The Relationship between Serum Vitamin D Levels, C- Reactive Protein, and Anxiety Symptoms

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Objective The aim of study is to investigate the relationship between serum vitamin D, c-reactive protein (CRP) levels, and anxiety symptoms.

Methods Serum vitamin D and CRP levels of 51,003 Korean adult participants were collected retrospectively. Anxiety symptoms were assessed using the Korean version of Beck Anxiety Inventory. Logistic regression was used to estimate the odds ratio (ORs) of anxiety symptoms by serum vitamin D and CRP levels. The regression was adjusted for covariates, and each model was adjusted mutually for vitamin D and CRP levels.

Results Compared with sufficient vitamin D levels (≥20 ng/mL), insufficient (10–19.99 ng/mL) and deficient (<10 ng/mL) vitamin D levels were significantly associated with risk of anxiety symptoms. Also, continuous vitamin D levels were negatively associated with the risk of anxiety symptoms. CRP levels did not affect the relationship between vitamin D levels and risk of anxiety symptoms.

Conclusion Insufficient (10–19.99 ng/mL) and deficient (<10 ng/mL) vitamin D levels were significantly associated with risk of anxiety symptoms. After adjusting for CRP levels, the results were not changed, and no evidence of interaction between vitamin D and CRP levels was found. CRP levels did not account for the association between vitamin D levels and risk of anxiety symptoms.

INTRODUCTION

Anxiety is a common psychiatric symptom in the general population. The global current prevalence of anxiety disorders has been reported to be 7.3–28%. When anxiety disorders are considered as comorbid as well as primary diagnoses, the prevalence is over three-quarters of all psychiatry disorders. The first line of treatment for ‘any’ anxiety disorder involves both antidepressant medication and cognitive-behavioral therapy. However, most clinical trials have reported that response rates of anxiety disorders are at least 50–60%. Therefore, there is a need for novel therapeutic and preventive strategies.

Recently, vitamin D has drawn attention as a new biomarker or supplement in the psychiatric field. According to the recent meta-analyses and systematic review studies, vitamin D deficiency is reported to be associated with psychiatric disorders including depression, neurocognitive disorders, psychotic disorders, autism spectrum disorders, and obstructive sleep apnea. However, few studies have investigated the relationship between serum vitamin D levels and anxiety. Armstrong et al. reported that vitamin D deficiency was associated with a higher level of anxiety and depressive symptoms in patients with fibromyalgia. A study by de Koning et al. found that individuals with lower levels of vitamin D showed significantly more symptoms of anxiety in old age. However, when they examined the relationship between vitamin D levels and anxiety separately from depressive symptoms, results were no
longer statistically significant. Wu et al.\textsuperscript{20} reported that serum vitamin D status is related to the occurrence of anxiety in post-stroke patients. However, these studies were limited by various methodological challenges; some only included certain subgroups of the population,\textsuperscript{6,10} while others used a small sample.\textsuperscript{6,10} Furthermore, considerations of possible confounding factors have differed widely across studies. Therefore, it is important to investigate the relationship between serum vitamin D levels and anxiety in a large sample of general adults.

In terms of the biological mechanisms for the association between serum vitamin D levels and anxiety, since vitamin D is known to upregulate anti-inflammatory mediators and reduce inflammatory molecules in inflammatory and immune cells,\textsuperscript{11} it is important to identify whether inflammation functions as a mediator or moderating factor in the relationship between serum vitamin D levels and anxiety. Besides, current studies examining the relationship between inflammation and anxiety suggest an association between elevated levels of CRP and anxiety.\textsuperscript{12-14}

Therefore, this study aimed to examine the relationship between vitamin D levels, CRP levels, and anxiety symptoms, and to investigate whether vitamin D and CRP levels mediate each other’s association with anxiety symptoms.

**METHODS**

**Study population**

Our study was a part of the Kangbuk Samsung Health Study in which participants were South Korean adults aged 18 years and older who had undergone an annual or biennial health screening examination at the health promotion centers of Kangbuk Samsung Hospital in Seoul and Suwon, South Korea. All data of the Kangbuk Samsung Health Study were collected naturalistically and retrospectively.

In South Korea, the Industrial Safety and Health Law guarantees all workers annual or biennial health examinations free of charge. Therefore, employees of various companies and their family members accounted for over 80% of this study’s participants. The remaining participants (less than 20%) were individuals taking health screening examinations voluntarily. In addition, since all data of the Kangbuk Samsung Health Study are collective naturalistically.

**Selection of a “healthy” sample**

The current study used a cross-sectional design with 53,478 persons aged above 18 years who visited Kangbuk Samsung Hospital’s health screening centers between January 2012 and December 2016. Considering the possibility of reverse causation, data were used to select a “healthy” sample without any evidence of current physical illness or psychiatric disorder. Those who had psychiatric disorders or took psychiatric medication were excluded. Since physical illness is an independent risk factor of mental disorders and results in physical inactivity which can disturb vitamin D synthesis from sun exposure, those who had chronic medical illnesses (stroke, brain hemorrhage, Alzheimer dementia, Parkinson’s disease, rheumatoid arthritis, inflammatory bowel disease, emphysema, chronic bronchitis and asthma) and acute infection (CRP ≥10 mg/L) were also excluded.\textsuperscript{12,13} The number of final eligible participants was 51,003 (Figure 1).

The study protocol was approved by the Institutional Review Board of Kangbuk Samsung Hospital (KBSMC 2019-01-042). The requirement for informed consent was waived because we used de-identified data that was routinely collected during the health screening visits.

**Serum 25-hydroxyvitamin D levels**

Blood specimens were obtained after a 10-hour fast. Serum levels of 25(OH) D were measured by electrochemiluminescence immunoassay (ECLIA) using Modular E170 (Roche diagnostics, Tokyo, Japan). During the study period, the coefficients of variation of the low-level and high-level Quality Control materials were 3.21–5.65% and 2.10–4.03%, respectively. According to the recent clinical guidelines, serum 25(OH) D levels were categorized into three groups: vitamin D deficiency (<10 ng/mL), insufficiency (10–19.99 ng/mL), and sufficiency (≥20 ng/mL).\textsuperscript{16-18}

**C-reactive protein**

Blood specimens were obtained after a 10-hour fast. Serum CRP levels were estimated by particle-enhanced turbidimetric immunoassay using Modular P800 (Roche diagnostics, Tokyo, Japan). During the study period, the coefficients of variation of the low-level and high-level Quality Control materials were 1.05–2.02% and 1.34–4.14%, respectively. According to the previous studies examining the relationship between serum levels of CRP and mental disorders, serum CRP levels were categorized into three groups: low (≤3 mg/L), high (3.01–10 mg/L), and abnormal (>10 mg/L). Since the CRP levels higher than 3 mg/L are defined as low-grade inflammation, we set the cut-off at 3 mg/L for distinguishing between low and high levels.\textsuperscript{13}

**Assessment of anxiety symptoms**

Anxiety symptoms were measured using Beck Anxiety Inventory (BAI). Internal consistency and test-retest reliability of the Korean BAI have been reported as 0.91–0.93 and 0.84, respectively.\textsuperscript{19,21} According to the previous studies, BAI scores of 16 and above were defined as clinically anxious state and caseness.\textsuperscript{21,22}
Potential confounding variables at baseline

Data about socio-demographic characteristics and anthropometric measurements were collected from health screening examinations. Socio-demographic factors including age, sex, location of the health screening center (Seoul or Suwon), marital status, education, income, alcohol habit, smoking status, medication history, and personal medical history were assessed via a self-report questionnaire. Problematic alcohol consumption was assessed using the Korean version of the Alcohol Use Disorders Identification Test (AUDIT). Body mass index (BMI) was included as a covariate because obesity is associated with increased CRP levels, anxiety, depression, and decreased vitamin D levels. Also, considering that vitamin D levels can increase from exposure to sunlight, seasonal data were included as covariate: spring (March–May), summer (June–August), fall (September–November) and winter (December–February). Furthermore, since anxiety and depressive symptoms were highly correlated, depressive symptoms were measured using the Center for Epidemiologic Studies Depression Scale (CES-D) and adjusted accordingly.

Statistical analysis

Descriptive statistics were used to summarize the baseline characteristics according to case-level anxiety symptoms. Besides, the t-test and chi-square tests were used to examine differences between anxious and non-anxious groups by categorical and continuous vitamin D and CRP levels. Logistic regression was used for calculating the odds ratio (OR) for case-level anxiety symptoms (BAI score ≥16) by categorical and continuous levels of serum vitamin D and CRP levels, with adjustments for age, sex, center (Seoul or Suwon), marital status, education, income, alcohol consumption, smoking status, season of data collection, BMI, and CES-D score. The regression models were also adjusted for categorical vitamin D levels and CRP status mutually to examine whether they affected each other’s relationship with anxiety symptoms. In-
teractions between serum vitamin D and CRP levels were also examined. The statistical analyses were performed using STATA version 14.0 (StataCorp LLC, College Station, TX, USA). All p-values were two-tailed. The p-values of <0.05 were considered statistically significant.

RESULTS

The characteristics of the healthy sample of 51,003 individuals are summarized in Table 1. Among 51,003 respondents, 3,818 (7.5%) were anxious and 47,185 (92.5%) were not anxious. The mean ages were 36.44±8.57 years (range: 18–84 years) and 38.10±8.31 years (range: 18–80 years) in the anxious and control group, respectively. Anxious participants accounted for 1,711 (5.5%) and 2,107 (10.7%) among men (31,301) and women (19,702), respectively. The non-anxious participants were more likely to be married, more likely to be smokers, have a higher education level, have lower income levels, lower AUDIT, and CES-D scores than the anxious participants.

As shown in Table 2, the mean level of vitamin D was lower in anxious than in non-anxious individuals (16.26±6.81 vs. 15.13±6.70 ng/mL, p<0.001). There were differences in the proportions of individuals with vitamin D sufficiency, insuf-
Table 2. The levels of total vitamin D and CRP according to anxiety status

|                        | Without case-level anxiety symptoms (N=47,185) | With case-level anxiety symptoms (N=3,818) | P      |
|------------------------|-----------------------------------------------|--------------------------------------------|--------|
| **Total vitamin D**    |                                               |                                            |        |
| Continuous level, mean±SD, ng/mL | 16.26±6.81                                   | 15.13±6.70                                | <0.001 |
| Categorical level, N (%) |                                             |                                            | <0.001 |
| Sufficiency, ≥20 ng/mL  |                                              |                                            |        |
| Insufficiency, 10–19.99 ng/mL | 11.634 (24.66)                              | 754 (19.75)                               |        |
| Deficiency, <10 ng/mL   | 7.842 (16.62)                                 | 866 (22.68)                               |        |
| **C-reactive protein (CRP)** |                                           |                                            | 0.799  |
| Continuous level, mean±SD, mg/L | 0.11±0.27                                   | 0.11±0.28                                 |        |
| Categorical level, N (%) |                                             |                                            | 0.383  |
| Low, ≤3 mg/L            | 47,103 (99.83)                               | 3,809 (99.76)                             |        |
| High, 3.01–10 mg/L      | 82 (0.17)                                    | 9 (0.24)                                  |        |

Table 3. Association between serum vitamin D levels and the presence of anxiety symptoms

| Total vitamin D            | Crude model OR (95% CI) p-value | Model 1 OR (95% CI) p-value | Model 2 OR (95% CI) p-value |
|---------------------------|---------------------------------|-----------------------------|----------------------------|
| Categorical level         |                                 |                             |                            |
| Sufficient, ≥20 ng/mL     | 1 [Reference]                  |                             |                            |
| Insufficient, 10–19.999 ng/mL | 1.224 (1.124–1.333)            | <0.001                      |                            |
| Deficient, <10 ng/mL      | 1.704 (1.539–1.886)            | <0.001                      |                            |
| Continuous level, ng/mL   | 0.974 (0.969–0.979)            | <0.001                      |                            |

Table 3 presents the association between serum vitamin D levels and anxiety symptoms. Categorical vitamin D status was negatively related to the risk of anxiety symptoms (BAI≥16). In the crude model, the OR of anxiety symptoms was increased in individuals with vitamin D insufficiency (OR=1.224, 95% CI=1.124–1.333) and was more strongly increased in those with vitamin D deficiency (OR=1.704, 95% CI=1.539–1.886). In model 1 with adjustment for age, sex, center (Seoul or Suwon), marital status, education, income, AUDIT, smoking status, season of data collection, BMI and CES-D, the results were virtually unchanged (vitamin D insufficiency: OR=1.116, 95% CI=1.013–1.229; vitamin D deficiency: OR=1.704, 95% CI=1.539–1.886). Table 4 presents the relationship between serum CRP levels and anxiety symptoms. Categorical CRP levels were not related to the presence of anxiety symptoms (crude model: OR=1.357, 95% CI=0.681–2.703; model 1: OR=1.258, 95% CI=0.572–2.767; model 2: OR=1.247, 95% CI=0.567–2.743). In model 2, additional adjustment for categorical CRP levels did not affect the inverse association between vitamin D levels and anxiety symptoms (vitamin D insufficiency: OR=1.116, 95% CI=1.013–1.229; vitamin D deficiency: OR=1.277, 95% CI=1.128–1.445). Continuous vitamin D levels were negatively associated with the risk of anxiety symptoms in the crude model (OR=0.974, 95% CI=0.969–0.979) and the statistical significance remained in both model 1 (OR=0.987, 95% CI=0.981–0.993) and model 2 (OR=0.987, 95% CI=0.981–0.993).

DISCUSSION

The results of the present study demonstrated a significant association between vitamin D deficiency (serum vitamin D level <10 ng/mL) and case-level anxiety symptoms (BAI≥16).
However, CRP level was not significantly associated with the presence of anxiety symptoms. In terms of the relationship between serum vitamin D and CRP levels, no interactions or mediating effects were found.

The results showed that vitamin D deficiency was associated with case-level anxiety symptoms. The continuous vitamin D levels also showed a negative association with the risk of anxiety symptoms. Armstrong et al. reported a significant association of vitamin D deficiency (<10 ng/mL) with higher levels of anxiety and depressive symptoms in fibromyalgia patients. Wu et al. found that low levels of vitamin D (<15 ng/mL) were related to the occurrence of anxiety symptoms in post-stroke patients. However, the sample sizes of these studies were small and the analyses included only certain subgroups. Therefore, generalizability can be impaired. The study by de Koning et al. found no association between serum vitamin D and anxiety symptoms in older people. However, considering older people have a range of medical diseases and lower levels of physical activity and sun exposure than general adults, the results of de Koning et al.'s study are difficult to apply to healthy adults. As far as we know, our study has the largest sample size (n=51,003), including adults above 18 years, and the analyses were also adjusted for many potential confounders.

There is a set of plausible biological mechanisms explaining the association between low serum vitamin D levels and anxiety symptoms. First, vitamin D receptor (VDR) and vitamin D activating enzyme 1a-hydroxylase, that are known to mediate vitamin D to function appropriately, are widely distributed in neuronal and glial cells of the human brain. A previous study reported that VDR knock-out mice showed reduced exploratory behavior and increased stress-related grooming on anxiety tests. Therefore, it is speculated that defects in the vitamin D-VDR system may cause the development of anxiety symptoms. Second, calcitriol [1, 25(OH) 2D, the active metabolite of vitamin D] is known to stimulate the synthesis of serotonin through activating the transcription of the serotonin-synthesizing gene tryptophan hydroxylase 2 (TPH2). Finally, as a potent anti-inflammatory agent, vitamin D might decrease the anxiety symptoms through its mediation of inflammation. Recent studies report that elevated levels of interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), interferon (IFN) and CRP are associated with the maintenance of fear- and anxiety-based symptoms by affecting the activity and connections in regions of the brain implicated in the etiology, including the amygdala, hippocampus, insula, medial prefrontal cortex, and the anterior cingulate. However, the results of our study showed that serum CRP levels did not affect the relationship between serum vitamin D levels and the risk of anxiety symptoms. This lack of association could be due to various reasons. We analyzed health screening data which included relatively healthy adults, and participants with severe anxiety symptoms might have been excluded. Besides, among several inflammatory markers known to be related to vitamin D levels, we only used serum CRP levels as a measure of inflammation in our study. Therefore, it is necessary to investigate whether serum vitamin D levels are related to other inflammation markers such as IL-1, IL-6, TNF, and IFN.

The ORs of anxiety symptoms did not differ between low (≤3 mg/L) and high (3.01–10 mg/L) CRP levels significantly. The results of recent studies examining the relationship between inflammation and anxiety symptoms/disorders have been mixed. As aforementioned, since our data included generally healthy adults, there were possibilities that individuals with severe anxiety symptoms were excluded. We also excluded participants with acute infection (CRP >10 mg/L) and chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease or asthma, which might have led to the selection of only healthy adults. This might cause the lack of association between CRP levels and anxiety symptoms. Lastly, it is possible that CRP levels do not sufficiently represent inflammatory status, and therefore, it is also essential to examine the relationship between other inflammatory markers and anxiety symptoms/disorders.

The results of this study should be interpreted with caution owing to several potential limitations. First, since the design of our study was cross-sectional, it could lead to a potential
for inflated associations and reverse causality. Since the result of our study was derived from a large sample, there was a possibility that although there is only a small effect size, the statistical significance could be easily reached. Second, this study defined a caseness based on BAI scores and cut-offs, not on clinical diagnosis. Furthermore, BAI is a self-report questionnaire; the results could be affected by response bias. Third, even though we adjusted for various explanatory confounding variables, there were still confounding factors such as outdoor exercise, dietary habits, other medical illnesses (e.g., renal disease, malignant disease), and usage of medication related to inflammation and vitamin D levels.

There were several strengths in this study. First, to the best of our knowledge, this study used the largest study sample to identify the association between serum vitamin D levels and anxiety symptoms. Second, in terms of the proportions of participants with an anxiety disorder and with lower than 20 ng/mL vitamin D levels, our data were obtained from a representative sample. The proportion of participants with anxiety symptoms (7.5%) was consistent with the previously reported prevalence of anxiety disorder in South Korean adults in 2016 (9.3%). According to a recent study based on the Korea National Health and Nutrition Examination Survey, the proportions of individuals with suboptimal vitamin D levels (<20 ng/mL) were 75.3% in men and 76.7% in women. Consistent with these, 69.2% of men and 86.0% of women in our study had lower than 20 ng/mL vitamin D levels.

Compared with sufficient vitamin D levels (≥20 ng/mL), insufficient (10–19.99 ng/mL) and deficient (<10 ng/mL) vitamin D levels were significantly associated with risk of anxiety symptoms. After adjusting for CRP levels, the results were not changed, and no evidence of interaction between vitamin D and CRP levels was found. CRP levels did not account for the association between vitamin D levels and risk of anxiety symptoms.

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We dedicate this paper to the spirit of the departed, Professor Se-Won Lim.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Sun-Young Kim. Data curation: Sun-Young Kim. Formal analysis: Sun-Young Kim. Funding acquisition: Young-Chul Shin, Sang-Won Jeon. Investigation: Sun-Young Kim, Young-Chul Shin. Methodology: Sun-Young Kim. Project administration: Young-Chul Shin, Weon-Jeon Lim, Sang-Won Jeon. Resources: Young-Chul Shin, Weon-Jeon Lim, Sun-Gwon Shin. Software: Sun-Young Kim. Supervision: Young-Chul Shin, Weon-Jeon Lim, Sang-Won Jeon, Kang-Seob Oh, Jae-Hyun Park, Dong-Won Shin, Sung-Joon Cho. Validation: Sun-Young Kim. Visualization: Sun-Young Kim. Writing—original draft: Sun-Young Kim. Writing—review & editing: all authors.

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