiber  
intake reduces the risk of colorectal cancer and obesity; and low physical activity may reduce

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3 plasma 25(OH)D concentration, thereby increasing the risk of colorectal cancer.<sup>42</sup> For every  
4 1kg/m<sup>2</sup> increase in BMI of colorectal cancer patients, serum Vitamin D level decreased  
5 significantly (0.46 ng/mL).<sup>67</sup> Most variation in Vitamin D levels usually comes from  
6 exposure to the sun, which is an essential source of Vitamin D for people who get more from  
7 fish, even in Japan.<sup>68</sup>  
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12 This meta-analytic comparison revealed a statistically significant beneficial effect of  
13 blood circulating Vitamin D for colorectal cancer. However, the diversity of the studies and  
14 the presence of moderate heterogeneity in the overall analysis of blood circulating Vitamin D  
15 levels ( $I^2=53.9\%$ ,  $P_{\text{heterogeneity}}=0.034$ ) may preclude making meaningful conclusions from the  
16 pooled estimate because it may not reflect the true underlying effect. The subgroup analysis  
17 did not explain much of the heterogeneity.  
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23 Potential reasons for the heterogeneity in the strength of the association may include  
24 the following. Firstly, food consumption and vitamin supplements varied according to the  
25 specific dietary habits and lifestyle in each Asian subregion. A systemic review studied  
26 correlations between various diet types, food or nutrients and colorectal cancer risk among  
27 Asians, and suggested that red meats, processed meats, preserved foods, saturated/animal  
28 fats, cholesterol, high sugar foods, spicy foods, tubers or refined carbohydrates have a  
29 positive association with colorectal cancer risk.<sup>69</sup> Besides diet, other personal and lifestyle  
30 factors (e.g., exposure to sunlight, obesity, smoking and drinking habit) may alter the strength  
31 of the association and contribute to the heterogeneity of the association in Asian countries.  
32 The numbers of studies from the different Asian subregions included in our meta-analysis  
33 was imbalanced, so we were cautious in interpreting results. For example, our meta-analysis  
34 included seven studies from Eastern Asia, only one from Western Asia, and none from  
35 Central, Southern or South-eastern Asia. Our study revealed no evidence of publication bias,  
36 and most of the studies included in our meta-analysis verified the diagnosis of colorectal  
37 cancer for cases. Histologic confirmation of cancer diagnoses for cases was an optimal  
38 validation for the case-control design in our meta-analysis.  
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51 Possible confounders for the association between colorectal cancer and blood  
52 circulating Vitamin D levels include sex, age, family history of colorectal cancer, smoking,  
53 alcohol drinking, body mass index, and diabetes. Most studies in our meta-analysis provided  
54 risk estimates that were adjusted for age,<sup>24 26-30 36</sup> sex,<sup>24 26 27 29 30 36</sup> body mass index,<sup>24-30 36</sup>  
55 smoking,<sup>24-30 36</sup> drinking,<sup>24-26 29 36</sup> physical activity,<sup>30</sup> alcohol consumption,<sup>24-30</sup> and family  
56 history of colorectal cancer.<sup>24 25 29</sup> Fewer were adjusted for folate,<sup>24 37</sup> energy intake,<sup>24 27</sup>  
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3 hypertension,<sup>36</sup> vitamin D binding protein,<sup>36</sup> blood collection,<sup>24 27</sup> or for vitamin supplement  
4 use.<sup>24</sup> For these studies, the observed reduced risk of colorectal cancer associated with  
5 Vitamin D levels is likely confounded by one or more of these factors.  
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9 In the overall analysis for both adenoma and carcinoma that is part of our subgroup  
10 analysis, we report a statistically significant association; yet, in the stratified analysis by  
11 colorectal adenoma or colorectal cancer separately, the association was not statistically  
12 significant. The results, however, show that associations were in the same direction (ORs<1  
13 indicating an inverse relationship). Uncontrolled confounders (e.g. dietary sources of Vitamin  
14 D, consumption of fish/fibres containing 25(OH)D, exposure to the sun, folate/calcium intake,  
15 Vitamin D supplement use etc.) in the original studies that are part of our meta-analysis may  
16 be responsible for these differences. Of note, only one of the 8 studies in our meta-analysis  
17 adjusted for these confounders.<sup>24</sup> The presence of negative confounders in original colorectal  
18 cancer studies (OR's are closer to 1), and positive confounders in original colorectal adenoma  
19 studies (OR's are further from 1) as well as some effect modification may also be responsible  
20 for the statistical significance difference noted. We used a random effects model in our  
21 analysis to reduce this effect. Further investigation of the subgroup analysis show that the  
22 weight of the studies could also be contributory. Two studies<sup>25 30</sup> contribute a large weight in  
23 the subgroup meta-analysis, but a smaller weight in the overall analysis.  
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35 Our analysis had the following limitations. First, the number of included studies is not  
36 sufficient to provide a robust estimate of the association of blood circulating Vitamin D  
37 levels and colorectal cancer risk, so the analysis and results should be interpreted in the  
38 context of the limitations of the available data. Second, heterogeneous definitions of blood  
39 circulating Vitamin D categories were used across studies. The variability in definitions could  
40 limit comparability between studies. Third, our study included seven case-control studies; the  
41 study design implying that the measurement of blood circulating Vitamin D is in individuals  
42 already diagnosed with colorectal cancer. The results from this study design need to be  
43 interpreted cautiously because of the potential for reverse causation. The time of blood  
44 sampling in relation to outcome ascertainment also varied among studies. Such cross-  
45 sectional measurements may not accurately reflect an individual's Vitamin D status across  
46 time. Fourth, some studies included in our meta-analysis did not adjust for potentially  
47 relevant confounders which may have led to residual confounding and may explain some of  
48 the observed heterogeneity. Fifth, the difference in the method used for measuring blood  
49 circulating Vitamin D levels may also be a source of heterogeneity between the included  
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3 studies. Sixth, in the dose-response analysis, the literature selected listed the median Vitamin  
4 D value, instead of the original Vitamin D value, which could also lead to inaccurate results.  
5 Seventh, although we assessed the quality of the observational studies in this meta-analysis  
6 with the NOS quality assessment tool, Bae<sup>70</sup> suggested that it is more reasonable to control  
7 for quality level by performing subgroup analysis according to study design rather than by  
8 using the NOS tool. In this context, however, we did not perform subgroup analysis  
9 according to study design since the included 8 studies were case-control and nested case-  
10 cohort studies. Finally, a recent meta-analysis investigated the association between blood  
11 circulating Vitamin D levels and survival of colorectal cancer and found that the pooled  
12 hazard ratios (95% confidence intervals) were 0.68 [0.55–0.85] and 0.67 [0.57–0.78]  
13 respectively for overall and colorectal cancer-specific survival comparing highest versus  
14 lowest categories of blood Vitamin D.<sup>15</sup> Our study did not explore the association of blood  
15 circulating Vitamin D levels and colorectal cancer mortality however, and this association in  
16 Asian countries is one we would encourage future studies to examine.  
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## 30 CONCLUSION

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33 Blood circulating Vitamin D levels is inversely associated with colorectal cancer prevalence  
34 in the Asian population. Our findings on the inverse association between blood circulating  
35 Vitamin D and the risk of colorectal cancer in the Asian population suggest the need for  
36 Asians to improve their nutritional status and maintain higher blood circulating Vitamin D  
37 levels. This meta-analysis provides valuable information for future research on the  
38 association between blood circulating Vitamin D and colorectal cancer risk in the Asian  
39 population. A multinational, population-based study in Asian countries may resolve the issue  
40 of heterogeneity and generate detailed information on blood circulating Vitamin D levels and  
41 the risk of colorectal cancer. Further studies may also focus on evaluating the association of  
42 Vitamin D levels with colorectal cancer mortality.<sup>60</sup>  
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## Authors Contributions

LZ: Study concept and design; acquisition of data; statistical analysis; interpretation of data; drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

HZ: Interpretation of data; drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

YZ: Interpretation of data; drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

CH: Drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

AA: Drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

XQ: Interpretation of data; drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

PJ: Interpretation of data; drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

YJ: Study concept and design; acquisition of data interpretation of data; drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

ZQ: Study concept and design; acquisition of data; interpretation of data; drafting and critical review of the manuscript for important intellectual content; approval of the final version of the manuscript.

## Figure legends/captions

**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

**Figure 2.** Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D. F, female; M, male;

**Figure 3.** Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modelled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line,  $P_{\text{non-linearity}}=0.11$ ). Lines with short dashes represent the linear trend.

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**Table 1.** Summary of characteristic for 8 studies included in the meta-analysis

Author(year)	Study design	Country	Study period	Outcome	Outcome ascertainment	Sex	Sample size (n); characteristics				Blood sample type	Vitamin D testing method
							Case control	or	No.	Male (%)		
Budhathoki et al. (2018) <sup>30</sup>	Nested case-cohort	Japan	1990-2009	colorectal cancer	NA	All	case control	637	52	56.2(7.5)	plasma	chemiluminescent enzyme immunoassay
Choi et al. (2015) <sup>37</sup>	case-control	Korea	2011-2012	colorectal adenoma	colonoscopy	All	case control	112	51	60.3 (5.3)	serum	chemiluminescent enzyme immunoassay
						Men	case control	112	51	59.5 (5.4)	serum	
						Women	case control	57	100	NA	serum	
							case control	57	100	NA	serum	
Hong et al. (2012) <sup>26</sup>	case-control	Korea	2009-2010	colorectal adenoma	colonoscopy	All	case control	143	68	58.7 (6.0)	serum	direct competitive electrochemiluminescence immunoassay
							control	143	68	58.7 (6.0)		
Otani et al. (2007) <sup>24</sup>	case-control	Japan	1990-2003	colorectal cancer	pathologically confirmed	All	case control	323	52	NA	plasma	competitive protein-binding assay
						Men	case control	621	52	NA		
						Women	case control	163	100	56.9		
							case control	324	100	56.9		
Takahashi et al. (2010) <sup>25</sup>	case-control	Japan	1997-2004	colorectal adenoma	colonoscopy	Men	case control	656	100	52.0 (1.4)	plasma or serum	radioimmunoassay method
							control	648	100	51.8 (1.5)		
Yamaji et al. (2012) <sup>27</sup>	case-control	Japan	2004-2005	colorectal adenoma	colonoscopy	All	case control	737	67	NA	plasma	radioimmunoassay method
Ying et al. (2015) <sup>36</sup>	case-control	China	2010-2014	colorectal cancer	colonoscopy	All	case control	703	65	NA	plasma	direct competitive enzyme-linked immunosorbent assay
							case control	212	NA	63(56-73)		
Yurekli et al. (2015) <sup>29</sup>	case-control	Turkey	2012	colorectal cancer	colonoscopy	All	cases control	96	67	57.8(10.0)	serum	NA
							control	195	54	51.2(12.9)		

**Abbreviations:** SD, Standard deviation; NA, Not available;  
All\*: all participants, including men and women

**Table 2.** Summary of risk estimate for 8 studies included in the meta-analysis

Author(year)	sex	Vitamin D level cut points (ng/ml)	Vitamin D medium level (ng/ml)	Number of cases	Number of controls	N	OR	LCI	UCI	Adjusted variables
Budhathoki et al. (2018) <sup>30</sup>	All	1st Q, 12.5-16.5 ng/ml	14.7	134	1004	1138	1			age, sex, BMI, smoking, alcohol use, physical activity, family history of cancer, and history of diabetes
		2nd Q, 17.6-21.6 ng/ml	19.9	165	1000	1165	1.08	0.84	1.39	
		3rd Q, 21.2-25.6 ng/ml	21.3	160	1016	1176	0.96	0.74	1.26	
		4th Q, 25.8-33.0 ng/ml	26.5	178	1024	1202	0.95	0.73	1.23	
Choi et al. (2015) <sup>37</sup>	All	1st Q, 10.3 ng/ml	10.3	37	28	65	1			age, sex, BMI, alcohol drinking, smoking status, folate intake; women: additional menopausal status and hormone replacement use
		2nd Q, 14.3 ng/ml	14.3	26	28	54	0.72	0.31	1.66	
		3rd Q, 17.9 ng/ml	17.9	28	28	56	0.73	0.29	1.82	
		4th Q, 24.2 ng/ml	24.2	21	28	49	0.49	0.19	1.27	
	Men	1st Q, 11.1 ng/ml	11.1	15	14	29	1			
		2nd Q, 14.6 ng/ml	14.6	13	14	27	1.31	0.36	4.82	
		3rd Q, 17.7 ng/ml	17.7	17	15	32	1.26	0.4	8.12	
		4th Q, 22.7 ng/ml	22.7	12	14	26	1.8	0.19	8.12	
	Women	1st Q, 9.5 ng/ml	9.5	16	14	30	1			
		2nd Q, 13.5 ng/ml	13.5	22	14	36	0.89	0.26	3.1	
		3rd Q, 18.4 ng/ml	18.4	8	14	22	0.54	0.13	2.16	
		4th Q, 25.4 ng/ml	25.4	9	13	22	0.22	0.04	1.15	
Hong et al. (2012) <sup>26</sup>	All	1st Q, <14.3 ng/ml	10.1	31	42	73	1			age, sex, BMI, smoking, alcohol drinking, physical activity, corrected calcium level
		2nd Q, 14.3-18.5 ng/ml	16.4	29	42	71	0.87	0.43	1.74	
		3rd Q, 18.6-23.8 ng/ml	21.2	41	31	72	0.4	0.2	0.82	
		4th Q, >=23.9 ng/ml	29.1	42	28	70	0.38	0.18	0.8	
Otani et al. (2007) <sup>24</sup>	Men	1st Q, <22.9 ng/ml	18.3	43	74	117	1			age, sex, BMI, smoking, alcohol consumption, physical exercise, Vitamin supplement use, family history of colorectal cancer, total energy intake, dietary fiber intake, folate intake, calcium intake, Vitamin D intake, n-3 fatty acid intake, red meat intake, fish intake
		2nd Q, 22.9-27.5 ng/ml	25.2	40	85	125	0.76	0.42	1.4	
		3rd Q, 27.6-32.0 ng/ml	29.8	36	85	121	0.76	0.39	1.5	
		4th Q, >32.1 ng/ml	36.5	44	80	124	0.73	0.35	1.5	
	Women	1st Q, <18.7 ng/ml	15.2	41	77	118	1			
		2nd Q, 18.7-22.2 ng/ml	20.5	34	73	107	1	0.55	1.9	
		3rd Q, 22.3-26.9 ng/ml	24.6	44	71	115	1.2	0.65	2.3	
		4th Q, >27.0 ng/ml	31.6	41	76	117	1.1	0.5	2.3	
Takahashi et al. (2010) <sup>25</sup>	Men	1st Q, <22 ng/ml	20	128	142	270	1			BMI, hospital, rank in the Self Defense Forces, smoking, alcohol drinking, parental history of colorectal cancer, physical activity, type of blood sample, the month of blood drawing
		2nd Q, 23-25 ng/ml	24	162	156	318	1.21	0.86	1.7	
		3rd Q, 26-29 ng/ml	27.5	223	208	431	1.21	0.87	1.69	
		4th Q, >30 ng/ml	33	143	142	285	1.25	0.85	1.84	
Yamaji et al. (2012) <sup>27</sup>	All	1st Q, 14-19 ng/ml	16.5	145	129	274	1			age, sex, BMI, screening periods, the season of blood collection, smoking, alcohol drinking, family history of colorectal cancer, nonsteroidal anti-inflammatory drug use, height, daily energy intake
		2nd Q, 20-23 ng/ml	21.5	132	128	260	0.86	0.6	1.24	
		3rd Q, 24-26 ng/ml	25	157	145	302	0.91	0.64	1.29	
		4th Q, 27-31 ng/ml	29	175	144	319	1.03	0.73	1.46	
		5th Q, 31-34 ng/ml	32.5	128	157	285	0.64	0.45	0.92	
Ying et al. (2015) <sup>36</sup>	All	1st Q, <7.29 ng/ml	3.65	80	53	133	1			age, sex, BMI, smoking, drinking, history of diabetes, hypertension, vitamin D binding protein
		2nd Q, 7.29-14.61 ng/ml	10.95	49	53	102	0.62	0.35	1.12	
		3rd Q, 14.61-28.84 ng/ml	21.73	46	53	99	0.67	0.38	1.20	
		4th Q, >=28.84 ng/ml	35.96	37	53	90	0.53	0.29	0.98	
Yurekli et al.(2015) <sup>29</sup>	All	1st Q, <8.6 ng/ml	7.1	24	48	72	1			age, sex, BMI, smoking, alcohol intake
		2nd Q, 8.6-10.1 ng/ml	9.35	20	53	73	0.48	0.24	0.98	
		3rd Q, 10.1-14.5 ng/ml	12.3	25	48	73	0.51	0.25	1.03	
		4th Q, >=14.5 ng/ml	18.9	27	46	73	0.69	0.36	1.36	

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**Abbreviations:** NA, Not available; OR: odds ratio; LCI: 95% CI's lower confidence interval; UCI: 95% CI's upper confidence interval; BMI: body mass index

All\*: all participants, including men and women

For peer review only

**Table 3.** Summary of subgroup analysis for the associations of blood circulating Vitamin D and the risk colorectal cancer and adenoma by outcome, sex, the subregion of Asia, and blood sample type.

Subgroup analysis	Number of estimates	Random effect The summary OR (95% CI)	Ratio of ORs (95% CI)	$I^2$ (%)	$P$ value
<b>Outcome</b>					
Colorectal adenoma	4	0.67 (0.40-1.14)			
Colorectal cancer	4	0.83 (0.66-1.06)	1.11(0.54-2.28)	59.48	0.73
<b>Sex</b>					
Women	2	0.59 (0.13-2.74)			
Men	3	1.13(0.81-1.58)	1.46 (0.03-63.5)	45.89	0.70
<b>Subregion</b>					
Eastern Asia	7	0.75 (0.57-1.00)			
Western Asia	1	0.69 (0.36-1.34)	0.92 (0.28-2.99)	59.79	0.87
<b>Blood sample type</b>					
Serum	3	0.52 (0.34-0.80)			
Plasma	4	0.77 (0.59-1.00)	1.49 (0.73-3.03)	22.67	0.21
<b>Range</b>					
≤15 ng/mL	4	0.93 (0.70-1.25)			
>15 ng/mL	4	0.62 (0.47-0.83)	0.65(0.39-1.07)	28.48	0.08

Abbreviations: OR, Odds ratio;  $I^2$ , Percent residual variation due to heterogeneity; CI, Confidence interval.  
\* $I^2$  and  $P$  value related to subgroup differences.

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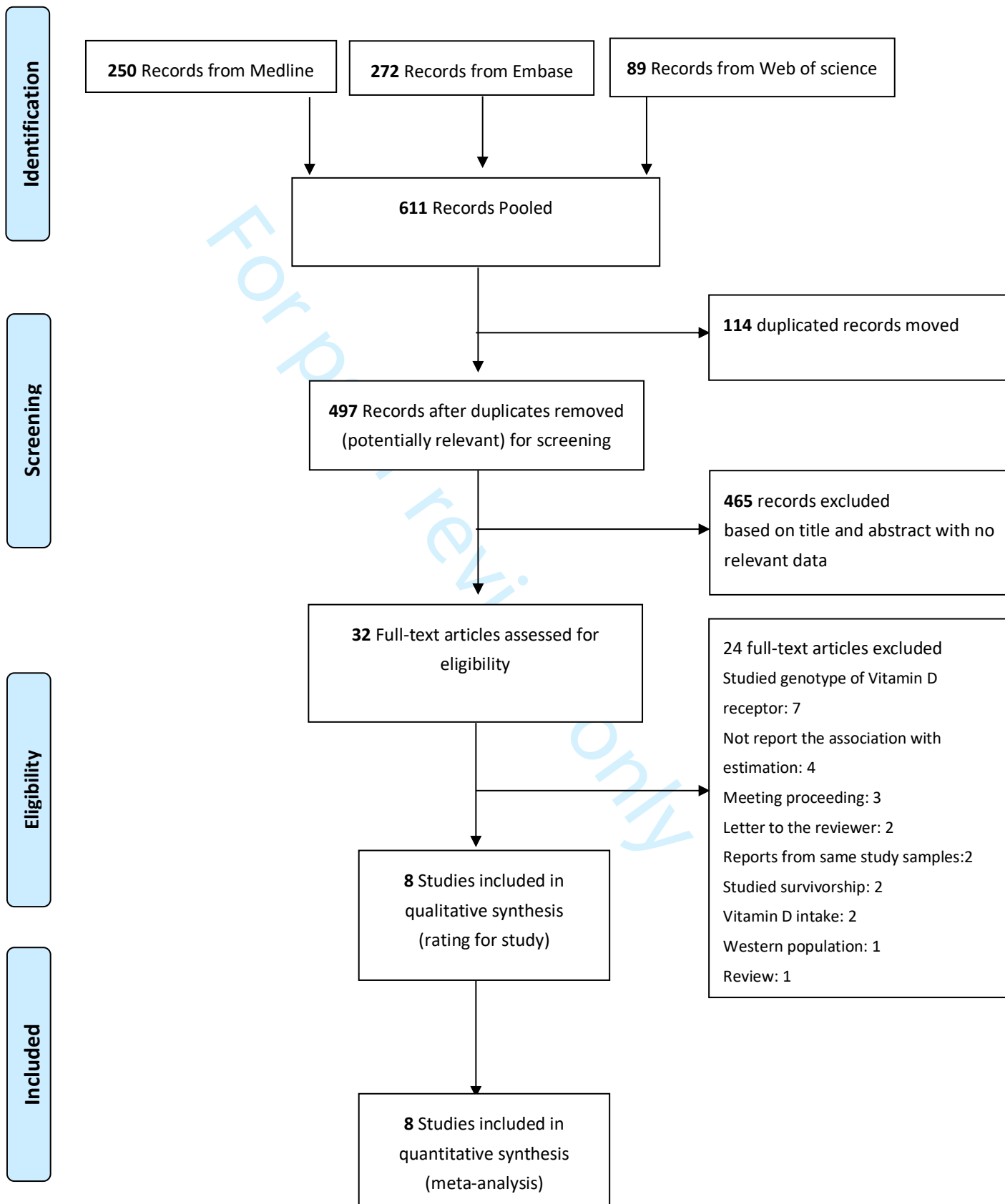
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For peer review only

**Figure 1.** PRISMA flow diagram of study selection for meta-analysis

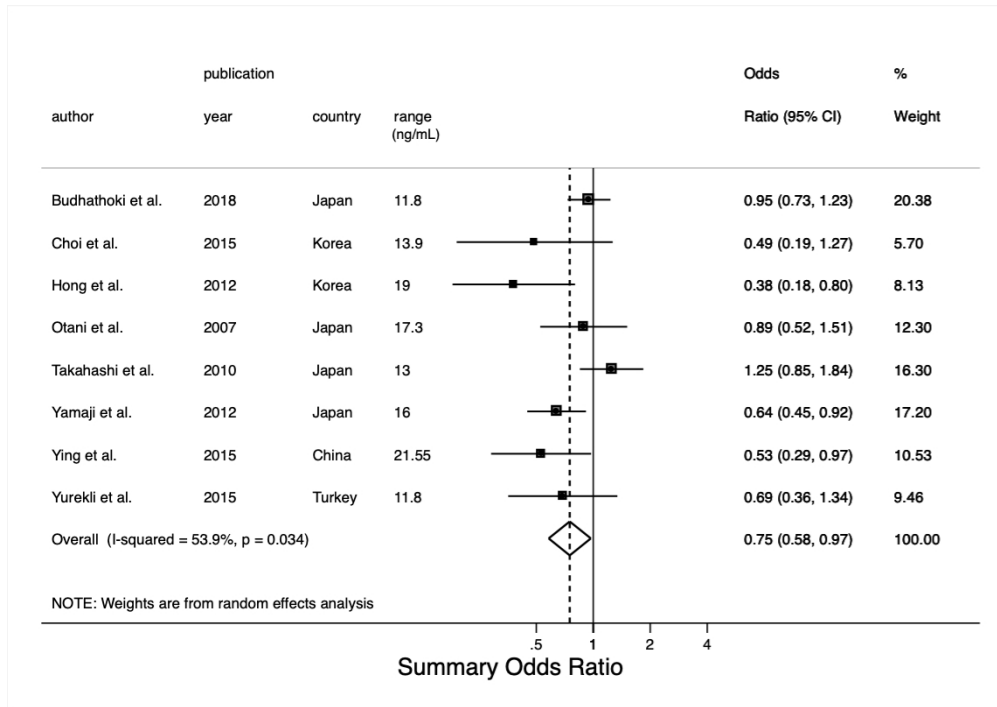


Figure 2. Meta-analysis of association between blood circulating Vitamin D and the risk of colorectal cancer. The adjusted odds ratios were included of colorectal cancer for the highest versus lowest categories of blood Vitamin D level from each study. The size of each square is proportional to the weight of the study. The range is the difference in the midpoint between the highest and lowest categories of blood Vitamin D.

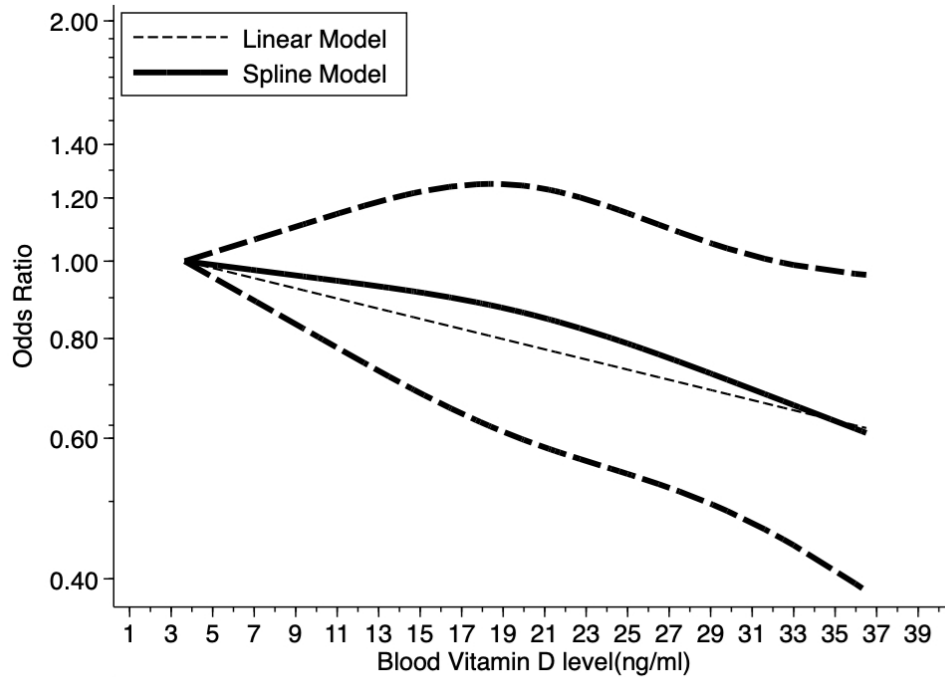


Figure 3. Dose-response relationship between blood Vitamin D levels and the ORs of colorectal cancer. Adjusted Odds Ratio and 95% CIs (dashed lines) are reported. Blood Vitamin D levels were modeled with a linear and spline model in a random-effects meta-regression model. The median value of the lowest reference interval (3.65 ng/mL) was used to estimate all Odds ratios. The vertical axis is on a log scale. Lines with long dashes represent the pointwise 95% confidence intervals for the fitted spline model with a nonlinear trend (solid line,  $P_{\text{non-linearity}}=0.11$ ). Lines with short dashes represent the linear trend.

## Supplementary Material

**Table S1.** Literature search results from Medline, Embase, and Web of Science.

	Medline	Results
1	exp colorectal neoplasms/	187315
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	2688871
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	80293
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1074938
5	1 or 2	2690742
6	3 and 4 and 5	258
7	limit 6 to English language	250

	Embase	Results
1	exp colorectal neoplasms/	26250
2	(colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	1075151
3	(25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	137427
4	(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or	1562807



	Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	
5	1 or 2	1075151
6	3 and 4 and 5	279
7	limit 6 to English language	272

	<b>Web of science</b>	<b>Results</b>
1	TS= (colorectal neoplasm or colorectal cancer* or colorectal or neoplasm* or colon cancer* or colon neoplasm* or bowel cancer* or colorectal adenoma* or colorectal adenocarcinoma* or colorectal polyp*)	460,541
2	TS= (25-OH-D or cholecalciferol or calcidiol or calcitriol or 25-hydroxyVitamin D or hydroxycholecalciferols or 25-hydroxyVitamin D3 or 1-alpha-hydroxylase or Vitamin D)	99,758
3	TS=(Asia* or Afghanistan or Armenia or Azerbaijan or Bahrain or Bangladesh or Bhutan or Brunei or Cambodia or China or Cyprus or Georgia or India or Indonesia or Iran or Iraq or Israel or Japan or Jordan or Kazakhstan or Kuwait or Kyrgyzstan or Laos or Lebanon or Malaysia or Maldives or Mongolia or Myanmar or Burma or Nepal or North Korea or Oman or Pakistan or Palestine or Philippines or Qatar or Russia or Saudi Arabia or Singapore or South Korea or Sri Lanka or Syria or Taiwan or Tajikistan or Thailand or Timor-Leste or Turkey or Turkmenistan or United Arab Emirates or Uzbekistan or Vietnam or Yemen)	2,184,572
4	#1 AND #2 AND #3	106
5	(#4) AND LANGUAGE: (English) AND DOCUMENT TYPES: (Article)	89

**Table S2.** List and details of the excluded studies from the meta-analysis

Reason for exclusion	Author	Year	Study title	Journal
Studied genotype of Vitamin D receptor(n=7)	Wong HL, et al.	2003	Vitamin D receptor start codon polymorphism and colorectal cancer risk: Effect modification by dietary calcium and fat in Singapore Chinese	Carcinogenesis
	Li C, et al.	2009	Vitamin D receptor gene polymorphisms and the risk of colorectal cancer in a Chinese population	Digestive Diseases and Sciences
	Kang JW et al.	2015	Role of vitamin D and local vitamin D receptor expression in colon carcinogenesis	Journal of Gastroenterology and Hepatology
	Takehige N, et al.	2015	Associations between vitamin D receptor (VDR) gene polymorphisms and colorectal cancer risk and effect modifications of dietary calcium and vitamin D in a Japanese population	Asian Pac J Cancer Prev
	Aumpansub P, et al.	2016	Strong association of single nucleotide polymorphisms of vitamin D receptor gene, BSML, and colorectal cancer in Asian population	Gastroenterology
	Budhathoki S, et al.	2016	Vitamin D receptor gene polymorphism and the risk of colorectal cancer: A nested case-control study	PLoS ONE
	Gong C, et al.	2017	Dietary factors and polymorphisms in vitamin D metabolism genes: the risk and prognosis of colorectal cancer in northeast China	Scientific Reports
Not report the association with estimation(n=4)	Mizoue T, et al.	2005	Dietary patterns and colorectal adenomas in Japanese men - The self-defense forces health study.	American Journal of Epidemiology
	Atoum MF, et al.	2014	Association between circulating vitamin D, the TaqI vitamin D receptor gene polymorphism and colorectal cancer risk among Jordanians. Asian Pacific journal of cancer prevention	Asian Pacific Journal of Cancer Prevention
	Kumagai Y, et al.	2014	Dietary patterns and colorectal cancer risk in Japan: the Ohsaki Cohort Study.	Cancer Causes & Control
	Hessami Arani S, et al.	2017	Rising rates of colorectal cancer among younger iranians: Is diet to blame?	Current Oncology
Meeting proceeding (n=3)	Grant WB, et al.	2011	Ecological study findings regarding vitamin D and cancer.	Anticancer Research
	Li K, et al.	2014	The association between serum vitamin D concentrations and colorectal cancer.	Clinical Chemistry and Laboratory Medicine
	Ozer C, et al.	2015	The relationship between serum 25-hydroxy vitamin D levels and insulin resistance in breast and colon cancer.	Clinical Chemistry and Laboratory Medicine
Letter to the reviewer (n=2)	Dunnigan MG, et al.	1990	Serum 25-hydroxyvitamin D and colon cancer.	Lancet
	Qu B, et al.	2017	Role of Circulating and Supplemental Calcium and Vitamin D in the Occurrence and Development of Colorectal Adenoma or Colorectal Cancer.	J Clin Gastroenterol
Reports from same study sample (n=2)	Byeon JS, et al.	2007	Colorectal neoplasm in asymptomatic Asians: a prospective multinational multicenter colonoscopy survey.	Gastrointestinal Endoscopy
	Shin A, et al.	2011	Site-specific risk factors for colorectal cancer in a korean population.	PLoS ONE
Studied survivorship (n=2)	Wesa KM, et al.	2015	Serum 25-hydroxy vitamin D and survival in advanced colorectal cancer: a retrospective analysis.	Nutr Cancer
	Woo KW, et al.	2015	Vitamin D deficiency in Hong Kong advanced cancer patients: Result of first 50 patients.	Annals of Oncology
Vitamin D intake (n=2)	Mizoue T, et al.	2008	Calcium, dairy foods, vitamin D, and colorectal cancer risk: The Fukuoka colorectal cancer study.	Cancer Epidemiology Biomarkers and Prevention
	Ishihara J, et al.	2008	Dietary calcium, vitamin D, and the risk of colorectal cancer.	American Journal of Clinical Nutrition
Western population (n=1)	Sy AM, et al.	2013	Association between serum vitamin D levels and colonic carcinomatous polyps.	Journal of Gastrointestinal Cancer
Review (n=1)	Grant WB, et al.	2009	Ecological Studies Of Ultraviolet B, Vitamin D And Cancer Since 2000.	Annals of Epidemiology

**Table S3.** Newcastle-Ottawa Scale for assessing the quality of studies in the systematic review

Author (Year)	Country	Region	Selection				Comparability		Exposure			Total Score
			S1	S2	S3	S4	C1	C2	E1	E2	E3	
Budhathoki et al. (2018)[1]	Japan	Eastern Asian	★	★	★	★	★	★	★	★		8
Choi et al. (2015)[2]	Korea	Eastern Asian	★				★	★	★	★		5
Hong et al. (2012)[3]	Korea	Eastern Asian	★	★	★	★	★	★	★	★		8
Otani et al. (2007)[4]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Takahashi et al. (2010)[5]	Japan	Eastern Asian	★	★	★	★		★	★	★	★	8
Yamaji et al. (2012)[6]	Japan	Eastern Asian	★	★	★	★	★	★	★	★	★	9
Ying et al. (2015)[7]	China	Eastern Asian	★			★	★	★	★	★	★	7
Yurekli et al.(2015)[8]	Turkey	Western Asian	★				★	★	★	★		5

**Guidelines for review***Selection:*

S1, Case definition adequacy: ★a) requires independent validation (>1 person/record/time/process to extract information or reference to primary record sources such as colonoscopy or medical/hospital records); b) record linkage or self-report with no reference to primary record; c) no description

S2, Representativeness of the cases: ★a) consecutive or representative series of cases; b) potential for selection biases or not stated

S3, Selection of controls: ★a) community controls; b) hospital controls, within same community as cases; c) no description

S4, Definition of controls: ★a) no history of colorectal cancer or adenoma; b) no description of the source

*Comparability:*

C1, ★ Study adjusted for confounder, such as age and sex (the most important factors);

C2, ★ Study adjusted for any additional confounders (1> additional factors, e.g., BMI, drinking or smoking)

*Exposure:*

E1, Ascertainment of exposure: ★a) secure record (e.g., medical records); ★b) structured interview were blind to case/control status; c) interview not blinded to case/control status; d) written self-report or medical record only; e) no description

E2, Same method of ascertainment for cases and controls: ★a) yes; b) no

E3, Non-response rate: ★a) the same rate for both groups; b) non-respondents described; c) rate different and no designation

**Figure S1.** Subgroup meta-analysis, including stratified by outcome (colorectal cancer or colorectal adenoma, **S1A**), sex (women, men or both, **S1B**), blood sample type (serum or plasma, **S1C**), range ( $\leq 15$  or  $>15$  ng/mL **S1D**), subregion (Eastern Asia or Western Asia, **S1E**).

**Figure S1A**

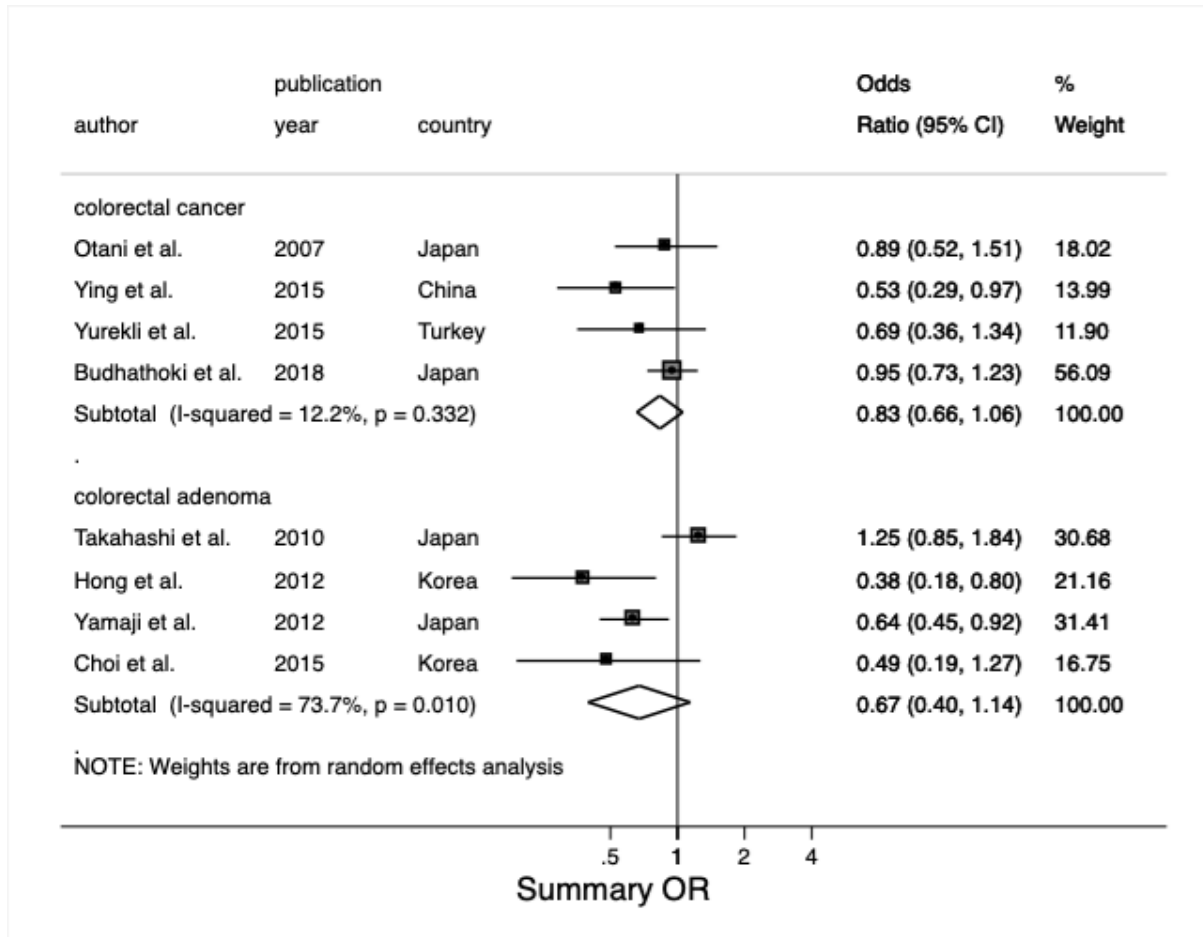


Figure S1B

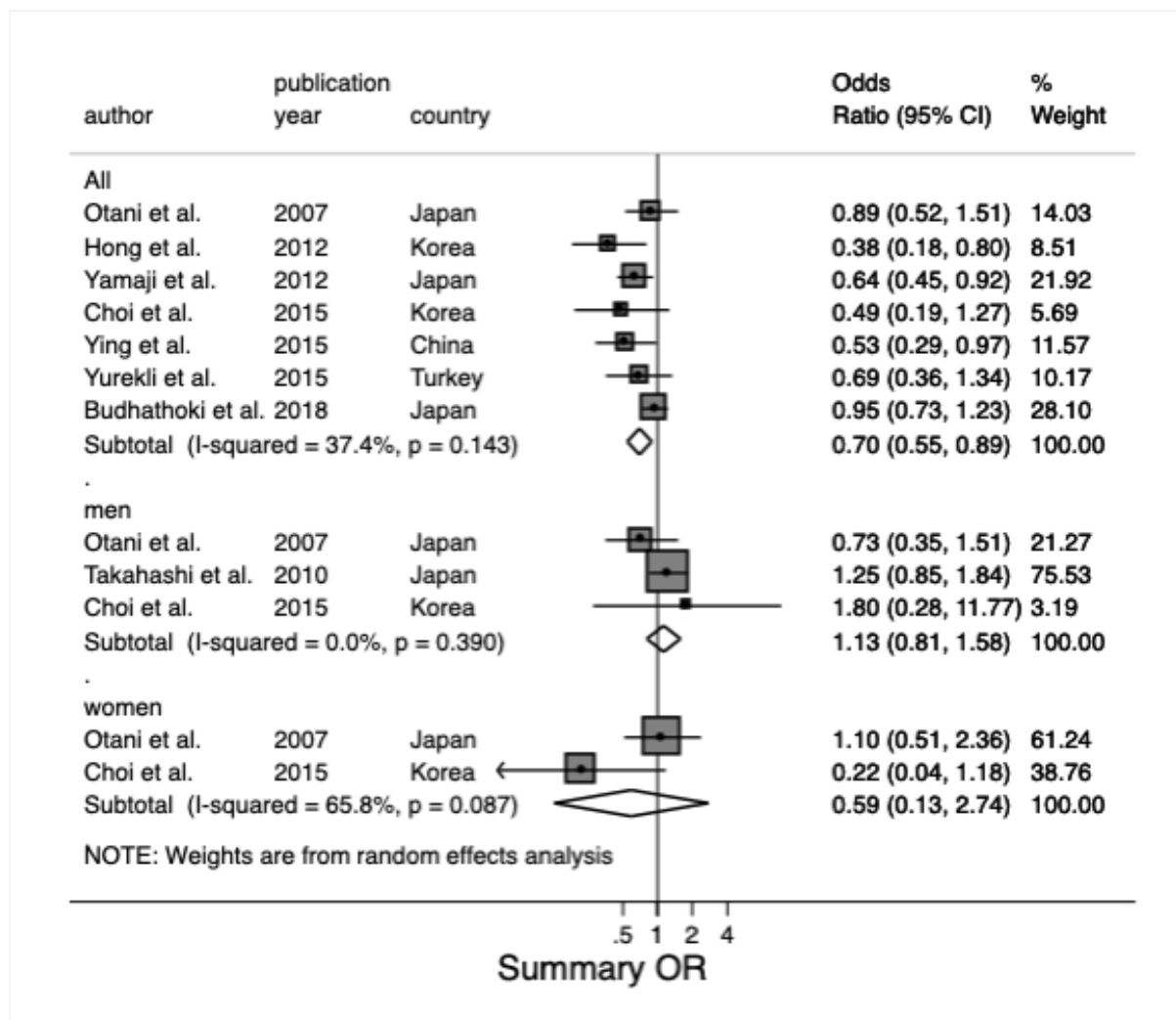


Figure S1C

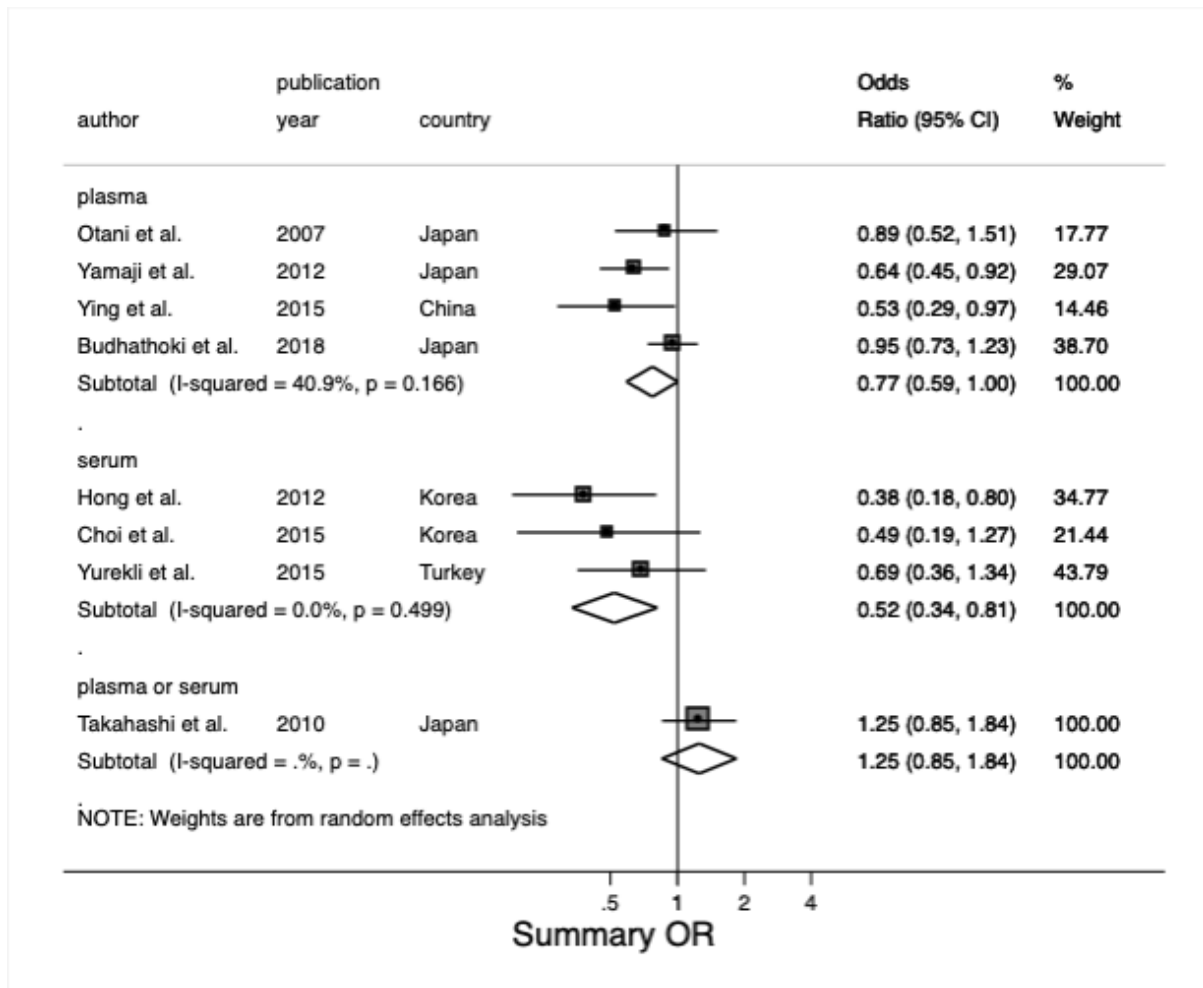


Figure S1D

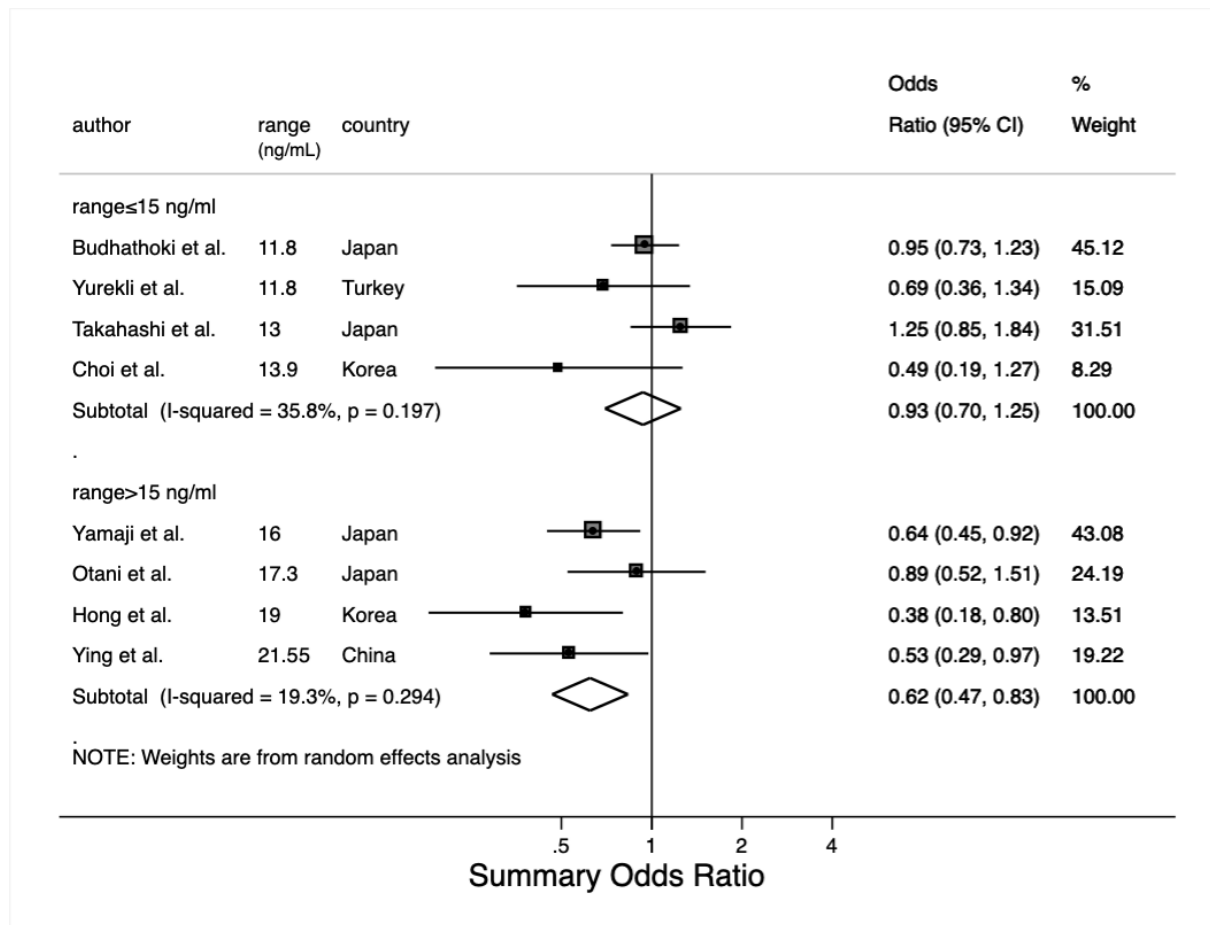
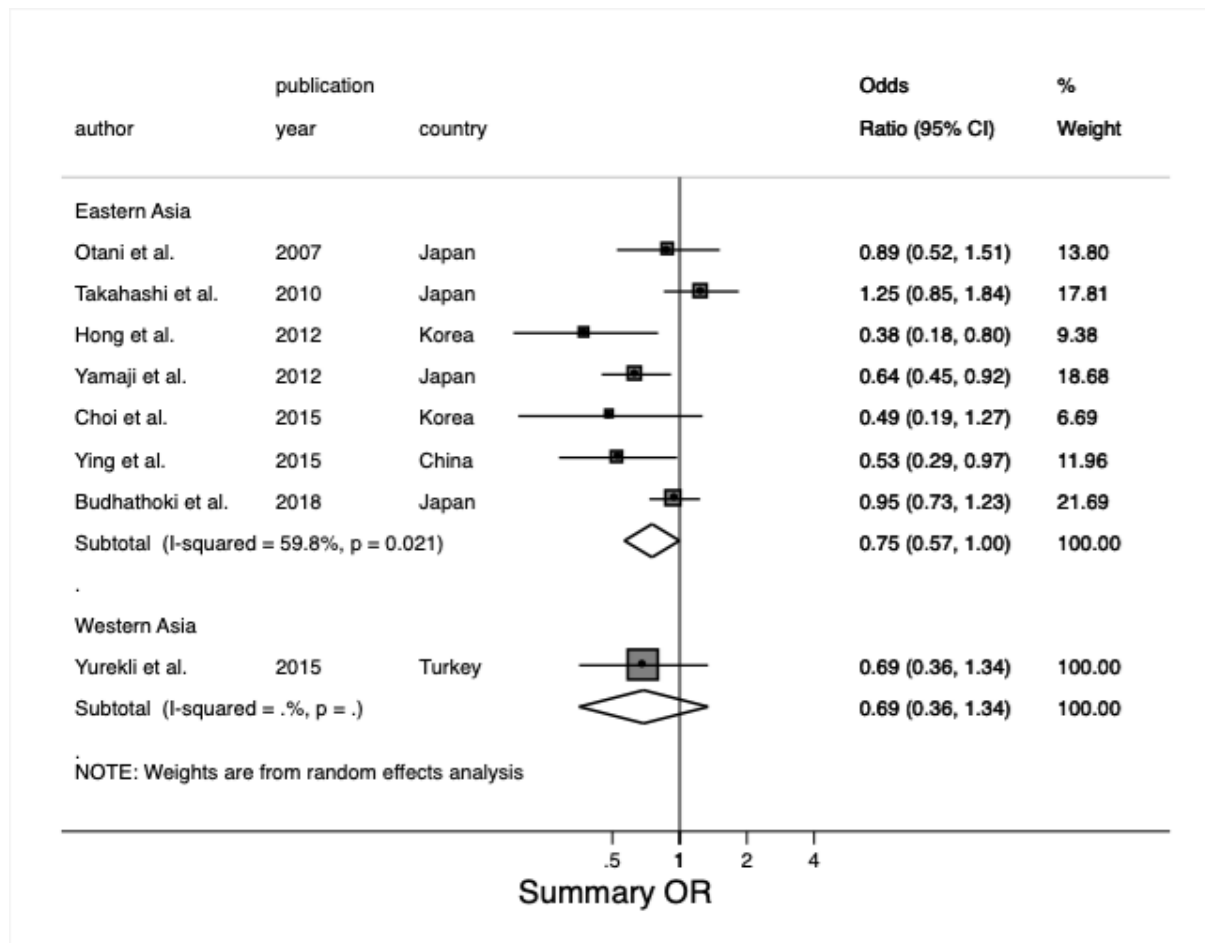
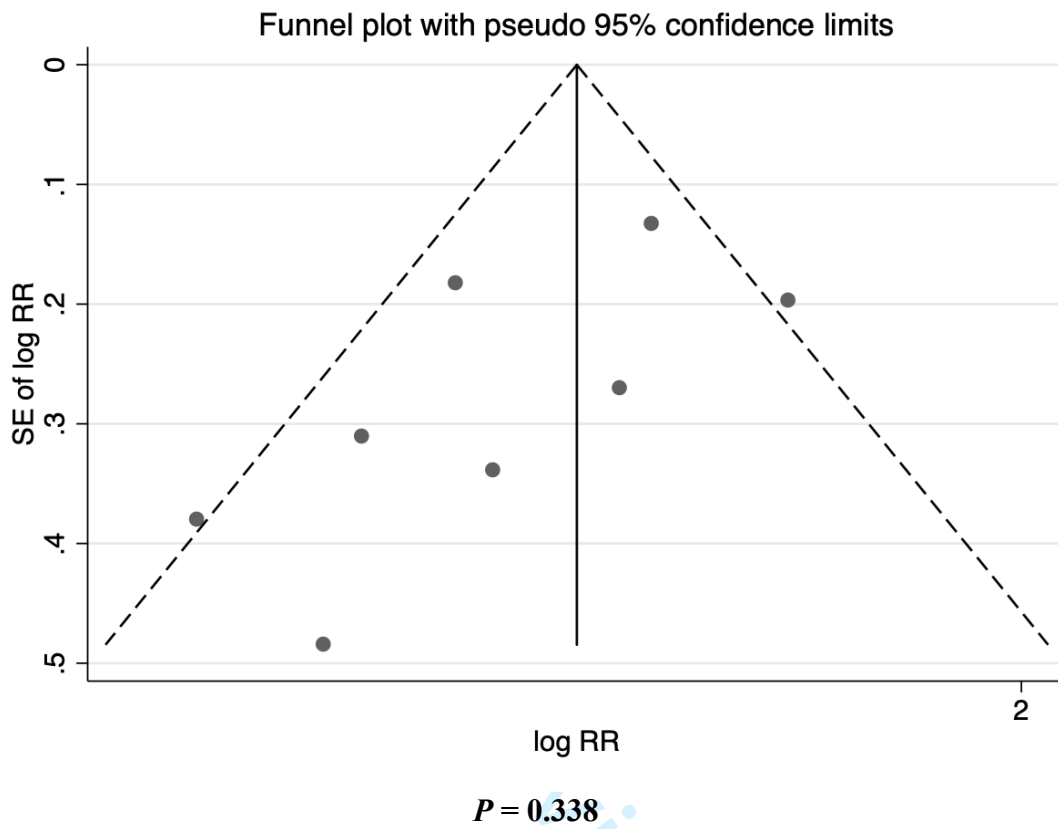


Figure S1E





**Figure S2.** Funnel plot for all studies included in the meta-analysis and between blood circulating Vitamin D and the colorectal cancer risk in Asian countries





# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Page 1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Page 3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Page 5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 6
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Page 5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Page 7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Page 7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Page 7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Page 8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Page 8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Page 8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Page 9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I <sup>2</sup> ) for each meta-analysis.	Page 9



# PRISMA 2009 Checklist

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Page 10
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 11 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Page 11, Table 1 and Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Page 11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Page 11 and Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Page 11 and 12
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Page 12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Page 12 and 13
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Page 14 to Page 16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Page 17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 18
<b>FUNDING</b>			
For peer review only - <a href="http://bmjopen.bmj.com/site/about/guidelines.xhtml">http://bmjopen.bmj.com/site/about/guidelines.xhtml</a>			



# PRISMA 2009 Checklist

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Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Page 2
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From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).

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