Vitamin B12 as a novel risk biomarker of spinal fractures
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1. Introduction
Osteoporosis is a disorder of decreased bone mass, microarchitectural deterioration, and fragility fractures.[1] Osteoporosis is a common and frequent disease among the elderly.[2] As the disease progresses, bone cell-mediated bone remodeling would emerge, reducing bone mass, damaging bone microstructure, leading to osteoporosis and increasing the risk of spinal fractures.[3] The prevalence of osteoporosis in the elderly is rising year over year due to the contemporary aging society.[4] Due to the deterioration of physical movement of the elderly, walking is unstable, and patients are prone to falls or collisions, and often accompanied by spinal fractures. In recent years, despite advances in surgical techniques and molecular targeted therapy and immunotherapy, the prognosis of osteoporosis is still unsatisfactory.[5,6]

A spinal fracture is a common orthopedic injury. The incidence of fracture is 5% to 6%, with the highest incidence of thoracolumbar fracture, followed by cervical and lumbar vertebrae, thoracic vertebral least, often complicated with spinal cord or cauda equina nerve injury. An indirect external force causes a spinal fracture; for the hip or sufficient ground when falling, impact external force is transmitted to the thoracolumbar segment to produce fracture. The clinical manifestations are deformity and pain of the spine after trauma, which can often be complicated by spinal cord injury. Life expectancy after spinal fracture is significantly reduced, and the decline is more significant in men than in women.[7] Spinal fractures have resulted in several adverse medical outcomes, including reduced lung function, cardiac output, immune impairment, muscle degeneration, chronic back pain, and impaired functional ability. In psychosocial terms, spinal fractures not only reduce the quality of life but also have a considerable impact on society.

Vitamin B12, also known as cobalamin or cyanocobalamin, is a B vitamin composed of cobalt-containing porphyrin compounds.[8,9] It consists of cobalt as the central atom and a corrin ring that encloses the metal atom.[10] Vitamin B12, the latest B vitamin discovered to date, is a water-soluble vitamin. Vitamin B12 is a light red needle-like crystal, soluble in water and ethanol, and most stable in pH 4.5 ~ 5.0 weak acid conditions, strong acid (pH < 2) or alkaline solution decomposition, heat can have a certain degree of damage.[11] However, a short time of high-temperature...
disinfection loss is small and easy to be destroyed in solid light or ultraviolet light. The failure of the usual cooking process is about 30%. Vitamin B12 is widely found in animal foods.\textsuperscript{[11]} The best source is meat and milk from ruminants because the natural bacteria synthesize vitamin B12 in these animals.\textsuperscript{[10]} And it cannot be absorbed by the body. In addition, vitamin B12 is the only vitamin that contains essential minerals because it contains cobalt and red, called red, a few colored vitamins. However, the relationship between vitamin B12 and the incidence of spinal fracture in patients with osteoporosis is unclear. Therefore, this study hypothesized that during the occurrence and development of osteoporosis, the higher the vitamin B12, the higher the risk of spinal fracture and the shorter the time to spinal fracture recurrence. Based on the above hypothesis, 105 patients with osteoporosis were recruited. The results might reveal vitamin B12 as a new risk biomarker for spinal fractures, providing fresh ideas for the molecular mechanism of the occurrence and development of spinal fractures.

## 2. Methods

### 2.1. Patients and ethics

One hundred five patients diagnosed with osteoporosis (or without spinal fractures) were recruited at the Second Central Hospital of Baoding from March 2015 to June 2020. Osteoporosis diagnosis: normal cardiopulmonary and clotting functions; were our inclusion criteria. Exclusion criteria: poor pulmonary, cardiac, and liver function; Patients and their families did not agree to participate in the trial.

The Ethics Committee approved this study of the Second Central Hospital of Baoding. Written informed consent was obtained from all patients.

### 2.2. Parameters in the research

Based on clinical information of, patients were classed according to sex (Male/Female), age (≤60/>60), Vitamin B2 (Low/High), Vitamin A (Low/High), Vitamin B12 (Low/High), Vitamin B9 (Low/High), and Spinal fractures (No/Yes). And the patients were followed up for 5 years, and the maintenance time from recovery to recurrence (MTRR) was recorded.

### 2.3. The detection of relative blood parameters

Venous blood samples were immediately sent for examination and then tested by tyrosine decarboxylase for vitamin B12, B2, vitamin A and vitamin B9 levels.

### 3. Results

#### 3.1. Associations between spinal fractures and correlative factors based on the $\chi^2$ test

Table 1 summarizes the associations between vitamin B12 and the related clinical factors according to Pearson’s chi-squared test. Among the individuals, vitamin B12 ($P < .001$) and vitamin B9 ($P = .011$) were markedly related to spinal fractures. However, no significant associations were found between sex ($P = .231$), age ($P = .722$), vitamin B2 ($P = .184$), vitamin A ($P = .656$) and spinal fractures (Table 1).

#### 3.2. Further associations between spinal fractures and correlative factors by Spearman’s correlation test

A further correlation analysis was performed to confirm whether the potential characteristics of vitamin B12 and vitamin B9 factors played an important role on spinal fractures. Spearman’s correlation coefficient displayed that spinal fractures were significantly correlated with the vitamin B12 ($\rho = 0.507, P < .001$), and vitamin B9 ($\rho = 0.247, P = .011$). However, there was no significant correlation between sex ($\rho = 0.117, P = .235$), age ($\rho = -0.035, P = .725$), Vitamin B2 ($\rho = 0.130, P = .187$), Vitamin A ($\rho = -0.043, P = .660$) and spinal fractures (Table 2).

#### 3.3. Univariate logistic regression analysis of odds ratios between spinal fractures and correlative factors

In addition, our study used binary logistic regression (including univariate logistic regression and multivariate logistic regression) to determine the association between correlative

### Table 1

| Parameters | Spinal fractures | χ² test |
|-----------|----------------|--------|
| Sex       |                |        |
| Male      | No  36 (34.3%) | 27 (25.7%) | .231 |
| Female    | 34 (32%)       | 22 (20.1%) |
| Age       |                |        |
| ≤60       | No  50 (47%)   | 29 (27.6%) | .722 |
| >60       | 25 (23.7%)     | 21 (20.0%) |
| Vitamin B2 |                |        |
| Low       | No  28 (26.7%) | 19 (18.1%) | .184 |
| High      | 25 (23.8%)     | 21 (20.0%) |
| Vitamin A |                |        |
| Low       | No  27 (25.7%) | 31 (29.5%) |
| High      | 34 (32.4%)     | 33 (31.4%) | .656 |
| Vitamin B12 |            |        |
| Low       | No  21 (20.0%) | 17 (16.2%) | <.001* |
| High      | 15 (14.3%)     | 30 (28.6%) |
| Vitamin B9 |                |        |
| Low       | No  15 (14.3%) | 11 (10.5%) | .011* |
| High      | 30 (28.6%)     | 29 (27.6%) |

*P < .05.
parameters and spinal fractures, odds ratios (ORs) and 95% confidence intervals (95% CIs) to determine further the risk factors and risk groups of spinal fractures. Table 3 describes the ORs and 95% CI of the study subjects at the univariate level using univariate logistic regression and concludes that the expression of vitamin B12 (OR = 9.455, 95% CI: 3.866–23.123, \( P < .001 \)) and vitamin B9 (OR = 2.955, 95% CI: 1.258–6.941, \( P = .013 \)) have a clear correlation with whether the patients have spinal fractures. However, there was no significant correlation between sex (OR = 1.614, 95% CI: 0.735–3.543, \( P = .233 \)), age (OR = 0.869, 95% CI: 0.401–1.882, \( P = .185 \)), Vitamin B2 (OR = 1.692, 95% CI: 0.777–3.684, \( P = .656 \)), Vitamin A (OR = 0.834, 95% CI: 0.375–1.854, \( P = <.001^* \)), and spinal fractures (Table 3).

3.4. Multivariate logistic regression analysis for correlative factors and spinal fractures

Table 4 applied multivariate logistic regression to describe the OR and 95% CI of the study subjects at the multivariate level. In terms of multivariate logistic regression level, vitamin B12 (OR = 10.266, 95% CI: 3.866–27.264, \( P < .001 \)) was significantly associated with spinal fractures, whereas sex (OR = 2.158, 95% CI: 0.787–5.915, \( P = .135 \)), age (OR = 0.631, 95% CI: 0.242–1.645, \( P = .346 \)), vitamin B2 (OR = 1.542, 95% CI: 0.577–4.272, \( P = .405 \)), vitamin A (OR = 0.740, 95% CI: 0.268–2.041, \( P = .561 \)) and vitamin B9 (OR = 2.020, 95% CI: 0.747–5.460, \( P = .166 \)) showed no significant associations with spinal fractures (Table 4).

3.5. Univariate cox regression for the proportional hazard analysis of spinal fractures

Table 5 presented the univariate hazard ratios (HRs) and 95% CI for patients who underwent osteoporosis. For overall survival, subjects with high vitamin B12 had lower MTRR than those with low vitamin B12 levels, and the HR was 7.985 (95% CI: 5.146–15.044, \( P < .001 \)). However, sex (HR = 0.811, 95% CI: 0.527–1.249, \( P = .342 \)), age (HR = 1.255, 95% CI: 0.825–1.908, \( P = .289 \)), Vitamin B2 (HR = 0.802, 95% CI: 0.514–1.251, \( P = .330 \)), Vitamin A (HR = 1.234, 95% CI: 0.790–1.927, \( P = .355 \)) and Vitamin B9 (HR = 1.037, 95% CI: 0.662–1.626, \( P = .873 \)) have no significant correlation with MTRR (Table 5).

3.6. Analysis of MTRR based on multivariate cox regression for the proportional hazards of related characteristics

All factors were incorporated into the multivariate cox regression model to control the influence of confounding factors effectively. Table 6 shows the result of the multivariate cox proportional regression analysis. Vitamin B12 gene level (HR = 9.930, 95% CI: 5.594–17.626, \( P < .001 \)) was significantly associated with MTRR. However, sex (HR = 0.799, 95% CI: 0.501–1.273, \( P = .345 \)), age (HR = 1.126, 95% CI: 0.705–1.798, \( P = .621 \)), vitamin B2 (HR = 0.732, 95% CI: 0.460–1.163, \( P = .186 \)), vitamin A (HR = 1.531, 95% CI: 0.941–2.491, \( P = .087 \)) and vitamin B9 (HR = 1.037, 95% CI: 0.662–1.626, \( P = .873 \)) have no significant correlation with MTRR (Table 5).
vitamin B9 (HR = 0.690, 95% CI: 0.423–1.126, P = .137) have no significant correlation with MTRR (Table 6).

4. Discussion

Based on the Pearson χ² and Spearman correlation tests, there exist strong relation between the expression level of vitamin B12 and spinal fractures. The expression of vitamin B12 has a clear correlation with spinal fractures via the univariate and multivariate logistic regression analysis. In addition, the expression level of vitamin B12 was significantly associated with MTRR of patients with osteoporosis.

Vitamins are low molecular organic substances indispensable for maintaining normal metabolism and some special physiological functions.[12] Although the human body has a small demand for vitamins, once the human body lacks vitamins, the corresponding metabolic reaction will be problematic, resulting in vitamin deficiency. Many people misunderstand vitamin B, thinking that vitamin B is a simple element, and it is not the case, and vitamin B is a family. Vitamin B also contains many vitamins, the general name for B vitamins. When we supplement vitamin B, we should see clearly what we need to supplement and which kind of vitamin B to achieve targeted to receive good results. B vitamin supplements are not suitable for all populations.

Vitamin B12 status in humans depends on intake.[13,14] Folic acid is one of the B complex vitamins.[15] And it is an umbrella term for different forms of water-soluble vitamins.[16] Folic acid is found mainly in vegetables or added to staple foods as folic acid fortification.[17] But too much vitamin B12 can also lead to a deficiency of folic acid,[18] essential for the body to use sugars and amino acids and for cell growth and reproduction. Folic acid plays a critical role in cell division and development and synthesizing nucleic acid, amino acid and protein. Lack of folic acid in the body can lead to abnormal red blood cells and an increase in immature cells.[19] In vivo, folic acid acts as tetrahydrofolic acid, which is involved in synthesizing and converting purine nucleic acids, and pyrimidine nucleotides in vivo play a key role in DNA synthesis.[20] And folic acid plays an essential role in manufacturing nucleic acids (RNA, deoxyribonucleic acid). Folic acid helps protein metabolism, thus increasing the supply of nutrients in the body. The lack of folic acid in the body cannot provide energy for bone modeling in osteoporosis, leading to further destruction and erosion of bone tissue, reducing bone mass and bone fragility.[21] Almost any bone can fracture as a result of the increased bone fragility of osteoporosis. Consequently, there is a greater chance of a clinical spinal fracture and a shorter recovery period after a spinal fracture.

In a study published by Harvard University in the United States, vitamin B12 intake of 30 micrograms a day or more was associated with a 25 per cent increased risk of fracture over a 30-year follow-up period, compared with <5 μg a day. High doses of vitamin supplements may cause unexpected adverse reactions, and a higher intake of vitamins B6 and B12 is associated with a higher risk of postmenopausal fractures in women.[22] So, vitamin B group to maintain human health, prevention and treatment of a variety of diseases have a particular role, but in the supplement of their lack of vitamin B, by all means, avoid a large number of supplements blindly.

However, this study also has some defects. Although clinical specimens were tested and analyzed, the molecular mechanism of vitamin B12 on osteoporosis and spinal fractures was not verified in animal models. Therefore, future studies should focus on animal experiments to explore the molecular functions of vitamin B12 and find its molecular pathways and mechanisms in osteoporosis and spinal fractures.

5. Conclusions

In summary, vitamin B12 is significantly associated with poor prognosis, spinal fracture incidence and recurrence time in patients with osteoporosis. The higher the vitamin B12, the higher the risk of spinal fracture and the shorter the time to spinal fracture recurrence. Vitamin B12 is a new risk biomarker for spinal fractures, providing fresh ideas for the molecular mechanism of the occurrence and development of spinal fractures.

Table 5

| Characteristics | HR   | 95% CI     | P   |
|----------------|------|------------|-----|
| Sex           |      |            |     |
| Male          | 63   | 1          | .342|
| Female        | 42   | 0.811      | 0.527-1.249 | .289|
| Age           |      |            |     |
| ≤65           | 59   | 1          | .825-1.908 | .330|
| >65           | 46   | 1.255      | .514-1.251 | .735|
| Vitamin B2    |      |            |     |
| Low           | 47   | 1          | .790-1.927 | <.001*|
| High          | 58   | 0.802      | .514-1.251 | .355|
| Vitamin A     |      |            |     |
| Low           | 67   | 1          | .790-1.927 | <.001*|
| High          | 38   | 1.234      | .790-1.927 | .355|
| Vitamin B12   |      |            |     |
| Low           | 51   | 1          | .790-1.927 | <.001*|
| High          | 54   | 1.234      | .790-1.927 | .355|
| Vitamin B9    |      |            |     |
| Low           | 36   | 1          | .790-1.927 | <.001*|
| High          | 69   | 1.037      | .662-1.626 | .137|

95% CI = 95% confidence interval, HR = hazard ratio, MTRR = maintenance time from recovery to recurrence.

*P < .05.

Table 6

| Characteristics | HR   | 95% CI     | P   |
|----------------|------|------------|-----|
| Sex           |      |            |     |
| Male          | 0.799| 0.501-1.273| .345|
| Female        | 1.126| 0.705-1.798| .621|
| Age           |      |            |     |
| Vitamin B2    | 0.732| 0.460-1.163| .186|
| Vitamin A     | 1.331| 0.941-2.491| .087|
| Vitamin B12   | 9.930| 5.594-17.626| <.001*|
| Vitamin B9    | 0.690| 0.423-1.126| .137|

95% CI = 95% confidence interval, HR = hazard ratio, MTRR = maintenance time from recovery to recurrence.

*P < .05.

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