Retrospective Study

Relationship between Vitamin D and Nonspecific Low Back Pain May Be Mediated by Inflammatory Markers

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Background: Vitamin D deficiency has been linked to nonspecific low back pain (Ns-LBP); however, the role of inflammation as a possible mediator between vitamin D levels and Ns-LBP is not well understood.

Objective: To explore the mediating effects of inflammatory markers on the relationship between vitamin D levels and pain outcomes.

Study Design: A retrospective study.

Setting: Department of Spinal Surgery of a hospital affiliated to a medical university.

Methods: In this cross-sectional study, we selected patients with non-specific acute low back pain (Ns-ALBP, n = 60) and non-specific chronic low back pain (Ns-CLBP, n = 78), as well as 60 people without Ns-LBP as controls, from January 2018 to January 2019. Serum 25(OH)D and inflammatory marker levels were examined. Regression and causal mediation analysis were used to evaluate the mediating effects of inflammatory markers on the association between vitamin D and pain.

Results: Mean serum concentrations of vitamin D in the control, Ns-ALBP, and Ns-CLBP groups were 25.70 ± 10.04, 21.44 ± 8.46 and 18.25 ± 8.05 ng/mL, respectively ($P < 0.001$). After adjustment for clinical factors, vitamin D deficiency was associated with Ns-LBP ($P < 0.05$); however, when the interleukin 6 (IL-6) level was added to the multivariable models, the association was no longer significant in Ns-CLBP patients. Mediation analysis estimated the overall mediated effect as –0.461 ($P < 0.001$) in Ns-CLBP patients, and the intermediary effect of IL-6 was 0.045.

Limitations: A retrospective study may include inevitable bias. More sensitive biomarkers were not investigated in this study. Pain intensity evaluation using the visual analogue scale is inevitably subjective.

Conclusion: Patients with Ns-LBP had lower vitamin D and higher inflammatory marker levels. This association between hypovitaminosis D and Ns-CLBP may be mediated by IL-6. Therefore, large-scale clinical trials are warranted to investigate the clinical efficacy of vitamin D supplementation for decreasing inflammation and relieving Ns-LBP.

Key words: Deficiency; hypovitaminosis D; inflammatory markers; interleukin; mediation; non-specific low back pain; vitamin D

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Low back pain (LBP) is defined as a syndrome mainly manifested by pain located in the lower back, lumbosacral, and hip regions, with or without radiation pain in the lower extremities, which can be divided into 3 types: acute, subacute, and chronic (1). Studies have shown that approximately 80% of people will have different degrees of LBP in their lifetime (2). The pain may be caused by disorders of the muscle, ligaments, joints, intervertebral discs, vertebral body, and nerve function, or infection or inflammation. However, the most common manifestation of LBP is nonspecific low back pain (Ns-LBP) (3), which cannot be associated with any underlying pathology. People with non-specific acute low back pain (Ns-ALBP) may develop non-specific chronic low back pain (Ns-CLBP), which can lead to high medical costs, unemployment, depression, and other undesirable effects on personal and social development (4).

A high incidence of hypovitaminosis D has become a public health issue worldwide (5). A meta-analysis (6) reported that hypovitaminosis D is associated with LBP, with especially stronger associations observed in women and in those < 60 years old. However, the association between vitamin D levels and pain intensity is controversial. Vitamin D regulates the metabolism of calcium and bone. Vitamin D also plays an essential role in the inflammatory/immune response mechanism of inflammatory diseases (7) (such as atherosclerosis, asthma, inflammatory bowel disease, and chronic kidney disease). Recent evidence shows that 1,25-$(OH)_2D_3$ inhibits the generation of inflammatory cytokines, interleukin (IL)-6, and tumor necrosis factor (TNF)-α in human monocytes (8). Consequently, hypovitaminosis D may aggravate chronic inflammatory pain through enhanced inflammatory cell infiltration and inflammatory cytokine release.

Different pain-related pathologies, such as LBP, headache and fibromyalgia, are linked with elevated serum levels of inflammatory biomarkers, such as IL-6, TNF-α and C-reactive protein (CRP) (9). The relationship between the levels of these inflammatory cytokine and Ns-LBP has already been confirmed. For example, Heffner et al (10) showed a significantly higher IL-6 serum concentration in patients with LBP compared with that in a matched control group; higher IL-6 concentration was associated with pain and poorer sleep quality. Wang et al (11) found a significantly higher proportion of TNF-α-positive subjects in patients with CLBP, than in a healthy control group without LBP (44.6% versus 12.3%) at a 6-month follow-up. CRP is an acute-phase protein used as an indicator to detect systemic inflammation. Briggs et al (12) reported 15,322 patients with increased CRP (> 3.0 mg/dL) had a greater risk of experiencing LBP. This phenomenon may be due to the important role of inflammatory cytokines in neuronal remodeling and enhancement of injurious sensory transduction (13).

The possible causal nature of the relationship between vitamin D, pro-inflammatory biomarkers, and Ns-LBP, however, has not yet been investigated. Considering that LBP resulting from vitamin D deficiency may be mediated by inflammatory biomarkers, we designed a study to evaluate vitamin D status and levels of inflammation in Ns-ALBP and Ns-CLBP patients. The data gathered will guide vitamin D assessment and show whether supplementation to relieve LBP is appropriate.

Methods

Patient Population

This was an observational cross-sectional study to examine whether the effect of vitamin D levels on Ns-LBP is mediated through inflammatory biomarkers. After applying strict inclusion and exclusion criteria, 198 patients with Ns-ALBP (n = 60) and Ns-CLBP (n = 78) were enrolled through the spine surgery unit in our hospital. Healthy controls (n = 60) matched for age, gender, socioeconomic status, and other basic demographic characteristics were enrolled during health checkups in our hospital. The sampling procedure began in January 2018 and continued until January 2019. The study was approved by our hospital ethics committee and written informed consent was obtained from each patient. Definitions of acute and subacute LBP do not specify the cutoff to divide the 2; therefore, we referenced guidelines (14) that defined acute LBP as LBP of less than 12 weeks duration and chronic LBP as LBP of more than 12 weeks duration.

The inclusion criteria applied in this study were Ns-ALBP and Ns-CLBP meeting the diagnostic criteria for LBP (15) (pain between the costal angles and gluteal folds that may radiate down one or both legs). Included patients were over 18 years of age and participated on a voluntary basis with provision of written informed consent to use their complete information. Included patients also had to live at a latitude between 30° and 40° north to ensure roughly the same exposure to light intensity. The exclusion criteria were: 1) specific etiology of LBP diagnosed based on signs, symptoms, and using radiographic/magnetic resonance imaging

(MRI) examination, including various neoplastic diseases, trauma/fracture, inflammatory systemic disease or infection (e.g., systemic lupus erythematosus, rheumatoid arthritis, and symptomatic osteoarthritis of the hip, knee, and ankle), and spine-related diseases (e.g., spondylodiscitis, lumbar disc herniation, spinal stenosis); 2) serious underlying cardio-cerebrovascular, blood, and digestive system disease and/or severe hepatorenal function damage; 3) severe osteoporosis; 4) pregnant or currently breastfeeding; 5) receiving treatment with estrogen, vitamin D supplementation, nonsteroidal anti-inflammatory drugs, or corticosteroids; 6) CRP > 10 mg/L, because this is considered to be consistent with acute infection and tissue damage.

Data Collection Tools and Evaluation Criteria

Blood samples were collected when patients visited the orthopedic clinic or health examination center and the serum concentration of 25(OH)D, CRP, neutrophils, white blood cells (WBCs), TNF-α, IL-6, and IL-1 were determined using an autoanalyzer device (Cobas e 602; Roche, Basel, Switzerland). The 25(OH)D levels ≤ 20 ng/mL, 21-29 ng/mL and ≥ 30 ng/mL were defined as vitamin D deficiency, insufficiency, and sufficiency, respectively. The total lumbar and hip region bone mineral density was determined using dual-energy X-ray absorptiometry (Prodigy GE Healthcare, Chicago, IL, USA). Patients with a T score of <–2.5 were diagnosed with osteoporosis (16). Lumbar spine MRI imaging was obtained with an Achieva 1.5-T dual MRI imaging system (Philips, Netherlands).

A visual analogue scale (VAS) score was used to assess the severity of LBP (17). The Modified Oswestry Disability Questionnaire (MODQ) (18) was used for the evaluation of functional disability and quality of life in patients with LBP. Both scoring methods were based on a 100-point scale, with 0 indicating no pain or healthy and 100 indicating severe pain or dysfunction. Interviews and questionnaires were used to acquire the patients' age, drinking status (alcohol drinking ≥ one standard drink at least once a month: yes/no) (19), smoking status (smoked ≥ 400 cigarettes in lifetime: yes/no) (20), body mass index (BMI), hypertension status, diabetes status, vitamin D supplementation, level of education, physical activity (exercise to the degree of sweating in the last week: < 2 days or ≥ 2 days) and pain severity.

Statistical Analysis

General demographics and inflammatory marker plasma levels were compared between 3 groups using the chi-squared test and analysis of variance followed by a least significant difference test. The relationship between 25(OH)D and inflammatory cytokine levels and pain intensity was analyzed using Spearman's correlation. We performed an analysis based on Baron and Kenny's (21) guidelines to confirm whether the relationship between vitamin D and pain intensity may be mediated by the inflammatory cytokines in the study. Multiple regression analysis was also used to examine their underlying mediatory role. In the first model, we adjusted for predetermined maternal factors considered to be clinically relevant to the outcomes, including age, BMI, gender ratio, hypertension, diabetes, drinking, smoking, season, level of education, and physical activity. In the next model, we examined their potential mediatory role after adjusting for inflammatory markers to identify whether a significant vitamin D-pain intensity coefficient became non-significant. All data analysis was performed using IBM SPSS Statistics ver. 25 (Armonk, NY, USA). A *P*-value < 0.05 was considered statistically significant.

Results

Patient Characteristics

The characteristics of the included study population are shown in Table 1. The 198 enrolled patients had a mean age of 63.42 ± 11.26 years, ranging from 33 to 80 years. No significant difference was found between groups with respect to age, BMI, gender ratio, hypertension, diabetes, drinking, smoking, season, level of education, or physical activity ($P > 0.05$); however, there were significant differences in CRP, TNF-α, IL-6 and 25(OH)D levels ($P < 0.05$). Mean serum 25(OH)D concentrations in the control, Ns-ALBP and Ns-CLBP groups were 25.70 ± 10.04, 21.44 ± 8.46, and 18.25 ± 8.05 ng/mL, respectively; the difference was statistically significant (control versus Ns-ALBP, $P < 0.05$; control versus Ns-CLBP, $P < 0.05$). CRP, TNF-α, and IL-6 levels were higher in the Ns-LBP group than in the control group ($P < 0.05$, Table 1).

Association between Vitamin D Concentration, Inflammatory Cytokine Levels, and Pain Outcomes

Among the whole study population, 97 (48.99%), 62 (31.31%), and 39 (19.70%) patients were assigned to the deficient, insufficient, and sufficient vitamin D groups, respectively. Patients in the group with suf-

Table 1. *Demographic characteristics of the 3 groups based on pain status.*

Characteristic	No pain (n = 60)	Ns-ALBP (n = 60)	Ns-CLBP (n = 78)	*P*
Age (year)	62.31 ± 11.06	64.46 ± 10.45	63.17 ± 12.49	0.531
Body mass index (kg/m^2)	24.80 ± 2.90	23.94 ± 2.93	23.64 ± 3.22	0.090
Gender (Men/Women)	28/32	17/43	27/51	0.104
Diabetes (Yes)	20 (33.3%)	17 (28.3%)	19 (24.4%)	0.510
Hypertension (Yes)	19 (31.7%)	20 (33.3%)	24 (30.8%)	0.949
Smoking (Yes)	8 (13.3%)	10 (16.7%)	16 (20.5%)	0.537
Drinking (Yes)	11 (18.3%)	15 (25%)	17 (21.8%)	0.675
Season test				
Fall–winter	32 (53.3%)	32 (53.3%)	46 (59%)	0.737
Spring–summer	28 (46.7%)	28 (46.7%)	32 (41%)	
Level of education				0.416
< High school	31 (51.7%)	34 (56.7%)	49 (62.8%)	
> High school	29 (48.3%)	26 (43.3%)	29 (37.2%)	
Level of physical activity				0.898
< 2 times/week	49 (81.7%)	47 (78.3%)	62 (79.5%)	
≥ 2 times/week	11 (18.3%)	13 (21.7%)	16 (20.5%)	
VAS	-	38.64 ± 9.26	35.28 ± 11.56	
MODQ	-	33.81 ± 9.47	31.38 ± 8.37	
CRP, ng/mL	4.43 ± 0.52	5.99 ± 2.56[a]	5.14 ± 2.36[a, b]	< 0.001
Neutrophils, ng/mL	3.61 ± 2.05	4.38 ± 6.94	4.00 ± 2.07	0.628
WBC, ng/mL	5.82 ± 2.44	5.86 ± 1.84	6.15 ± 2.42	0.659
TNF-α, ng/mL	4.20 ± 1.49	5.13 ± 2.24[a]	5.64 ± 2.65[a]	0.002
IL-6, ng/mL	1.48 ± 0.64	1.84 ± 0.87[a]	1.80 ± 0.66[a]	0.013
IL-1, ng/mL	0.11 ± 0.05	0.11 ± 0.08	0.14 ± 0.22	0.516
25(OH)D levels	25.70 ± 10.04	21.44 ± 8.46[a]	18.25 ± 8.05[a, b]	< 0.001
Deficiency (≤ 20 nmol/L)	19 (31.7%)	30 (50.0%)	48 (61.5%)	
Insufficient (20-30 nmol/L)	17 (28.3%)	21 (35.0%)	24 (30.8%)	
Normal (≥ 30 nmol/L)	24 (40%)	9 (15%)	6 (7.7%)	

Values are presented in mean ± standard error (SE) or percentages. All *P* values were calculated with the χ^2/ANOVA. [a] Pairwise comparisons to group of no pain, $P < 0.05$. [b] Pairwise comparisons to group of Ns-ALBP, $P < 0.05$. Nonspecific acute low back pain (Ns-ALBP), Non-Specific Chronic low back pain (Ns-CLBP), Visual analog scale (VAS), Modified Oswestry Disability Questionnaire (MODQ), C-reactive protein (CRP), White blood cell (WBC), Tumor necrosis factor TNF, Interleukin (IL).

ficient vitamin D levels had lower IL-6 levels than the groups with deficient and insufficient vitamin D levels (1.21 ± 0.44 versus 1.98 ± 0.79 or 1.71 ± 0.71 ng/mL, $P < 0.001$, respectively; Table 2). Levels of other inflammatory markers (CRP, neutrophils, WBC, TNF-α, and IL-1) were not significantly different between the 3 groups ($P > 0.05$). Spearman's correlation analysis revealed a negative correlation between vitamin D and IL-6 levels in the Ns-ALBP (r = –0.158, $P = 0.027$) and Ns-CLBP groups (r = -0.426, $P < 0.001$). CRP, neutrophil, WBC, TNF-α, and IL-1 levels were unrelated to vitamin D levels ($P > 0.05$). VAS and MODQ scores negatively correlated with vitamin D levels in patients with Ns-CLBP (r = –0.317 and –0.310, $P < 0.001$), but not Ns-ALBP (r = –0.123 and –0.106, $P > 0.05$, Table 3).

Exploratory Analysis of Potential Role of Inflammatory Markers

When age, BMI, gender ratio, hypertension, diabetes, drinking, smoking, season, level of education, and physical activity were included as covariates in the multiple logistic regression, vitamin D was a significant predictor of Ns-ALBP (OR = 0.93, $P = 0.002$) and Ns-CLBP (OR = 0.95, $P = 0.017$). After adjusting for inflammatory markers, such as CRP, neutrophils, WBC, TNF-α, IL-1, and IL-6, the relationship between 25(OH)D levels and pain at baseline became insignificant only when IL-6 was as a variable added to the above model of Ns-CLBP (OR = 0.96, $P > 0.121$, Table 4). Table 5 shows the mediation analysis results for vitamin D and pain intensity. It was assumed that IL-6 (M) was the mediating variable between vitamin D levels (X) and pain intensity (Y_1 = Ns-ALBP, Y_2 = Ns-CLBP). Regression analysis indicated no significant moderating effect between vitamin D levels and pain intensity in patients with Ns-ALBP (β_1 = -0.102, $P = 0.372$, Fig. 1); however, IL-6 levels played a completely mediating role in the relationship between vitamin D levels and pain intensity in patients with Ns-CLBP. Vitamin D levels accounted for –0.102 of the overall effect on pain intensity in patients with Ns-CLBP, while the intermediary effect of IL-6 was 0.045, which accounted for 44.12% of the overall effect.

Discussion

The main findings of our study are that there is a significant negative correlation between 25(OH)D levels and pain intensity in Ns-CLBP patients, and that IL-6 is an effective mediator of this relationship, such that IL-6 has a stronger negative correlation with pain

Table 2. *Differences in inflammatory markers according 25(OH)D levels.*

	Deficiency (≤ 20 nmol/L, n = 97)	Insufficient (20 -30 nmol/L, n = 62)	Normal (≥ 30 nmol/L, n = 39)	*P*
CRP, ng/mL	5.55 ± 2.46	5.26 ± 2.00	4.66 ± 1.63	0.083
Neutrophils, ng/mL	3.81 ± 2.00	3.77 ± 2.14	4.85 ± 9.08	0.414
WBC, ng/mL	6.02 ± 2.16	5.94 ± 2.45	5.76 ± 1.97	0.814
TNF-α, ng/mL	4.94 ± 2.25	4.91 ± 2.07	5.27 ± 2.53	0.675
IL-6, ng/mL	1.98 ± 0.79a	1.71 ± 0.71a	1.21 ± 0.44	< 0.001
IL-1, ng/mL	0.14 ± 0.19	0.10 ± 0.03	0.11 ± 0.04	0.292

All *P* values were calculated with the ANOVA. [a] Pairwise comparisons to group of normal vitamin D levels, $P < 0.05$.

Table 3. *Univariable associations between 25(OH)D concentrations and inflammatory markers and pain.*

Variable	Ns-ALBP		Ns-CLBP	
	Correlation coefficient (r)	*P*	Correlation coefficient (r)	*P*
CRP, ng/mL	-0.153	0.243	-0.085	0.461
Neutrophils, ng/mL	-0.107	0.418	-0.046	0.688
WBC, ng/mL	-0.08	0.542	-0.124	0.281
TNF-α, ng/mL	0.092	0.484	0.117	0.307
IL-6, ng/mL	-0.158	0.027	-0.426	< 0.001
IL-1, ng/mL	0.071	0.588	-0.144	0.21
VAS	-0.123	0.285	-0.317	0.013
MODQ	-0.106	0.357	-0.310	0.016

P = significance of associations with 25(OH)D using spearman's correlations for continuous variables.

intensity or vitamin D levels. To our knowledge, this is the first study evaluating the association between vitamin D deficiency and LBP through the intermediary of inflammatory cytokines.

The higher prevalence of hypovitaminosis D is becoming a worldwide problem, and it is linked to an increased risk of developing infection, breast cancer, atherosclerosis, chronic renal disease, and musculoskeletal pain (22,23). Recently, increasing numbers of studies have focused on the association between hypovitaminosis D and Ns-LBP. Panwar et al (24) investigated a total of 250 patients with CLBP, 177 patients with SLBP, and 248 healthy controls and found that the proportion of individuals with vitamin D deficiency was significantly higher among patients with CLBP and SLBP.

Table 4. *Result from multivariate logistic regression analysis for the associations between vitamin D and inflammatory markers in people with different pains.*

Variable	Relationship between Vitamin D and Ns-ALBP*	*P*	Relationship between Vitamin D and Ns-CLBP*	*P*
Vitamin D only	0.93 (0.89-0.98)	0.002	0.95 (0.91-0.99)	0.017
Vitamin D (with CRP)	0.94 (0.90-0.99)	0.009	0.95 (0.91-0.99)	0.03
Vitamin D (with IL-6)	0.95 (0.90-0.99)	0.029	0.96 (0.92-1.01)	0.121
Vitamin D (with IL-1)	0.93 (0.89-0.98)	0.002	0.95 (0.91-0.99)	0.019
Vitamin D (with TNF-α)	0.93 (0.89-0.97)	0.002	0.94 (0.90-0.98)	0.008

*Adjusted for age, body mass index, gender ratio, hypertension, diabetes, drinking, smoking, season, level of education, and physical activity.

Table 5. *Estimates for mediating effect of IL-6 on Ns-ALBP and Ns-CLBP patients.*

	Standardized Regression Equation (β)	SE	t	*P*
Ns-ALBP				
Step 1	$Y_1 = -0.102X$	0.133	-0.898	0.372
Step 2	$M = -0.409X$	0.012	-3.903	< 0.001
Step 3	$Y_1 = -0.111X - 0.02M$	0.147	-0.878	0.383
		1.333	-0.156	0.876
Ns-CLBP				
Step 1	$Y_2 = -0.461X$	0.087	-3.958	< 0.001
Step 2	$M = -0.462X$	0.012	-3.962	< 0.001
Step 3	$Y_2 = -0.316X + 0.314M$	0.168	-2.513	0.015
		1.565	2.5	0.015

Y_1: Ns-ALBP, Y_2: Ns-CLBP, X: vitamin D levels, M: IL-6, SE: standard error.

Moreover, higher VAS and MODQ scores were related to a greater risk of having vitamin D deficiency. Ghai, et al (25) found that after 6 months of supplementation with 60,000 IU of oral 25(OH)D_3, 68 CLBP patients had alleviation of pain and improved functional ability (MODQ) in the follow up. However, there are some controversies regarding the correlation between serum 25(OH)D level and CLBP. Thörneby et al (26) found that vitamin D levels were no different between patients with CLBP (n = 44) and healthy controls (n = 44), and hypovitaminosis D was not a risk factor for CLBP. The

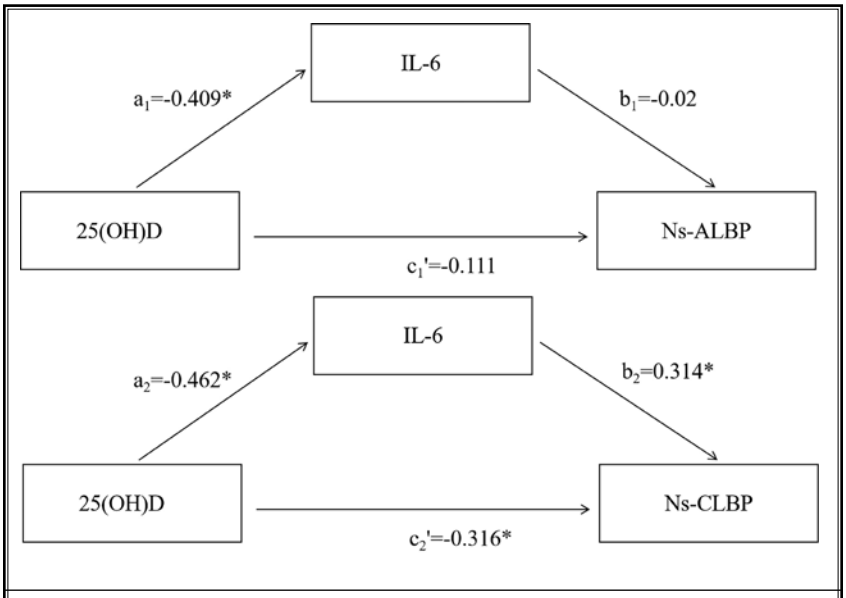

Fig. 1. *Mediation models with standardized path coefficients for (A) Ns-ALBP and (B) Ns-CLBP. * Non-zero ordered confidence intervals and $P < 0.05$. a_1 = path coefficients of 25(OH)D on IL-6; b_1 = path coefficients of IL-6 on Ns-ALBP; c_1 = direct effect estimates of 25(OH)D on Ns-ALBP after controlling for IL-6. a_2, b_2, c_2 represent the correlation between 25(OH)D, IL-6, and Ns-CLBP.*

small sample size and lack of categorical comparison based on the severity of vitamin D deficiency may interfere with the accuracy of their results. Ghai, et al (27) found that among 328 patients, 217 (66%) of patients with CLBP had vitamin D deficiency among north Indians; however, the study lacked a demographically matched control group, therefore, it is difficult to draw firm conclusions regarding prevalence. A recent meta-analysis has found existing lower quality evidence to support the relationship between vitamin D deficiency and Ns-CLBP (28). Therefore, it is of great importance to perform large sample, prospective, randomized controlled studies to explore the effect of serum vitamin D level on Ns-ALBP and Ns-CLBP, avoiding confounding factors, such as latitude and race. Our study may help provide evidence further supporting this relationship.

Vitamin D plays a key role in the etiology and progression of various chronic pain conditions through its anatomical, hormonal, neurological, and immunological effects on the body (29,30). First, von Känel et al (31) found that lower vitamin D concentrations were associated with increased peripheral and central pain sensitivity during mechanical stimulation in patients with CLBP. This may be because vitamin D has a beneficial effect on neurite and astrocyte outgrowth and relieves neuropathic pain by modulating opioid signaling (32,33). The more generally accepted view is that the beneficial effects of vitamin D on pain are mediated by inflammatory cytokines. A recent systematic review reported that the concentration CRP and IL-6 positively correlates with the severity of Ns-LBP, and TNF-α positively correlates with the presence of Ns-LBP (34). The association between vitamin D and inflammatory cytokines has also been confirmed by some studies. In an observational study of 957 Irish adults aged > 60 years, a higher concentration of IL-6 and lower concentration of IL-10 were found in the vitamin D deficient group, compared with those in the vitamin D sufficient group (35). Ngo et al (36) found that in 253 healthy people aged 51-77 years had vitamin D levels which negatively correlated with high-sensitivity C-reactive protein (hsCRP) levels. This relationship is similar to that between TNF-α and vitamin D (37).

Our results show a negative association between vitamin D and IL-6 concentrations; IL-6 may be the most important intermediary factor of the relationship between vitamin D and pain among the numerous inflammatory cytokines. The association between hypovitaminosis D, IL-6, and systemic inflammation is particularly significant in chronic inflammation and metabolic dysfunction. In a large sample prospective study of 3,258 patients with cardiovascular disease, low vitamin D levels negatively correlated with IL-6 and CRP concentrations (38). Furthermore, recent studies have shown that skeletal muscle is also an endocrine organ and that muscle fibers can produce and release cytokines, especially IL-6. During physical exercise, skeletal muscle contraction leads to elevated cytosolic Ca^{2+} levels and activation of p38 MAPK, which results in the production of IL-6 (39). IL-6 is an inflammatory cytokine that plays an important role in pathophysiology of inflammatory pain, cancer pain, and nerve pain. The IL-6 complex with membrane-bound IL-6 receptor (mIL-6R) then associates with transducing membrane protein gp130 and can activate "classical IL-6 signaling" (eg,

JAK/STAT, MAPK/ERK, PI3K/Akt) to regulate mechanical allodynia and thermal hyperalgesia (40). A systematic review (41) of eligible studies showed that only the serum concentration of IL-6 was consistently higher in patients with fibromyalgia syndrome than in controls. Therefore, the increased IL-6 levels may mainly be the result of skeletal and muscle fiber disorder in patients with Ns-LBP.

The molecular mechanism of the relationship between vitamin D and IL-6 is that vitamin D regulates the pathway of the proinflammatory transcription factor nuclear factor κB (NFκB), inhibiting the expression of the downstream IL-6 (42). In vitamin D receptor (VDR)-knockout mice, abolished VDR/P65 binding led to elevated NFκB activity and increased IL-6 serum levels (43); however, we failed to find any relationship between vitamin D and other inflammation cytokines, such as TNF-α, CRP, and IL-1. These results are understandable because the differences in study population and confounding factors cannot be totally avoided. De Vita, et al (44) investigated 867 older adults aged ≥ 65 years to overcome this specific issue. In a more sophisticated analysis, they tested the association between 25(OH) D and soluble IL-6 receptors (sIL6r and sgp130) and soluble TNF-α receptors (sTNFR1 and sTNFR2), which have been proven to be more sensitive than IL-6 and TNF-α. The results showed that vitamin D negatively correlated with IL-6 and positively correlated with sIL6r. There was no relationship between 25(OH)D, TNF-α and sTNFR, indicating that vitamin D might not influence the TNF-α system.

We observed that the mediating effects of IL-6 on the associations between vitamin D and pain were significant in Ns-CLBP patients and not in Ns-ALBP patients. Moreover, vitamin D levels were lower in Ns-CLBP patients than in Ns-ALBP patients and healthy patients. The proposed mechanism of hypovitaminosis D in these patients is that Ns-CLBP reduces appetite, outdoor sports activity, and sunshine exposure, which leads to a long-term reduction in vitamin D intake and synthesis (45). It is a long process for acute LBP to develop into chronic LBP, with changes in various organs' metabolism and function. Vania Apkarian, et al (46) have used functional MRI (fMRI) to explore the neural mechanisms that mediate the transition from acute to chronic pain. They found that nociceptive information, perhaps distorted by peripheral and spinal cord sensitization processes, impinges on the limbic circuitry. The interaction of the limbic circuitry with prefrontal processes determines the level at which a certain pain condition transitions to a more emotional state. Therefore, vitamin D supplementation may induce different cortical reorganization for Ns-CLBP and Ns-ALBP. Furthermore, it is necessary to perform further studies examining the modulation of neural signals using fMRI in response to vitamin D supplementation in the context of human models of Ns-CLBP and Ns-ALBP, providing an opportunity to restrain the transition to chronic pain and reducing the resulting healthcare burden.

Our study also had several limitations. First, the cross-sectional nature of the study limits its ability to detect direct associations between vitamin D, inflammation cytokines, pain intensity and functional disability. However, we cannot overlook that low vitamin D levels may be suggested to be a biomarker of poor healthy resulting from inflammatory processes. Second, some more sensitive biomarkers that were not available for our study included soluble IL-6 receptors (sIL6r and sgp130), soluble TNF-α receptors, (sTNFR1 and sTNFR2), and hsCRP levels. These cytokines may be better to clarify the relationship between vitamin D with multiple inflammatory biomarkers and the pain system. Last, evaluation of pain intensity using the VAS score system is inevitably subjective. Differences in latitude, race and dietary conditions may mean that the results cannot be generalized to widely different populations. Further studies are still required. Despite these limitations, our study was the first to show that inflammatory markers may mediate the relationship between vitamin D and Ns-LBP. We believe that our data are clinically useful for spinal surgeons and pain physicians evaluating vitamin D status and highlight new therapeutic approaches in patients with Ns-LBP patients in the future.

Conclusion

Our study showed that patients with Ns-LBP have a high prevalence rate of hypovitaminosis D and high levels of inflammatory cytokines. Hypovitaminosis D is also associated with higher levels of inflammatory cytokines and higher VAS scores. This association between hypovitaminosis D and Ns-CLBP may be mediated by IL-6. Therefore, large scale clinical trials are warranted to investigate the clinical efficacy of vitamin D supplementation to prevent and relieve Ns-LBP. However, its benefit for Ns-LBP cannot be taken for granted based on the mediation of the inflammatory pathways examined in this study. The mechanisms underlying this relationship should continue to be explored.

References

1. Hartvigsen J, Hancock MJ, Kongsted A, et al. What low back pain is and why we need to pay attention. *Lancet* 2018; 391(10137):2356-2367.
2. Joud A, Petersson IF, Englund M. Low back pain: Epidemiology of consultations. *Arthritis Care Res (Hoboken)* 2012; 64:1084-1088.
3. Maher C, Underwood M, Buchbinder R. Non-specific low back pain. *Lancet* 2017; 389(10070):736-747.
4. Thelin A, Holmberg S, Thelin N. Functioning in neck and low back pain from a 12-year perspective: A prospective population-based study. *J Rehabil Med* 2008; 40(7):555-561.
5. Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res* 2011; 31:48-54.
6. Zadro J, Shirley D, Ferreira M, et al. Mapping the association between vitamin D and low back pain: A systematic review and meta-analysis of observational studies. *Pain Physician* 2017; 20:611-640.
7. Murdaca G, Tonacci A, Negrini S, et al. Emerging role of vitamin D in autoimmune diseases: An update on evidence and therapeutic implications. *Autoimmun Rev* 2019; 18:102350.
8. Zhang Y, Leung DY, Richers BN, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol* 2012; 188:2127-2135.
9. Klyne DM, Barbe MF, Hodges PW. Systemic inflammatory profiles and their relationships with demographic, behavioural and clinical features in acute low back pain. *Brain Behav Immun* 2017; 60:84-92.
10. Heffner KL, France CR, Trost Z, Ng HM, Pigeon WR. Chronic low back pain, sleep disturbance, and interleukin-6. *Clin J Pain* 2011; 27:35-41.
11. Wang H, Schiltenwolf M, Buchner M. The role of TNF-alpha in patients with chronic low back pain-a prospective comparative longitudinal study. *Clin J Pain* 2008; 24:273-278.
12. Briggs MS, Givens DL, Schmitt LC, Taylor CA. Relations of C-reactive protein and obesity to the prevalence and the odds of reporting low back pain. *Arch Phys Med Rehabil* 2013; 94:745-752.
13. Kiguchi N, Kobayashi Y, Kishioka S. Chemokines and cytokines in neuroinflammation leading to neuropathic pain. *Curr Opin Pharmacol* 2012; 12:55-61.
14. Stochkendahl MJ, Kjaer P, Hartvigsen J, et al. National Clinical Guidelines for non-surgical treatment of patients with recent onset low back pain or lumbar radiculopathy. *Eur Spine J* 2018; 27:60-75.
15. Casazza BA. Diagnosis and treatment of acute low back pain. *Am Fam Physician* 2012; 85:343-350.
16. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone Miner Res* 1994; 9:1137-1141.
17. Haefeli M, Elfering A. Pain assessment. Eur Spine J 2006; 15 (Suppl 1):S17-24.
18. Fritz JM, Irrgang JJ. A comparison of a modified Oswestry Low Back Pain Disability Questionnaire and the Quebec Back Pain Disability Scale. *Phys Ther* 2001; 81:776-788.
19. Kann L, Kinchen S, Shanklin SL, et al. Youth risk behavior surveillance--United States, 2013. *MMWR Suppl* 2014; 63:1-168.
20. Nyberg F, Agudo A, Boffetta P, Fortes C, Gonzalez CA, Pershagen G. A European validation study of smoking and environmental tobacco smoke exposure in nonsmoking lung cancer cases and controls. *Cancer Causes Control* 1998; 9:173-182.
21. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986; 51:1173-1182.
22. Lockau L, Atkinson SA. Vitamin D's role in health and disease: How does the present inform our understanding of the past?. *Int J Paleopathol* 2018; 23:6-14.
23. Holick MF. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord* 2017; 18:153-165.
24. Panwar A, Valupadas C, Veeramalla M, Vishwas HN. Prevalence of vitamin D deficiency in chronic and subacute low back pain patients in India: A triple-arm controlled study. *Clin Rheumatol* 2018; 37:1367-1374.
25. Ghai B, Bansal D, Kanukula R, et al. Vitamin D supplementation in patients with chronic low back pain: An Open label, single arm clinical trial. *Pain Physician* 2017; 20:E99-e105.
26. Thorneby A, Nordeman LM, Johanson EH. No association between level of vitamin D and chronic low back pain in Swedish primary care: A cross-sectional case-control study. *Scand J Prim Health Care* 2016; 34:196-204.
27. Ghai B, Bansal D, Kapil G, Kanukula R, Lavudiya S, Sachdeva N. High prevalence of hypovitaminosis D in Indian chronic low back patients. *Pain Physician* 2015; 18:E853-862.
28. Zadro JR, Shirley D, Ferreira M, et al. Is Vitamin D supplementation effective for low back pain?: A systematic review and meta-analysis. *Pain Physician* 2018; 21:121-145.
29. Cutolo M, Paolino S, Sulli A, Smith V, Pizzorni C, Seriolo B. Vitamin D, steroid hormones, and autoimmunity. *Ann N Y Acad Sci* 2014; 1317:39-46.
30. Jesus CA, Feder D, Peres MF. The role of vitamin D in pathophysiology and treatment of fibromyalgia. *Curr Pain Headache Rep* 2013; 17:355.
31. von Kanel R, Muller-Hartmannsgruber V, Kokinogenis G, Egloff N. Vitamin D and central hypersensitivity in patients with chronic pain. *Pain Med* 2014; 15:1609-1618.
32. Brown J, Bianco JI, McGrath JJ, Eyles DW. 1,25-dihydroxyvitamin D3 induces nerve growth factor, promotes neurite outgrowth and inhibits mitosis in embryonic rat hippocampal neurons. *Neurosci Lett* 2003; 343:139-143.
33. Poisbeau P, Aouad M, Gazzo G, et al. Cholecalciferol (Vitamin D3) reduces rat neuropathic pain by modulating opioid signaling. *Mol Neurobiol* 2019; 56:7208-7221.
34. van den Berg R, Jongbloed EM, de Schepper EIT, Bierma-Zeinstra SMA, Koes BW, Luijsterburg PAJ. The association between pro-inflammatory biomarkers and nonspecific low back pain: A systematic review. *Spine J* 2018; 18:2140-2151.
35. Laird E, McNulty H, Ward M, et al. Vitamin D deficiency is associated with inflammation in older Irish adults. J Clin Endocrinol Metab 2014; 99:1807-1815.
36. Ngo DT, Sverdlov AL, McNeil JJ, Horowitz JD. Does vitamin D modulate asymmetric dimethylarginine and C-reactive protein concentrations?. *Am J Med* 2010; 123:335-341.
37. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. *J Inflamm (Lond)* 2008; 5:10.

38. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008; 168:1340-1349.
39. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: Focus on muscle-derived interleukin-6. *Physiol Rev* 2008; 88:1379-1406.
40. Zhou YQ, Liu Z, Liu ZH, et al. Interleukin-6: An emerging regulator of pathological pain. *J Neuroinflammation* 2016; 13:141.
41. Uceyler N, Hauser W, Sommer C. Systematic review with meta-analysis: Cytokines in fibromyalgia syndrome. *BMC Musculoskelet Disord* 2011; 12:245.
42. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension* 2011; 57:63-69.
43. Wu S, Liao AP, Xia Y, et al. Vitamin D receptor negatively regulates bacterial-stimulated NF-kappaB activity in intestine. *Am J Pathol* 2010; 177:686-697.
44. De Vita F, Lauretani F, Bauer J, et al. Relationship between vitamin D and inflammatory markers in older individuals. *Age (Dordr)* 2014; 36:9694.
45. Xu HW, Yi YY, Zhang SB, et al. Does vitamin D status influence lumbar disc degeneration and low back pain in postmenopausal women? A retrospective single-center study. *Menopause.* 2020; 27:586-592.
46. Apkarian AV, Baliki MN, Farmer MA. Predicting transition to chronic pain. *Curr Opin Neurol* 2013; 26:360-367