The relationship between standardized serum 25-hydroxyvitamin D (25(OH)D) concentration and incident anemia in the United States (U.S.) is unclear. The purpose of our study was to examine the association between serum 25(OH)D and anemia risk. We performed a cross-sectional analysis of the U.S. population participating in the National Health and Nutrition Examination Survey (NHANES) between 2001 and 2018. A generalized linear model and restricted cubic spline (RCS) plot curve were constructed to assess the relationship between serum 25(OH)D concentration and anemia incidence. Additionally, the association between serum 25(OH)D concentration and red blood cell (RBC) count and hemoglobin (HB) levels was investigated using generalized additive models with smooth functions. Subgroup analysis also was performed. A total of 29,933 individuals were included in our research. After adjusting for known confounding variables, compared with the lowest quartile, the odds ratios (ORs) with 95% confidence intervals (CIs) for association of serum 25(OH)D with anemia across the second, third, and fourth quartiles were 0.735 (0.651, 0.829), 0.527 (0.461, 0.602), and 0.696 (0.611, 0.792), respectively. Serum 25(OH)D concentration was associated with anemia risk in a U-shaped pattern, as shown by an RCS plot (P for nonlinearity <0.001). In addition, RBC count and HB levels initially increased and then decreased as serum 25(OH)D levels increased. Serum 25(OH)D concentration and risk of anemia were associated with a U-shaped curve in the U.S. general population. Serum 25(OH)D concentration in the range 59.7–70.3 nmol/l was associated with anemia incidence <1. Therefore, the risk of anemia can be reduced by close monitoring and appropriate vitamin D supplementation.

1. Introduction

Worldwide, anemia affects one out of every three people and is estimated to represent a greater global disease burden than major depression and chronic respiratory illnesses combined [1, 2]. Symptoms of anemia include fatigue, weakness, cognitive decline, and even death, and the condition is more common in women [3]. Anemia pathogenesis involves an imbalance between red blood cell loss and production, which may be due to ineffective or inadequate erythropoiesis and/or excessive red blood cell loss [1]. The most common conditions causing anemia are iron deficiency, thalassemia and hemoglobinopathies, folate deficiency, and parasitic diseases [4].

As a fat-soluble vitamin, vitamin D is naturally found in very few foods; the canonical role of vitamin D is to regulate the balance of calcium and phosphorus metabolism and minerals in bones [5]. Vitamin D deficiency has been linked
to anemia [6–9], since vitamin D is found in the bone marrow and can promote red blood cell production [10–12]. The main ring form of vitamin D, 25(OH)D, is created by hydroxylation of vitamin D, which can be taken in from exogenous sources or synthesized in the liver [13]. In recent years, the relationship between 25(OH)D and anemia has attracted increasing attention. Many previous studies have shown a correlation between 25(OH)D and anemia in chronic kidney disease as well as sickle cell anemia [14–18]; however, few recent studies have explored the link between serum 25(OH)D levels and anemia risk, and previous studies had small sample sizes or concomitant disease interference.

Due to the detrimental effects of anemia, recognizing risk factors for anemia development and devising measures to promptly avoid or control its negative consequences would be highly advantageous. According to epidemiological research, the relationship between serum 25(OH)D and anemia risk in the United States (U.S.) population remains unclear. Therefore, in this study, we analyzed data from the Nutrition and Health Examination Survey (NHANES) 2001–2018 to investigate the link between serum 25(OH)D and anemia incidence in the U.S. general population.

2. Materials and Methods

2.1. Study Population. NHANES is an American cross-sectional survey that collects data on the health and nutrition of the general population through stratified multistage random sampling (https://www.cdc.gov/nchs/nhanes/). Eight two-year cycles (2001–2018) of data from NHANES were used for this analysis. Participants with insufficient serum 25(OH)D or anemia data were excluded (n = 10632 and 146, respectively). Additionally, participants <18 years old and with missing demographic or biochemical data were excluded (n = 41606). Finally, this research included a total of 29933 individuals. Data on parathyroid hormone (PTH) levels, history of osteoporosis, and arthritis or rheumatism obtained by the affiliated hospital of Xuzhou Medical University between 2012 and 2022 were also included and analyzed in this study. NHANES was authorized by the National Center for Health Statistics study ethical review board, and each participant provided signed written informed permission [19]. All tests were taken at a mobile testing facility on-site.

2.2. Serum 25(OH)D Concentrations. Blood samples were collected during the examination, centrifuged, divided, and frozen at –70°C on-site, then shipped on dry ice to a central laboratory, where they were stored at –70°C until analysis. After acetonitrile-based extraction, a radioimmunoassay kit (DiaSorin, Stillwater, MN) was used to measure serum 25(OH)D levels at the National Center for Environmental Health (Atlanta, GA); however, due to concern about bias and imprecision of the DiaSorin radioimmunoassay (RIA), the Centers for Disease Control (CDC) developed regression equations to convert RIA values to liquid chromatography with tandem mass spectrometry (LC-MS/MS) equivalents for NHANES 2001–2006. In addition, the CDC laboratory analyzed serum 25(OH)D metabolites from 2007 to 2018, using LC-MS/MS, and calculated total serum 25(OH)D (nmol/L) as the sum of 25(OH)D3 and 25(OH)D2, excluding the C3-epi-25(OH)D3 metabolite. Detailed information and procedures are described on the NHANES website (https://www.cdc.gov/nchs/nhanes/vitamin/analyticalnote.aspx).

2.3. Covariates. The following covariates were included in the study: age, sex, race/ethnicity, family poverty income ratio (PIR), education level, marital status, hypertension, diabetes mellitus (DM), smoking status, and alcohol consumption status; history of coronary heart disease (CHD), congestive heart failure (CHF), angina pectoris, heart attack, stroke, chronic kidney diseases (CKD), and osteoporosis; body mass index (BMI); waist circumference; folic acid, vitamin B12, vitamin C; iron; and serum iron, calcium, phosphorus, and PTH; and number of days with arthritis or rheumatism. In addition, red blood cell (RBC) numbers and hemoglobin (Hb) levels of participants were collected. During the home interview, the following data were self-reported by participants: age, sex, ethnicity, education level, marital status, smoking status, alcohol consumption status, and dietary intake; history of CHD, CHF, angina pectoris, heart attack, stroke, CKD, and osteoporosis; and number of days with arthritis or rheumatism. Data on serum iron, calcium, phosphorus, and PTH were obtained from laboratory tests. More information about the variables analyzed in this research can be found at https://www.cdc.gov/nchs/nhanes/.

2.4. Anemia Ascertainment. The World Health Organization defines anemia as hemoglobin level <13 g/dL in men and <12 g/dL in women [20]. A Beckman Coulter DxH 800 instrument in the NHANES mobile examination center produces a complete blood count from blood specimens, which provided blood cell distributions for all participants. Refer to the Laboratory Method Files section for a detailed description of the laboratory methods used.

2.5. Statistical Analysis. All analyses were performed using R version 3.6.4 (R Foundation for Statistical Computing, Vienna, Austria) and Stata version 13.0 (Stata Corporation, College Station, TX, USA). Samples with missing covariate data were excluded from this study. P-value <0.05 was regarded as statistically significant. Serum 25(OH)D levels were divided into quartiles, and the lowest quartile served as the reference group (Q1). All estimates were calculated accounting for NHANES sample weights. Continuous variables are expressed as mean (standard deviation) and categorical variables are presented as numbers (%). Weighted linear regression models (continuous variables) and weighted chi-square tests (categorical variables) were used to assess the significance of differences. Multivariate logistic regression analysis was used to investigate the relationship between serum 25(OH)D levels and anemia risk. Model 1 was adjusted for age and sex. Model 2 was adjusted...
for model 1 variables plus race/ethnicity, education level, marital status, family PIR, smoking status, alcohol consumption status, history of hypertension, and DM. Finally, model 3 was adjusted for model 2 variables plus history of CHD, CHF, angina pectoris, heart attack, stroke, and CKD; BMI; waist circumference; folic acid, vitamin B12, vitamin C, iron; and serum iron, calcium, and phosphorus, as our core model.

### 3. Results

#### 3.1. Baseline Characteristics.

Table 1 shows the baseline characteristics of the research participants. The incidence of anemia in this group was 8.3%. The characteristics of the participants were subclassified based on serum 25(OH)D quartiles (Q1: 6.31–44.4 nmol/L; Q2: 44.5–60.6 nmol/L; Q3: 60.7–77.7 nmol/L; and Q4: 77.8–422 nmol/L). There were significant differences in age, sex, race, family PIR, education level, marital status, smoking status, alcohol consumption status, hypertension, DM, CHF, stroke, CKD, BMI, waist circumference, RBC, Hb, serum iron, calcium, phosphorus, mean energy intake, protein intake, folic acid intake, vitamin B12 intake, and iron intake among the Q1, Q2, Q3, and Q4 groups. Compared with Q1, Q3, and Q4 groups, the Q2 group had a lower proportion of participants with hypertension, heart attack, stroke, and CKD, as well as the highest RBC counts; however, compared with the Q1, Q2, and Q4 groups, a lower proportion of individuals in the Q3 group had DM, CHF, angina, and anemia, while they had the highest levels of Hb, mean energy intake, protein intake, folic acid intake, vitamin B12 intake, and iron intake. In addition, compared with Q1, Q2, and Q3, participants in Q4 were older and had the highest levels of family PIR, calcium, phosphorus, serum iron, and vitamin C intake, and had lower BMI and waist circumference. The basic characteristics of the 959 participants from the affiliated hospital of Xuzhou Medical University are shown in Supplementary Table 1, including the levels of PTH, history of osteoporosis, and number of days with arthritis or rheumatism.

#### 3.2. Association between Serum 25(OH)D and Anemia.

The findings of multivariate logistic regression analysis of the relationship between serum 25(OH)D and anemia are presented in Table 2. After adjusting for interfering factors, the odds ratios (ORs) with 95 percent confidence intervals (CIs) for association of serum 25(OH)D with anemia across quartiles two, three, and four were 0.735 (0.651, 0.829), 0.527 (0.461, 0.602), and 0.696 (0.611, 0.792), compared with Q1. As shown by the restricted cubic spline (RCS) plot, serum 25(OH)D was associated with anemia prevalence with a U-shaped curve ($P$ for nonlinearity <0.001, Figure 1). As serum 25(OH)D concentrations increased, the risk of anemia initially decreased significantly; risk of anemia was lowest when serum 25(OH)D concentrations reached 65.0 nmol/L, then the curve showed an upward trend. In the fully adjusted model including PTH, history of osteoporosis, and number of days with arthritis or rheumatism, the ORs and 95 percent CIs for anemia incidence across the serum 25(OH)D quartiles were 0.771 (0.449, 1.326), 0.499 (0.267, 0.932), and 0.596 (0.323, 1.097), compared with the first quartile of serum 25(OH)D (Supplementary Table 2). Additionally, RCS plots constructed after adjusting for PTH, history of osteoporosis, and number of days with arthritis or rheumatism as covariates also displayed U-shaped curves (Supplementary Figure 1).

#### 3.3. Associations of Serum 25(OH)D with RBC Count and Hb Level.

Generalized additive models with smooth functions suggested nonlinear relationships between serum 25(OH)D and risk of anemia. As serum 25(OH)D levels increased, both RBC count and Hb level first increased and then decreased (Figures 2(a) and 2(b)). Additionally, generalized additive models with smooth functions generated following adjustment for PTH, history of osteoporosis, and number of days with arthritis or rheumatism as covariates also showed an increase, after declining (Supplementary Figures 2(a) and 2(b)).

#### 3.4. Subgroup Analyses.

Subgroup analyses, stratified by age, sex, race/ethnicity, hypertension, DM, and obesity were conducted to estimate the associations between serum 25(OH)D and incident anemia. The results demonstrated U-shaped relationships between serum 25(OH)D and risk of anemia in individuals of all ages, male or female, who were Mexican American, Other Hispanic, Non-Hispanic Black, or Non-Hispanic White, without hypertension, without DM, and never participated, with or without hypertension, with or without diabetes, and whether or not obese (Table 3). There were significant interactions of age, sex, race/ethnicity, hypertension, DM, and obesity with the associations with serum 25(OH)D in subgroup analyses. In addition, we performed subgroup analysis stratified by age, sex, hypertension, DM, and obesity with the associations with serum 25(OH)D in subgroup analyses. In addition, we performed subgroup analysis stratified by age, sex, hypertension, DM, and obesity, to examine the correlation between serum 25(OH)D and the risk of anemia, after adjusting for variables, including PTH, history of osteoporosis, and number of days with arthritis or rheumatism (Supplementary Table 3).

### 4. Discussion

In recent years, studies of the relationship between 25(OH)D and anemia have focused on sickle cell anemia, and have demonstrated that 25(OH)D supplementation is helpful in treatment of sickle cell anemia [16, 18, 21–23]; however, the overall relationship between 25(OH)D and anemia, particularly its trend, has not been clarified. Our study revealed a U-shaped relationship between serum 25(OH)D levels and risk of anemia, and with increased 25(OH)D level, hemoglobin level, and RBC counts the association showed a downward trend, suggesting that caution should be applied in the use of 25(OH)D supplementation to treat anemia. Insufficient 25(OH)D levels can reduce local calcitriol production in the bone marrow and limit erythropoiesis. As calcitriol has a synergistic effect with endogenous erythropoietin, it can up-regulate the expression of erythropoietin receptors on erythrocyte progenitor cells [24–26]; however,
Table 1: Study population data according to serum 25(OH)D quartiles.

| Serum 25(OH)D | Total | Q1 | Q2 | Q3 | Q4 | P-value |
|---------------|-------|----|----|----|----|---------|
| Age, years    | 47.319 ± 0.218 | 44.127 ± 0.288 | 45.035 ± 0.323 | 47.076 ± 0.279 | 51.095 ± 0.294 | <0.001 |
| Gender, %     | 14830 (49.5%) | 3479 (11.6%) | 4020 (13.5%) | 4032 (13.5%) | 3299 (11.0%) | <0.001 |
| Race, %       | 4818 (16.1%) | 1505 (5.0%) | 1630 (5.4%) | 1141 (3.8%) | 542 (1.8%) | <0.001 |
| Marital status, % | 12822 (42.8%) | 3769 (12.9%) | 4020 (13.5%) | 5177 (17.3%) | 5047 (18.1%) | <0.001 |
| Hypertension, % | 17111 (57.2%) | 4243 (14.2%) | 4515 (15.1%) | 4383 (14.6%) | 3970 (13.3%) | <0.001 |
| DM, %         | 24826 (82.9%) | 6059 (20.2%) | 6202 (20.7%) | 6308 (21.1%) | 6257 (20.9%) | <0.001 |
| Alcohol user, % | 3991 (13.3%) | 1228 (4.1%) | 1030 (3.4%) | 851 (2.8%) | 872 (2.9%) | <0.001 |
| CKD, %        | 28680 (95.8%) | 7258 (24.2%) | 7175 (24.0%) | 7161 (23.9%) | 7086 (23.7%) | <0.001 |
| CHF, %        | 29054 (97.1%) | 7263 (24.3%) | 7278 (24.3%) | 7283 (24.3%) | 7230 (24.2%) | <0.001 |
| Angina, %     | 29089 (97.2%) | 7295 (24.4%) | 7267 (24.3%) | 7276 (24.3%) | 7251 (24.2%) | 0.166 |
| Heart attack, %| 28665 (95.8%) | 7209 (24.1%) | 7187 (24.0%) | 7138 (23.8%) | 7131 (23.8%) | 0.239 |
| Stroke, %     | 28877 (96.5%) | 7253 (24.2%) | 7256 (24.2%) | 7215 (24.1%) | 7153 (23.9%) | 0.001 |
| CKD, %        | 24689 (82.5%) | 6161 (20.6%) | 6321 (21.1%) | 6287 (21.0%) | 5920 (19.8%) | <0.001 |
| BMI, kg/m²    | 28.844 ± 0.072 | 30.960 ± 0.137 | 29.571 ± 0.132 | 28.492 ± 0.105 | 27.378 ± 0.091 | <0.001 |
| RBC, million cells/ul | 4.714 ± 0.007 | 4.724 ± 0.010 | 4.762 ± 0.009 | 4.750 ± 0.009 | 4.642 ± 0.010 | <0.0001 |
Table 1: Continued.

| Serum 25(OH)D | Total       | Q1          | Q2          | Q3          | Q4          | P-value |
|---------------|-------------|-------------|-------------|-------------|-------------|---------|
| Hb, g/dl      | 14.350 ± 0.023 | 14.093 ± 0.033 | 14.438 ± 0.028 | 14.504 ± 0.027 | 14.300 ± 0.034 | <0.0001 |
| Serum iron, ug/dl | 87.784 ± 0.348 | 80.342 ± 0.549 | 87.907 ± 0.594 | 88.667 ± 0.498 | 91.244 ± 0.591 | <0.001  |
| Calcium, mg/dl | 9.435 ± 0.006  | 9.390 ± 0.009  | 9.422 ± 0.008  | 9.439 ± 0.007  | 9.469 ± 0.009  | <0.001  |
| Phosphorus, mg/dl | 1.208 ± 0.002  | 1.204 ± 0.003  | 1.204 ± 0.003  | 1.208 ± 0.003  | 1.216 ± 0.003  | 0.005   |
| Mean energy intake, kcal | 2137.327 ± 7.357 | 2071.327 ± 13.574 | 2150.599 ± 13.724 | 2204.818 ± 12.138 | 2106.481 ± 12.942 | <0.001  |
| Protein intake, g | 83.081 ± 0.323 | 78.920 ± 0.512 | 83.956 ± 0.541 | 85.968 ± 0.571 | 82.309 ± 0.552 | <0.001  |
| Folic acid intake, mcg | 189.390 ± 1.525 | 168.985 ± 2.166 | 191.162 ± 2.509 | 200.219 ± 2.819 | 190.416 ± 2.712 | <0.001  |
| Vitamin B12 intake, mcg | 83.903 ± 0.872 | 82.746 ± 1.461 | 82.011 ± 1.264 | 84.748 ± 1.207 | 85.248 ± 1.470 | 0.189    |
| Iron intake, mg | 15.361 ± 0.072 | 14.070 ± 0.110 | 15.322 ± 0.111 | 16.057 ± 0.129 | 15.528 ± 0.123 | <0.001  |

Anemia, %

|                | No          | Yes         |
|----------------|-------------|-------------|
|                | 27438 (91.7%) | 2495 (8.3%) |
|                | 6583 (22.0%) | 931 (3.1%)  |
|                | 6898 (23.0%) | 583 (1.9%)  |
|                | 7043 (23.5%) | 421 (1.4%)  |
|                | 6914 (23.1%) | 560 (1.9%)  |

Table 2: Adjusted ORs for associations between serum 25(OH)D and the risk of anemia.

| Serum 25(OH)D | Model 1 OR (95% CI) | Model 2 OR (95% CI) | Model 3 OR (95% CI) |
|---------------|----------------------|----------------------|----------------------|
| Q1            | Ref.                 | 0.592 (0.530, 0.661) | 0.617 (0.552, 0.690) |
| Q2            | 0.396 (0.351, 0.448) | 0.440 (0.388, 0.498) | 0.527 (0.461, 0.602) |
| Q3            | 0.463 (0.413, 0.518) | 0.536 (0.476, 0.603) | 0.696 (0.611, 0.792) |

P for trend: <0.001 <0.001 <0.001

Q1, 6.31–44.4 nmol/L; Q2, 44.5–60.6 nmol/L; Q3, 60.7–77.7 nmol/L; Q4, 77.8–422 nmol/L; Serum 25(OH)D, serum 25-hydroxyvitamin D; Family PIR, family poverty income ratio; DM, diabetes mellitus; CHD, coronary heart disease; CHF, congestive heart failure; CKD, chronic kidney disease; BMI, body mass index; RBC, red blood cell; Hb, hemoglobin.

Figure 1: Restricted cubic spline plot of the association between serum 25(OH)D and the incidence of anemia.
Figure 2: Associations of serum 25(OH)D with hemoglobin levels and red blood cell counts. (a) Association between serum 25(OH)D and hemoglobin level. (b) Association between serum 25(OH)D and red blood cell count.

Table 3: Subgroup analysis for associations between serum 25(OH)D and the risk of anemia.

| Serum 25(OH)D | Q1 (OR, 95% CI) | Q2 (OR, 95% CI) | Q3 (OR, 95% CI) | Q4 (OR, 95% CI) | P for trend | P for interaction |
|---------------|----------------|----------------|----------------|----------------|-------------|------------------|
| Age           |                |                |                |                |             |                  |
| <60           | 1.00           | 0.689 (0.589, 0.806) ** | 0.470 (0.390, 0.565) *** | 0.476 (0.388, 0.583) *** | <0.001      |                  |
| ≥60           | 1.00           | 0.884 (0.726, 1.076) *** | 0.682 (0.557, 0.836) *** | 1.073 (0.892, 1.292) *** | <0.001      |                  |
| Gender        |                |                |                |                |             |                  |
| Male          | 1.00           | 0.759 (0.612, 0.940) * | 0.508 (0.404, 0.639) *** | 0.866 (0.697, 1.075) ** | <0.001      | <0.001           |
| Female        | 1.00           | 0.734 (0.631, 0.853) *** | 0.558 (0.471, 0.662) *** | 0.620 (0.524, 0.734) *** | <0.001      | <0.001           |
| Race          |                |                |                |                |             |                  |
| Mexican American | 1.00          | 0.905 (0.663, 1.236)  | 0.764 (0.527, 1.106)  | 1.117 (0.724, 1.723)  | 0.378       |                  |
| Other Hispanic | 1.00           | 0.783 (0.488, 1.256)  | 0.711 (0.428, 1.183)  | 0.874 (0.499, 1.532)  | 0.573       |                  |
| Non-Hispanic Black | 1.00         | 0.989 (0.817, 1.196)  | 0.742 (0.581, 0.948) * | 1.022 (0.802, 1.303)  | 0.092       | <0.001           |
| White         | 1.00           | 1.031 (0.774, 1.373)  | 0.982 (0.744, 1.296)  | 1.308 (1.005, 1.703) * | 0.019       |                  |
| Other race    | 1.00           | 1.195 (0.783, 1.824)  | 0.421 (0.243, 0.730) ** | 0.755 (0.463, 1.232)  | 0.002       |                  |
| Hypertension  |                |                |                |                |             |                  |
| No            | 1.00           | 0.675 (0.567, 0.804) ** | 0.471 (0.386, 0.574) *** | 0.505 (0.409, 0.625) *** | <0.001      | 0.001            |
| Yes           | 1.00           | 0.791 (0.668, 0.937) ** | 0.579 (0.482, 0.695) *** | 0.864 (0.730, 1.022) *** | <0.001      |                  |
| DM            |                |                |                |                |             |                  |
| No            | 1.00           | 0.680 (0.590, 0.783) ** | 0.467 (0.398, 0.547) *** | 0.624 (0.535, 0.729) *** | <0.001      | <0.001           |
| Yes           | 1.00           | 0.921 (0.728, 1.164)  | 0.728 (0.566, 0.936) * | 0.934 (0.733, 1.191)  | 0.088       |                  |
| Obesity       |                |                |                |                |             |                  |
| <30 kg/m²²    | 1.00           | 0.761 (0.647, 0.895) ** | 0.538 (0.453, 0.639) *** | 0.619 (0.523, 0.732) *** | <0.001      |                  |
| ≥30 kg/m²²    | 1.00           | 0.718 (0.597, 0.862) ** | 0.504 (0.405, 0.627) *** | 0.863 (0.701, 1.062) *** | <0.001      |                  |

Q1, 6.31–44.4 nmol/L; Q2, 44.5–60.6 nmol/L; Q3, 60.7–77.7 nmol/L; Q4, 77.8–422 nmol/L; Serum 25(OH)D, serum 25-hydroxyvitamin D; *, P < 0.05; **, P < 0.01; ***; P < 0.001; OR, odd ratio; CI, confidence interval. Analysis was adjusted for age, gender, race/ethnicity, education level, marital status, family poverty income ratio, hypertension, diabetes mellitus, smoke status, and drink status, the history of coronary heart disease, congestive heart failure, angina pectoris, heart attack, stroke, and chronic kidney diseases, body mass index, waist circumference, folic acid intake, Vitamin B12 intake, Vitamin C intake, iron intake, serum iron, calcium, and phosphorus.
with age, sex, race/ethnicity, hypertension, diabetes, obesity, and serum 25(OH)D. For the Hispanics (other) ethnic category, the 25(OH)D threshold was significantly lower than that for other races.

Immune diseases are a cause of anemia, and are often accompanied by abnormal metabolism of vitamin D and PTH, leading to osteoporosis [31, 32]. Further, low levels of 25(OH)D may increase the risk of immune-related diseases because they exert an immunomodulatory effect through nuclear vitamin D receptor expressed by antigen-presenting cells and activated T and B cells [33, 34]. Therefore, we screened the database for data from patients with immune diseases and hyperparathyroidism; after adjusting for these covariates, the results were generally consistent with those above, confirming that arthritis and rheumatism are not additional risk factors for reduced plasma 25(OH)D concentrations [35]. Notably, 25(OH)D levels in patients with arthritis and rheumatism were higher than those in the whole group when anemia risk was in the trough, which may be due to the effects of the pathological processes underlying arthritis or rheumatism on anemia, or the regulation of 25(OH)D by PTH; however, neither significantly affected the overall trend relationship between anemia and 25(OH)D. Therefore, the use of vitamin D supplementation to treat anemia needs to be personalized for different populations, particularly in patients with comorbidities that may affect vitamin D metabolism. In summary, our study shows that there is no simple linear relationship between 25(OH)D and risk of anemia, and that age, sex, race, hypertension, diabetes, arthritis, rheumatism, and obesity all interact with 25(OH)D levels. Further basic and prospective experiments are needed to further explore the relationship between 25(OH)D and the mechanism underlying anemia development.

Our study has some limitations. First, we used the NHANES public database for this analysis, which covers the years 2001 to 2018. To verify our findings, participants from other nations should be recruited. Second, a retrospective study has the disadvantage of introducing bias to some relevant results. Third, a study of the mechanisms underlying serum 25(OH)D levels and anemia incidence is also necessary.

5. Conclusion

In conclusion, the relationship between serum 25(OH)D and risk of anemia presents as a U-shaped curve. An inflection point for serum 25(OH)D was observed in our study; the incidence of anemia was lowest when the serum 25(OH)D level was 65.0 nmol/l, and anemia risk was <1 when serum 25(OH)D levels were between 59.7 and 70.3 nmol/l. Therefore, with close monitoring and adequate vitamin D supplementation, the risk of anemia can be reduced.

Data Availability

The survey data are publicly available on the Internet for data users and researchers throughout the world (https://www.cdc.gov/nchs/nhanes/).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Yang Zhang and Xiaotong Wang contributed to hypothesis development and manuscript preparation. Jia Wang contributed to study design. Weiwei Hu, Xiaolu Song, Ding Yuan, and Xianliang Yan undertook data analyses. Yang Zhang drafted and revised the manuscript. All authors approved the final draft of the manuscript for publication. Yang Zhang, Xiaotong Wang, and Jia Wang contributed equally to this work.

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Supplementary Materials

Supplementary Figure 1: Restricted cubic spline plot of the association between serum 25(OH)D and the incidence of anemia. Supplementary Figure 2: Associations of serum 25(OH)D with hemoglobin levels and red blood cell counts. (a) Association between serum 25(OH)D and hemoglobin level. (b) Association between serum 25(OH)D and red blood cell count. Supplementary Table 1: Study population data according to serum 25(OH)D quartiles. Supplementary Table 2: Adjusted ORs for associations between serum 25(OH)D and the risk of anemia. Supplementary Table 3: Subgroup analysis for associations between serum 25(OH)D and the risk of anemia. (Supplementary Materials)

References

[1] C. M. Chaparro and P. S. Suchdev, “Anemia epidemiology, pathophysiology, and etiology in low- and middle-income countries,” *Annals of the New York Academy of Sciences*, vol. 1450, no. 1, pp. 15–31, 2019.
[2] E. McLean, M. Cogswell, I. Egli, D. Wojdyla, and B. de Benoist, “Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993-2005,” *Public Health Nutrition*, vol. 12, no. 4, pp. 444–454, 2009.
[3] N. Abdo, S. Douglas, A. Batieha et al., “The prevalence and determinants of anaemia in Jordan,” *Eastern Mediterranean Health Journal*, vol. 25, no. 5, pp. 341–349, 2019.
[4] M. Dugdale, *Obstetrics and Gynecology Clinics of North America*, vol. 28, no. 2, pp. 363–382, 2001.
[5] Y. Yuan, Z. Cai, Y. Dai et al., “Association of maternal serum 25-hydroxyvitamin D concentrations with risk of gestational anemia,” *Cellular Physiology and Biochemistry*, vol. 43, no. 4, pp. 1526–1532, 2017.
[6] T. S. Perlstein, R. Pande, N. Berliner, and G. J. Vanasse, “Prevalence of 25-hydroxyvitamin D deficiency in subgroups of elderly persons with anemia: association with anemia of inflammation,” *Blood*, vol. 117, no. 10, pp. 2800–2806, 2011.
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[7] E. M. Smith, J. A. Alvarez, G. S. Martin, S. M. Zughai, T. R. Ziegler, and V. Tangri, “Vitamin D deficiency is associated with anaemia among African Americans in a US cohort,” British Journal of Nutrition, vol. 113, no. 11, pp. 1732–1740, 2015.

[8] M. A. Atkinson, M. L. Melamed, J. Kumar et al., “Vitamin D, race, and risk for anemia in children,” The Journal of Pediatrics, vol. 164, no. 1, pp. 153–158.e1, 2014.

[9] H. J. Jin, J. H. Lee, and M. K. Kim, “The prevalence of vitamin D deficiency in iron-deficient and normal children under the age of 24 months,” Blood Research, vol. 48, no. 1, pp. 40–45, 2013.

[10] H. Reichel, H. P. Koeffler, and A. W. Norman, “The role of the vitamin D endocrine system in health and disease,” New England Journal of Medicine, vol. 320, no. 15, pp. 980–991, 1989.

[11] A. W. Norman, “Vitamin D receptor: new assignments for an already busy receptor,” Endocrinology, vol. 147, no. 12, pp. 5542–5548, 2006.

[12] I. Blazsek, C. Farabos, P. Quittet et al., “Bone marrow stromal cell defects and 1 alpha, 25-dihydroxyvitamin D3 deficiency underlying human myeloid leukemias,” Cancer Detection and Prevention, vol. 20, no. 1, pp. 31–42, 1996.

[13] E. H. Yoo and H. J. Cho, “Prevalence of 25-hydroxyvitamin D deficiency in Korean patients with anemia,” Journal of Clinical Laboratory Analysis, vol. 29, no. 2, pp. 129–134, 2015.

[14] J. Kendrick, G. Targher, G. Smits, and M. Chonchol, “25-Hydroxyvitamin D deficiency and inflammation and their association with hemoglobin levels in chronic kidney disease,” American Journal of Nephrology, vol. 30, no. 1, pp. 64–72, 2009.

[15] V. A. Kumar, D. A. Kujubu, J. J. Sim, S. A. Rason, and P. S. Yang, “Vitamin D supplementation and recombinant human erythropoietin utilization in vitamin D-deficient hemodialysis patients,” Journal of Nephrology, vol. 24, no. 1, pp. 98–105, 2011.

[16] S. A. Adegoke, O. S. Smith, A. D. Adekile, and M. S. Figueiredo, “Relationship between serum 25-hydroxyvitamin D and inflammatory cytokines in paediatric sickle cell disease,” Cytokine, vol. 96, pp. 87–93, 2017.

[17] S. Deane, R. J. Schroth, A. Sharma, and C. Rodd, “Combined deficiencies of 25-hydroxyvitamin D and anemia in preschool children with severe early childhood caries: a case-control study,” Paediatrics and Child Health, vol. 23, no. 3, pp. e40–e45, 2018.

[18] S. A. Adegoke, O. S. Smith, A. T. Adeniyi, and A. D. Adekile, “Thrombospondin-1 and vitamin D in children with sickle cell anemia,” Journal of Pediatric Hematology, vol. 41, no. 8, pp. e525–e529, 2019.

[19] G. Zipf, M. Chiappa, K. S. Porter, Y. Ostchega, B. G. Lewis, and J. Dostal, “National health and nutrition examination survey: plan and operations,” 2013, https://pubmed.ncbi.nlm.nih.gov/25078429/.

[20] M. K. Lee, K. D. Han, J. H. Lee et al., “High hemoglobin levels are associated with decreased risk of diabetic retinopathy in Korean type 2 diabetes,” Scientific Reports, vol. 8, no. 1, p. 5538, 2018.

[21] H. H. Soe, A. B. Abas, N. N. Than et al., “Vitamin D supplementation for sickle cell disease,” Cochrane Database of Systematic Reviews, vol. 1, no. 1, 2017.

[22] S. A. Adegoke, J. A. P. Braga, A. D. Adekile, and M. S. Figueiredo, “The association of serum 25-hydroxyvitamin D with biomarkers of hemolysis in pediatric patients with sickle cell disease,” Journal of Pediatric Hematology, vol. 40, no. 2, pp. 159–162, 2018.

[23] O. Adekunle, A. O. Dada, F. O. Njokanma, A. U. Solarin, B. A. Animasahun, and M. O. Lamina, “Comparative effectiveness of a six-week treatment course of vitamin D2 and D3 in children with sickle cell anemia in steady state with hypovitaminosis D: a randomized clinical trial,” Journal Hematology, vol. 10, no. 3, pp. 114–122, 2021.

[24] G. Saab, D. O. Young, Y. Gincherman, K. Giles, K. Norwood, and D. W. Coyne, “Prevalence of vitamin D deficiency and the safety and effectiveness of monthly ergocalciferol in hemodialysis patients,” Nephron Clinical Practice, vol. 105, no. 3, 2007.

[25] B. Alon, C. Chaimovitz, A. Dvilansky et al., “Novel role of 1, 25(OH)(2)D(3) in induction of erythroid progenitor cell proliferation,” Experimental Hematology, vol. 30, no. 5, pp. 403–409, 2002.

[26] F. Accella, R. P. Scalzulli, G. Gatta, M. Vigilante, A. M. Carella, and C. Stallone, “Calcitriol increases burst-forming unit-erythroid proliferation in chronic renal failure,” Nephron Clinical Practice, vol. 95, no. 4, pp. c121–c127, 2004.

[27] M. Kikuchi, T. Inagaki, and N. Shinagawa, “Five-year survival of older people with anemia: variation with hemoglobin concentration,” Journal of the American Geriatrics Society, vol. 49, no. 9, pp. 1226–1228, 2001.

[28] J. B. Lanier, J. J. Park, and R. C. Callahan, “Anemia in older adults,” American Family Physician, vol. 98, no. 7, pp. 437–442, 2018.

[29] S. Killip, J. M. Bennett, and M. D. Chambers, “Iron deficiency anemia,” American Family Physician, vol. 75, no. 5, pp. 671–678, 2007.

[30] B. K. Saydam, R. E. Genc, F. Sarac, and E. C. Turfan, “Prevalence of anemia and related factors among women in Turkey,” Pakistan Journal of Medical Sciences, vol. 33, no. 2, pp. 433–438, 2017.

[31] P. P. Sainaghi, M. Bellan, A. Nerviani et al., “Superiority of a high loading dose of cholecalciferol to correct hypovitaminosis d in patients with inflammatory/autoimmune rheumatic diseases,” Journal of Rheumatology, vol. 40, no. 2, pp. 166–172, 2013.

[32] P. P. Sainaghi, M. Bellan, G. Antonini, G. Bellomo, and M. Pirisi, “Unsuppressed parathyroid hormone in patients with autoimmune/inflammatory rheumatic diseases: implications for vitamin D supplementation,” Rheumatology, vol. 50, no. 12, pp. 2290–2296, 2011.

[33] C. Sirbe, S. Rednic, A. Grama, and T. L. Pop, “An update on the effects of vitamin D on the immune system and autoimmune diseases,” International Journal of Molecular Sciences, vol. 23, no. 17, p. 9784, 2022.

[34] E. v Etten and C. Mathieu, “Immune regulation by 1, 25-dihydroxyvitamin D3: basic concepts,” The Journal of Steroid Biochemistry and Molecular Biology, vol. 97, no. 1-2, pp. 93–101, 2005.

[35] P. P. Sainaghi, M. Bellan, S. Carda et al., “Hypovitaminosis D and response to cholecalciferol supplementation in patients with autoimmune and non-autoimmune rheumatic diseases,” Rheumatology International, vol. 32, no. 11, pp. 3365–3372, 2012.