Vitamin D and liver cancer risk: a meta-analysis of prospective studies

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ABSTRACT

Background and Objectives: The association between circulating vitamin D and liver cancer risk has been controversial on the basis of epidemiological studies. The aim of this study was to quantitatively evaluate this association with prospective studies. **Methods and Study Design:** A systematic literature search was implemented in PubMed and Scopus databases up to June 2019. Using a random-effects model, the multivariate-adjusted relative risks (RRs) with corresponding 95% confidence interval (CI) were pooled for the highest versus lowest category. Trend estimation was conducted with a two-stage dose-response meta-analysis. **Results:** Six independent prospective studies (992 liver cancer events and 60,811 participants) were included for data synthesis. The summary estimate showed that a higher circulating vitamin D was associated with lower risk of liver cancer (Summary RR=0.78; 95% CI: 0.63, 0.95; I^2=53.6%, p=0.035). Dose-response analysis indicated that liver cancer was associated with 8% (95%CI: 0.89, 0.95) lower risk with a 10 nmol/L increment of circulating vitamin D concentration. **Conclusions:** The present study provides substantial evidence that a higher concentration of circulating vitamin D would have conferred protection against liver cancer.

Key Words: vitamin D, liver cancer, prospective study, meta-analysis, dose-response analysis

INTRODUCTION

As one of the most common malignancies, liver cancer is the sixth common cancer and ranks the second leading cause of cancer-related death worldwide.[1] Chronic virus infections such as hepatitis B virus (HBV) and hepatitis C virus (HCV), have been confirmed as the major risk factors of liver cancer events. Other than virus infections, smoking, drinking and exposure to aflatoxin B1 have shown to be associated with the risk of liver cancer.[2] Although approximate 85% events of liver cancer is occurred in developing countries, the incidence of liver cancer has risen in Western countries in parallel with obesity and non-alcoholic fatty liver disease, due to changes in lifestyles and environments.[3] As a highly malignant tumor, liver cancer, which is usually diagnosed as late stages, generally has an average of five-year survival rate < 15% because of limited treatment options.[4] Thus, it is necessary and urgent to identify the favourable factors for protection against liver cancer in regions where chronic virus infections and exposure to aflatoxins are less prevalent.

Accumulating evidence has suggested that dietary factors play critical roles for cancer prevention, and several protective factors have been identified to be associated with lower risk of liver cancer, such as vegetable, green tea and coffee, etc.[5-7] As a secosteroid hormone, the biologically active metabolite of vitamin D is attributed to 1,25-dihydroxyvitamin D [1,25(OH)2D], which is synthesized from the circulating 25-hydroxyvitamin D [25(OH)D] by 1a-hydroxylase (CYP27B1). It has been reported that 1,25(OH)2D exerts various kinds of biological actions after binding to vitamin D receptor. In addition to the functions of vitamin D on skeletal tissue and calcium homeostasis, growing evidence has shown that the benefits might be involved in immune modulation, cell differentiation and inflammation regulation as well.[8,9] Considering that liver is the main synthesis site of vitamin D-binding protein and 25(OH)D, we hypothesized that vitamin D might be directly responsible for liver-related diseases, including liver cancer. Cell line and animal models have indicated that vitamin D contributes to anti-proliferative, pro-differentiating and anti-inflammation effects on malignant cells, suggesting a chemopreventive role in carcinoma.[10,11] Regarding epidemiological studies, there has been growing interest in the relationship between circulating vitamin D and liver cancer risk. However, the findings have been still inconsistent and controversial.[8,12-16] Therefore, we conducted a systematic review and meta-analysis to quantitatively assess the association of circulating vitamin D with liver cancer risk. Furthermore, a dose-response analysis was performed trend estimation.

MATERIALS AND METHODS

Study selection

The criteria for Meta-analysis of Observational Studies in Epidemiology (MOOSE) were followed to conduct the present study. A systematic literature search was implemented with the databases of PubMed and Scopus up to June 2019. Vitamin D, VD, 25-hydroxyvitamin D, 25(OH)D, 1,25-dihydroxyvitamin D or 1,25(OH)2D were paired with liver cancer, hepatocellular carcinoma or cancer as search terms. Additionally, references of recent reviews and meta-analyses were also scrutinized to identify the potential publications.

Inclusive criteria

To be included in the meta-analysis, the studies had to meet the criteria as follows: 1) prospective studies which included nested case-control, case-cohort and prospective cohort studies; 2) serum/plasma and whole blood circulating 25(OH)D concentration as exposure; 3)

liver cancer as outcome of interest; 4) the eligible studies which provided the relative risk (RR) with corresponding 95% confidence interval (CI).

Data extraction

The data of included studies were independently extracted by two investigators (TZ and J-MH), and any discrepancy was resolved with the third investigator (Duo Li) to reach agreement. The following information of the eligible studies was extracted, including the surname of the first author, published year, region/nation, gender, duration of follow-up, mean age at baseline, liver cancer events, participants, adjusted covariates, and RR with corresponding 95% CI. The Newcastle-Ottawa Scale criteria with a 9-star system was adopted to perform quality assessment. The scoring system summarized 9 aspects of each eligible study. The full score was defined as 9 stars, and a study was classified as low, moderate and high-quality with stars of 0-3, 4-6 and 7-9, respectively.[17]

Statistical analysis

RR was regarded as the common risk estimate for the relationship between circulating vitamin D concentration and liver cancer risk. Multivariate-adjusted RRs with the corresponding 95% CIs for the highest versus lowest category were logarithm transformed, and the summary RR was calculated by using a random-effects model, as weighted by the inverse of their variance.[18] The I^2 statistic was used to evaluate the heterogeneity between studies, with values of 25%, 50% and 75% as cut-off points indicting low, moderate and high degree of heterogeneity.[19] The I^2 value >50% was regarded as indicative of heterogeneity according to the Cochrane Handbook.[20] To explore the sources of heterogeneity, stratified analysis was conducted based on region, gender and biospecimen determination. The eligible studies, which provided 3 or more categories, were available for trend estimation. The median concentration of circulating 25(OH)D assigned in respective quantiles was extracted. The midpoint of the lower and upper categories was regarded as the 25(OH)D concentration of the quantile if the median concentration was not provided. The concentration of 25(OH)D was defined as 1.2-fold of the highest boundary if the highest quantile was open-ended.[21] Meanwhile, the 25(OH)D concentration of the lowest quantile (the reference) was set as zero in each study.[21] Trend estimation was implemented with a two-stage random dose-response meta-analysis. By using a restricted cubic spline model, curvilinear trend was done by modelling the concentration of 25(OH)D with 3 knots at percentiles (25%, 50% and 75%) of the distribution.[22] A *p* value for curvilinear association was calculated by testing the null

hypothesis that the coefficient of the second spline was equal to zero.[23] In the presence of linear trend (p for nonlinearity >0.05), a linear dose-response meta-analysis was conducted for trend estimation by using the generalized least squares trend estimation as described by Greenland and Longnecker and Orsini et al.[24,25] If the studies did not provide the number of cases, number of participants or person years, dose-response estimation was performed with variance weighted least squares regression model. To examine whether any study exerted substantial influence on the pooled RR, sensitivity analysis was performed with deletion one study at a time, and the summary estimate was re-calculated. By using Egger's rank correlation test, publication bias was implemented with significant level at p<0.1.26 STATA 11.0 for windows (Stata Corp, College station, TX) was adopted for statistical analysis. Two-trailed p<0.05 was considered as statistically significant difference.

RESULTS

Literature search

The process of literature search is presented in Figure 1. There were 15,026 unique citations identified from PubMed, Scopus, and manual search with deletion of duplicates. After screening titles and abstracts, 14,988 studies were excluded, leaving 38 studies for full-text examination. Of these, 32 studies were not eligible for the present study because they did not meet the inclusive criteria (e.g., without provided detailed data and study design). Finally, 6 independent prospective studies were eligible for data synthesis.[8,12-16]

Study characteristics

The basic information of the eligible studies is listed in Table 1. Two prospective studies were conducted in Asia,[8,14] and remaining studies were performed in Western countries.[12,13,15,16] The majority of studies used chemiluminescence immunoassay to determine the concentration of 25(OH)D.[8,12,15,16] Besides, one study used enzyme immunoassay kit to measure the concentration of 25(OH)D,[14] and another study adopted liquid chromatography/tandem mass spectrometry (LC-MS/MS) method.[13] There were a total of 60,811 participants, of which 992 liver cancer events were diagnosed with 6-28 years of follow-up. On the basis of Newcastle-Ottawa scale criteria, three prospective studies were classified as high-quality,[8,13,14] and the remaining studies were classified as moderate-quality (Supplementary Table 1).[12,15,16]

Circulating vitamin D and liver cancer risk

As shown in Figure 2, the association of circulating vitamin D with liver cancer risk was pooled with six independent prospective studies, and a higher circulating vitamin D was associated with lower risk of liver cancer (Summary RR=0.78; 95%CI: 0.63, 0.95), with significant between-study heterogeneity (I^2=53.6%, p=0.035). Five independent studies provided available data to perform trend estimation between circulating vitamin D and liver cancer risk.[8,13-16] Non-significant curvilinear association was found between vitamin D concentration and liver cancer risk; however, a significant linear association was observed (p for trend <0.001) (Figure 3). Dose-response analysis indicated that a 10 nmol/L increment of circulating vitamin D concentration was associated with 8% lower risk of liver cancer (95% CI: 0.89, 0.95).

Subgroup analysis

The studies stratified by region indicated that a higher circulating vitamin D was inversely associated with a lower risk of liver cancer in Asia, but not in Western countries. Besides, the concentration of vitamin D, which was measured in serum, was associated with lower risk of liver cancer, but not in plasma. However, there was non-significant difference between groups with meta-regression analysis. Considering that limited studies were included for subgroup analysis, the findings should be explained with caution (Supplementary table 2).

Publication bias and sensitivity analysis

In a sensitivity analysis, each study was sequentially deleted at a time, and the remaining data were re-calculated. The results showed that the summary estimate was not substantially driven with exclusion of any one study (Supplementary figure 1). In publication bias analysis, non-significant publication bias was observed with Egger's rank correlation test (p=0.175).

DISCUSSION

To the best of our knowledge, the present meta-analysis was the first to evaluate the association of circulating vitamin D concentration with liver cancer risk. Convincing evidence indicated that a higher circulating vitamin D was associated with 22% (95% CI: 0.63, 0.95) reduction of liver cancer risk. A significant linear association was observed (p for trend <0.001), and dose-response analysis showed that liver cancer was 8% lower risk (95%CI: 0.89, 0.95), with a 10 nmol/L increment of circulating vitamin D concentration.

As the essential components of vitamin D signalling, 25(OH)D and vitamin D-binding protein are mainly synthesized in the liver. Therefore, we hypothesized that vitamin D insufficiency might be responsible for liver-related diseases. Although substantial cell lines and animal models showed that vitamin D exerted beneficial effects in the liver, including anti-proliferative, anti-inflammatory and pro-apoptotic properties, the associations of circulating vitamin D with liver cancer risk have been inconsistent in epidemiological studies.[8,12-16] The Linxian Nutrition Intervention Trials, which was the first cohort study, explored the association of vitamin D with liver cancer risk with 22 years of follow-up. Due to the high prevalence of chronic hepatitis infection and lower concentration of vitamin D in the study participants (median of 20.1 nmol/L), non-significant association was observed.[14] Additional cohort study, which was conducted in the Copenhagen City Heart Study, have shown null association between vitamin D and liver cancer risk, and the reason might be attributed to a small number of liver cancer events (n=55).[12] On the contrary, a nested case-control study from the European Prospective Investigation into Cancer and Nutrition cohort (EPIC) showed an inverse association between circulating vitamin D and liver cancer risk with an average of 6 years follow-up.[13] Recently, two prospective studies also found that a higher circulating vitamin D concentration was associated with lower risk of liver cancer.[8,16] Although the inclusive prospective studies have shown controversy and inconsistent associations, the summary estimate provided substantial evidence that a higher circulating vitamin D has beneficial effects in the prevention of liver cancer.

The underlying mechanisms why higher circulating vitamin D has conferred protection against liver cancer have summarized as follows. First, vitamin D through its active form 1,25(OH)2D regulates a variety of signalling pathways, and has demonstrated direct effects on cell proliferation, differentiation and cell death. Besides, vitamin D has been shown to have anti-inflammation, improve oxidative stress, regulate immune responses, and that might contribute to inhibiting the initiation and development of tumor cell.[11,27,28] Second, vitamin D-binding protein and 25(OH)D are mainly synthesized in the liver, thus vitamin D might have a direct effect on liver-related diseases, including liver cancer. Third, vitamin D has reported to prevent chromosomal aberrations and DNA stand breaks, and that might protect against liver cancer.[29] Fourth, chronic liver disease and insulin resistance have been reported to be associated with risk of liver cancer.[30] Accumulating correlation analyses indicated that a lower circulating concentration of vitamin D was associated with increased risk of chronic liver disease,[31] and supplemental vitamin D significantly improved fasting glucose and insulin

resistance in patients with diabetes and non-alcoholic fatty liver disease.[32-34] Thus, higher circulating vitamin D is beneficial for liver cancer prevention.

The strengths of the present study should be put forward. First, this is the first meta-analysis to explore the association of circulating vitamin D with risk of liver cancer. The relatively large sample-size with strong statistical power provided convincing evidence that a higher circulating vitamin D was inversely associated with risk of liver cancer. Second, the concentration of vitamin D was precisely measured with chemical method, thereby reducing the recall bias and recording errors by using food-frequency questionnaires. Third, the studies included in the present study were prospective studies with long period of follow-up and enough liver cancer events, thus this study has sufficiently powered to capture the inverse association between vitamin D and liver cancer risk. Additionally, the summary estimate was not substantially driven after deleting any one study at a time with sensitivity analysis, indicating stability of the pooled effect. Besides, there was non-significant publication bias with Egger's test, suggesting that unpublished studies or missing data did not exert the summary estimate. Simultaneously, the limitations of the study should be considered. First, although the multivariate-adjusted RRs were extracted for data synthesis, the report bias and residual confounding factors are inevitable to influence the summary estimate. Second, the inclusive studies used different methods to assess the concentration of vitamin D, including liquid chromatography/tandem mass spectrometry, chemiluminescent immunoassay and enzyme immunoassay kit. Measurement bias is unavoidable for the pooled effect, although the methodology of summary estimate is calculated with the highest versus the lowest category.

Conclusion

The present study provides strong evidence that a higher circulating vitamin D is inversely associated with the risk of liver cancer, and this association demonstrates a linear trend. Since the majority of the prospective studies are conducted in the Western countries, further prospective studies should be conducted in other regions and ethnic origin to confirm this association.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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Table 1. Characteristics of included studies for circulating vitamin D associated with liver cancer risk

First author	Publication year and region	Age (gender)	Subjects (cases)	Follow-up period, year	Exposure assessment	Diagnosis method	Exposure	Covariates adjusted
Afzal	2013, Denmark.	(M) 58	9,791 (55)	28	Chemiluminescence immunoassay	Registry	Plasma 25(OH)D	Age, sex, pack-years, BMI, alcohol intake, leisure time and work-related physical activity, duration of education, and month of blood sample
Budhathoki	2018, Japan	56.2±7.5 (Both)	3,301 (165)	19	Chemiluminescent immunoassay	Registry	Plasma 25(OH)D	Age, sex, BMI, smoking, alcohol intake, physical activity, family history of cancer, and reported history of diabetes
Fedirko	2014, European	59.9±7.3 (Both)	138 (138)	6	LC-MS/MS	Registry	Serum 25(OH)D	Smoking status, BMI, alcohol intake, and coffee intake
Lai	2018, Finnish	57 (M)	427 (202)	25	Chemiluminescence immunoassay	Registry	Serum 25(OH)D	Age, date of blood draw, BMI, history of diabetes, number of years smoked, and daily intake of alcohol and coffee
Wang	2013, China	55 (Both)	1,063 (226)	22	Enzyme immunoassay kit	Pathology	Serum 25(OH)D	Age, sex, smoking, drinking, BMI, season of blood draw, HBsAg, HBcAg, and HCsAg
Weinstein	2018, European	58 (Both)	4,616 (206)	28	Chemiluminescence immunoassay	Registry	Serum 25(OH)D	Age, BMI, number of cigarettes smoked per day, years of smoking, physical activity, serum cholesterol, history of diabetes, family history of cancer, systolic blood pressure, trial intervention group, calendar year of diagnosis, and prior cancer diagnoses

BMI: body mass index; LC-MS/MS: liquid chromatography/tandem mass spectrometry; 25(OH)D: 25-hydroxyvitamin D.

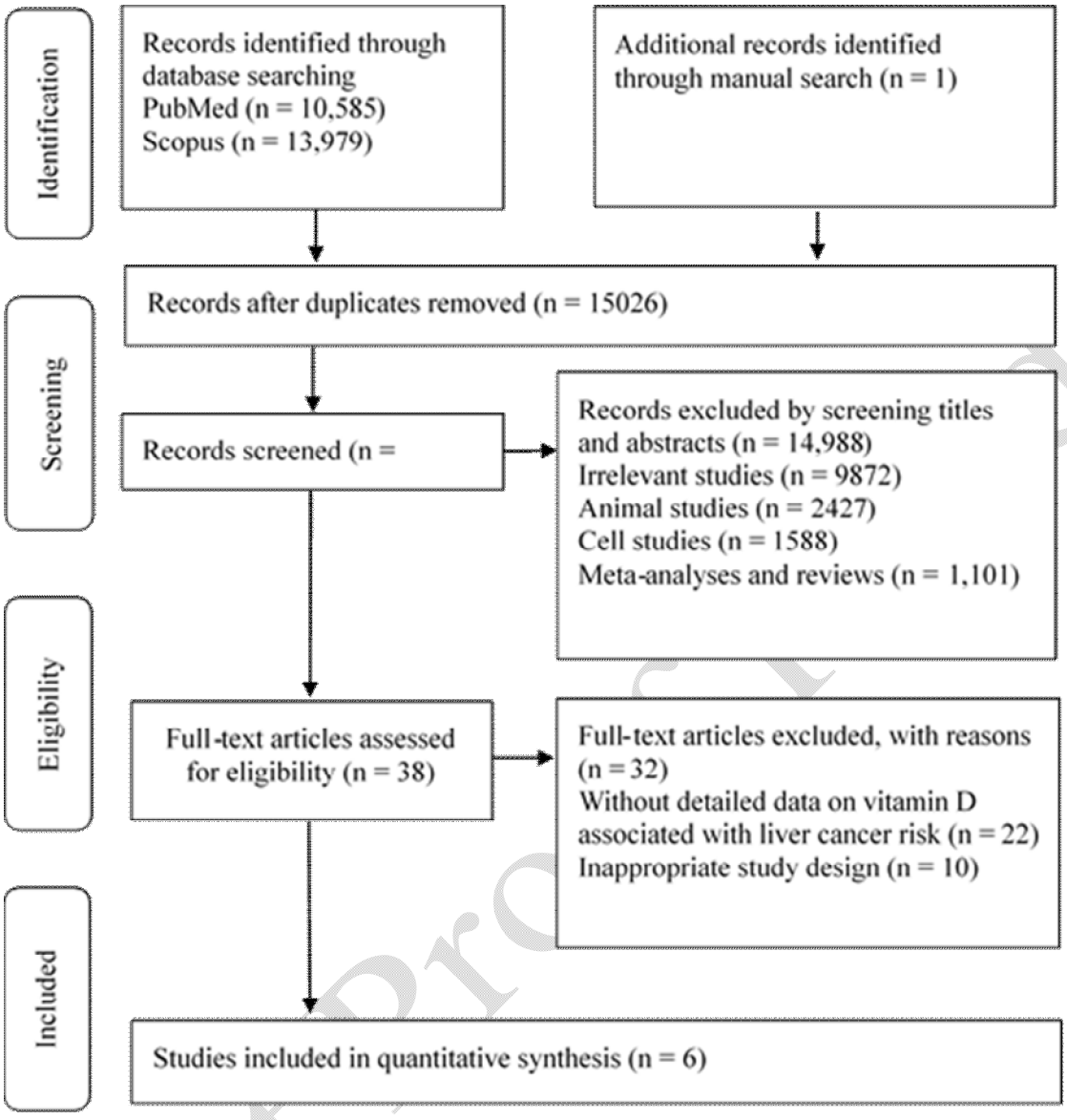

Figure 1. The flow diagram for detailed steps of literature search.

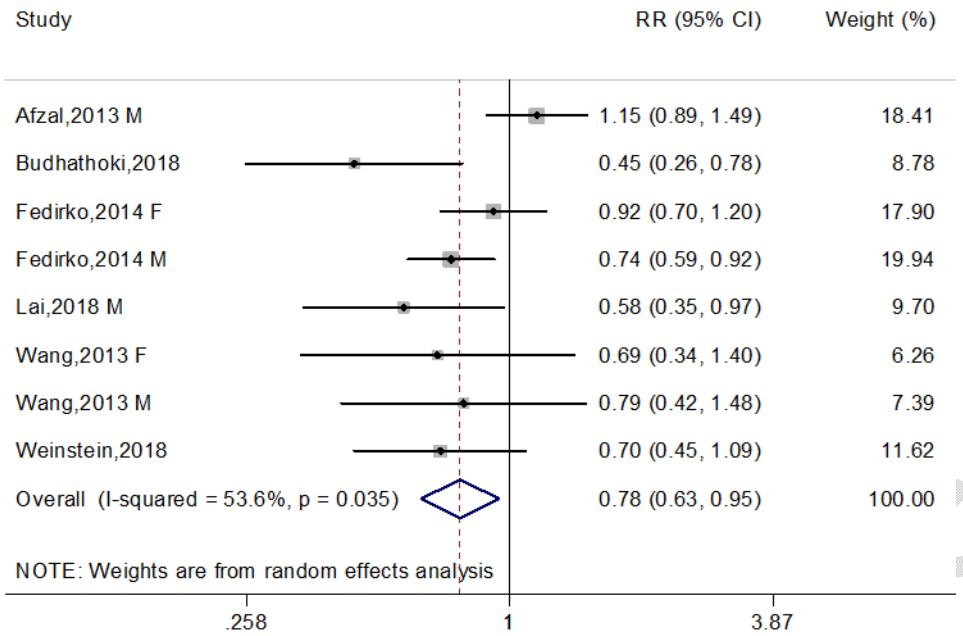

Figure 2. The association of circulating vitamin D with risk of liver cancer risk comparing the highest with lowest category. The size of the gray box representing each risk estimate was proportional to the weight. The diamonds represent summary relative risk.

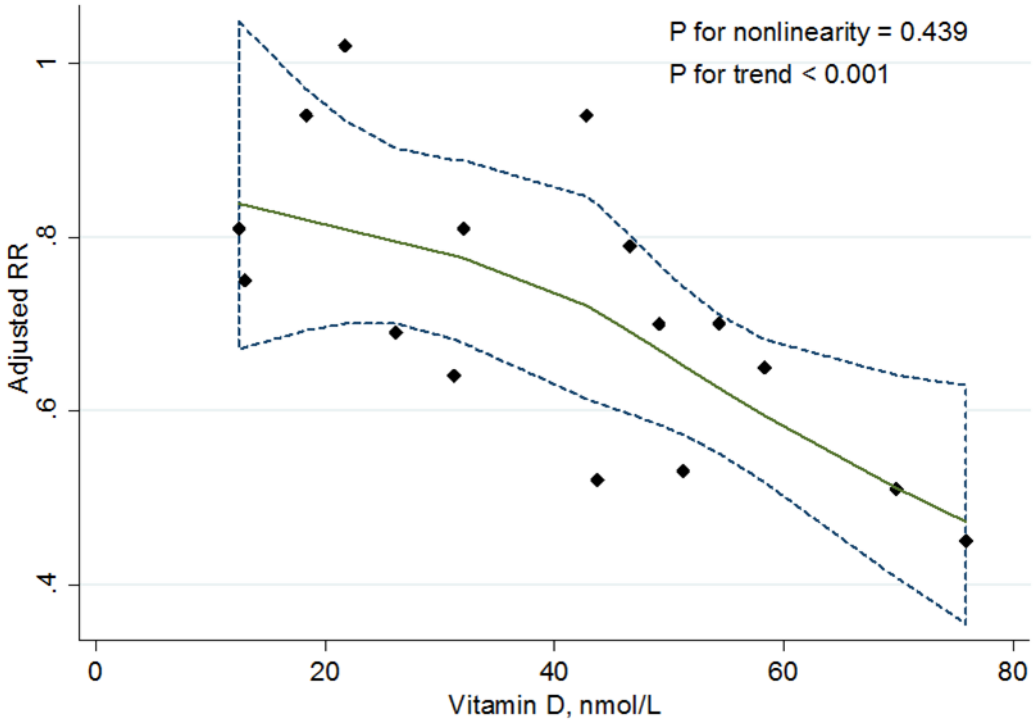

Figure 3. Trend estimation between circulating vitamin D and liver cancer risk. Adjusted RRs from all categories in each study were separately represented by the small black diamonds, and corresponding curvilinear association was represented by the navy short-dash line using restricted cubic splines model with three knots at fixed percentiles 25%, 50%, and 75% of the distribution.