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Vitamin B12 as a novel risk biomarker of spinal fractures

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Abstract
Spinal fractures are common intra-articular fractures. Osteoporosis is a common and frequent disease among the elderly with a poor prognosis and a high risk of spinal fractures. However, the underlying factors for spinal fractures in patients with osteoporosis are unclear. A total of 105 patients with osteoporosis were recruited. Clinical and followed-up information was recorded. And vitamin B12, vitamin B2, vitamin A, and vitamin B9 in the blood were tested. Pearson's chi-squared and spearman tests were performed to analyze the correlation between spinal fractures and relative parameters. Univariate and multivariate logistic regression, univariate and multivariate Cox proportional hazards regression analysis. There exists strong relation between the expression level of vitamin B12 and spinal fractures. Pearson's chi-square and Spearman correlation test showed a strong association between vitamin B12 and vitamin B9 and a spinal fracture. Univariate logistic regression analysis showed that vitamin B12 and vitamin B9 were significantly associated with a spinal fracture. Multivariate logistic regression analysis showed that vitamin B12 was associated considerably with a spinal fracture. In addition, Cox regression analysis showed that vitamin B12 expression was significantly associated with maintenance time from recovery to recurrence (MTRR) of spinal fractures in patients with osteoporosis. Enhanced vitamin B12 is significantly correlated with the poor prognosis of patients with osteoporosis and the increasing incidence of a spinal fracture. The higher the vitamin B12, the higher the risk of spinal fracture and the shorter the time to spinal fracture recurrence.

Abbreviations: 95% CIs = 95% confidence intervals, HRs = hazard ratios, MTRR = maintenance time from recovery to recurrence, ORs = odds ratios.

Keywords: osteoporosis, risk biomarker, spinal fractures, vitamin B12

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 vertebrae 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 cyanocobaltin, 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.[10] However, a short time of high-temperature

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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

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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.[11] The best source is meat and milk from ruminants because the natural bacteria synthesize vitamin B12 in these animals.[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.

2.4. Statistical methods

The data were expressed as a percentage of total and percentage. Associations between the clinical parameters and vitamin B12 were analyzed using Pearson's chi-squared test. The Spearman-rho test was executed to compare clinical data and vitamin B12 for the correlation analysis. Univariate and multivariate logistic regression analysis was used to calculate the OR of each variable for spinal fractures. Using univariate and multivariate Cox proportional hazards regression analysis, potential prognostic factors for MTRR were explored. A further illustration for MTRR was made by the Kaplan–Meier method.

All statistical analyses were conducted using SPSS software, version 21.0 (IBM Corp., Armonk, NY). A P value <.05 was considered statistically significant.

3. Results

3.1. Associations between spinal fractures and correlative factors based on the χ^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

Relevant characteristics of patients with Spinal fractures.

Parameters			Spinal fractures		P
			No	Yes	
Sex	Male	63	36(34.3%)	27(25.7%)	.231
	Female	42	19(18.1%)	23(21.9%)	
Age	≤60	59	30(28.6%)	29(27.6%)	.722
	>60	46	25(23.8%)	21(20.0%)	
Vitamin B2	Low	47	28(26.7%)	19(18.1%)	.184
	High	58	27(25.7%)	31(29.5%)	
Vitamin A	Low	67	34(32.4%)	33(31.4%)	.656
	High	38	21(20.0%)	17(16.2%)	
Vitamin B12	Low	51	40(38.1%)	11(10.5%)	<.001*
	High	54	15(14.3%)	39(37.1%)	
Vitamin B9	Low	36	25(23.8%)	11(10.5%)	.011*
	High	69	30(28.6%)	39(37.1%)	

Pearson's chi-squared test was used.

*$P < .05$.

Table 2

The relationship between characteristics of patients and Spinal fractures.

Characteristics	Spinal fractures ρ	P
Sex	0.117	.235
Age	-0.035	.725
Vitamin B2	0.130	.187
Vitamin A	-0.043	.660
Vitamin B12	0.507	<.001*
Vitamin B9	0.247	.011*

Spearman correlation test was used.
*P < .05

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).

Table 3

Correlative parameters' effect on spinal fractures based on univariate logistic proportional regression analysis.

Parameters			Spinal fractures OR	95% CI	P
Sex	Male	63	1		.233
	Female	42	1.614	0.735-3.543	
Age	≤65	59	1		.722
	>65	46	0.869	0.401-1.882	
Vitamin B2	Low	47	1		.185
	High	58	1.692	0.777-3.684	
Vitamin A	Low	67	1		.656
	High	38	0.834	0.375-1.854	
Vitamin 12	Low	51	1		<.001*
	High	54	9.455	3.866-23.123	
Vitamin B9	Low	36	1		.013*
	High	69	2.955	1.258-6.941	

95% CI = 95% confidence interval, OR = odds ratio.
*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, $P = .722$), Vitamin B2 (OR = 1.692, 95% CI: 0.777–3.684, $P = .185$), Vitamin A (OR = 0.834, 95% CI: 0.375–1.854, $P = .656$) 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).

3.6. 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

Table 4

The characteristics and their effect on spinal fractures based on multivariate Logistic proportional regression analysis.

Characteristics	Spinal fractures OR	95%CI	P
Sex	2.158	0.787-5.915	.135
Age	0.631	0.242-1.645	.346
Vitamin B2	1.542	0.577-4.272	.405
Vitamin A	0.740	0.268-2.041	.561
Vitamin B12	10.266	3.866-27.264	<.001*
Vitamin B9	2.020	0.747-5.460	.166

95% CI = 95% confidence interval, OR = odds ratio.
* < .05

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 χ^2 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 naturally in vegetables or added to staple foods as folic acid. Vitamin B12 in its natural state is found only in animal foods.[9] Vitamin B12 promotes protein biosynthesis in the form of a coenzyme that increases the availability of folic acid and promotes carbohydrate, fat and protein metabolism. The risks of folic acid intake >1 mg/day do not outweigh the benefits of 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 remodeling 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.

Table 5

Characteristics and their effect on MTRR based on univariate Cox proportional regression analysis.

			MTRR		
Characteristics			**HR**	**95% CI**	***P***
Sex	Male	63	1		.342
	Female	42	0.811	0.527-1.249	
Age	≤65	59	1		.289
	>65	46	1.255	0.825-1.908	
Vitamin B2	Low	47	1		.330
	High	58	0.802	0.514-1.251	
Vitamin A	Low	67	1		.355
	High	38	1.234	0.790-1.927	
Vitamin B12	Low	51	1		<.001*
	High	54	7.985	4.738-13.455	
Vitamin B9	Low	36	1		.873
	High	69	1.037	0.662-1.626	

95% CI = 95% confidence interval, HR = hazard ratio, MTRR = maintenance time from recovery to recurrence.
*P < 0.05.

Table 6

Characteristics and their effect on MTRR based on multivariate Cox regression analysis.

	MTRR		
Characteristics	**HR**	**95% CI**	***P***
Sex	0.799	0.501-1.273	.345
Age	1.126	0.705-1.798	.621
Vitamin B2	0.732	0.460-1.163	.186
Vitamin A	1.531	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 < 0.05.

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.

Author contributions

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