Journal of Korean Society of
Spine Surgery

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J Korean Soc Spine Surg 2016 Mar;23(1):15-24.
Originally published online March 31, 2016;
http://dx.doi.org/10.4184/jkss.2016.23.1.15

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pISSN 2093-4378 eISSN 2093-4386

Original Article

J Korean Soc Spine Surg. 2016 Mar;23(1):15-24. http://dx.doi.org/10.4184/jkss.2016.23.1.15

The Association of Low-energy Spine Fractures and Vitamin D Inadequacy: A Case-control Study

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Study Design: Retrospective study.
Objectives: To compare serum vitamin D levels in elderly patients with or without osteoporotic spinal compression fractures (OSCFs) and to identify relationships between the serum vitamin D level and other variables, such as age, bone mineral density (BMD), and bone turnover markers (osteocalcin and C-telopeptide).
Summary of Literature Review: Vitamin D plays a key role in calcium metabolism in the bone tissue. Vitamin D deficiency can lead to decreased BMD and an increased risk of falls and of osteoporotic fractures.
Materials and Methods: We retrospectively reviewed the medical records of 95 elderly patients (≥60 years) with OSCFs (fracture group) and 118 subjects who had been diagnosed with osteoporosis without OSCFs (control group). Serum vitamin D levels were contrasted between the two groups taking into account other factors such as patient age, sex, and seasonal variations. For all the patients, we also evaluated the correlation between the vitamin D level and the patient age, BMD, and bone turnover markers.
Results: The mean of the serum 25(OH) vitamin D_3 levels was significantly lower in the fracture group than in the control group. There were significant differences in the 25(OH) vitamin D_3 levels in autumn. In all patients, the mean serum 25(OH) vitamin D_3 levels were the highest in autumn and the lowest in spring. Furthermore, the mean serum 25(OH) vitamin D_3 levels were significantly correlated with patient age and BMD.
Conclusions: A low serum vitamin D level might be a risk factor of OSCFs in elderly patients.

Key Words: 1,25 Dihydroxyvitamin D_3, Spinal fracture, Osteoporosis, Bone mineral density

Introduction

Osteoporosis is characterized by a low bone mass and structural change of bony tissue.[4] Therefore, patients with osteoporosis are at increased risk of developing osteoporotic fracture. The World Health Organization (WHO) defines osteoporosis as a spinal or hip BMD of 2.5 standard deviations or more below the mean for healthy, young women (T-score of −2.5 or below) as measured by dual energy x-ray absorptiometry.[16] Moreover, with the increasing number of elderly people in our population, the prevalence of osteoporosis and osteoporotic fracture has also increased along with interest in the treatment of patients with osteoporosis.[1,2,3] In patients diagnosed with osteoporosis, the optimal treatment regimens include fall prevention, an appropriate intake of calcium and vitamin D and the use of various drugs containing bisphosphonate.[4] There has been an increased tendency towards the use of various therapeutic agents, such as bisphosphonate, parathyroid hormone, calcium and vitamin D to treat osteoporosis.[5,6] In particular, vitamin D plays a key role in calcium metabolism in bone tissue. Moreover, it is also one elemental nutrient that contributes to homeostasis and the stabilization of neural tissue.[7-10] Jang et al.[11] reported that serum vitamin D levels were significantly lower in post-menopausal Korean women with distal radial fracture as compared with their non-fracture controls. Other studies have showed that vitamin D deficiency can lead to decreased BMD and increased propensity to falls and increased osteoporotic fractures.[5] Moreover, the

Received: August 13, 2015
Revised: August 18, 2015
Accepted: November 29, 2015
Published Online: March 31, 2016
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risk of developing hip fracture and non-vertebral fracture can be reduced with the administration of vitamin D at a daily dosage of 700-800 IU.[13] To date, there have also been reports that the risk of vertebral fractures was decreased with the use of various drugs, including bisphosphonate.[14] However, few studies about the correlation between the use of vitamin D and the decreased incidence of vertebral fractures in patients with osteoporosis exist.[15]

Therefore, the purpose of this study was to investigate serum levels of vitamin D in elderly patients with OSCF diagnosed with osteoporosis. Serum levels of vitamin D were also compared between the two groups with consideration for age, sex and seasonal variations. In all patients, we also evaluated the correlation between serum levels of vitamin D and factors such as age, BMD and bone turnover markers.

Materials and Methods

Selection criteria

From October 2008 to December 2012, we evaluated the vitamin D and bone turnover markers of 1,033 patients aged 60 years or older who visited the outpatient clinic of our medical institution. We performed a retrospective analysis of these patients' medical records. All the patients were divided into two groups: the fracture group and the control group.

Exclusion criteria for the current study were as follows (Fig. 1): Patients with;

(1) Vertebral fracture due to high-energy trauma that is defined according to the fracture morphology and patient's history.

(2) Past history of taking drugs that may affect bone metabolism.

(3) Osteoporosis who were currently taking anti-osteoporotic drugs or vitamins or had a past history of taking such drugs.

(4) Underlying diseases associated with bone metabolism, such as a bone tumor or hematooncologic, renal, hepatic, rheumatoid, or endocrine diseases.

(5) Past history of fractures related to osteoporosis (distal radius, proximal femur, and spine).

(6) Past history of spine surgery.

(7) T-score of BMD higher than -2.5.

A diagnosis of spinal fracture was based on plain x-ray and computed tomography. Magnetic resonance imaging was used for further evaluation that assists to figure the appearance of spinal fracture. Thus, 95 patients were assigned to the OSCF group and 118 patients to the control group (Fig. 1).

In the fracture group, we noted the season of onset of fracture (March to May, June to August, September to November and December to February). In the control group, we categorized the season when serum vitamin D levels were measured. Vitamin D levels were obtained based on the serum levels of 25(OH) vitamin D_3 ($25(OH)D_3$). Although 1,25-dihydroxycholecalciferol ($1,25(OH)2D_3$, calcitriol) is the biologically active form of vitamin D, serum $25(OH)D_3$ levels are 1000 times those of calcitriol, which leads to more accurate measurements; they are also an indicator of the overall status of vitamin D that is produced in vivo or through dietary intake.[15]

In the fracture group, the blood sampling was performed between 9:30 a.m. and 12:00 p.m. on the next day of hospitalization. In the control group, it was also performed between 9:30 a.m. and 12:00 p.m. in the outpatient department. Taken samples were stored at 4 to 8 degree Celcious if it needed.

Based on a target therapeutic serum $25(OH)D_3$ Osteocalcin and C-telopeptide were also measured using blood sample at the same time. Blood samples were collected from patients in the fasting state. Quantification of serum $25(OH)D_3$ was done using ECLIA (Electrochemiluminescence immunoassay; Liaison®, DiaSorin, Stillwater, MN, USA) by MODULAR ANALYTICS E170 (Roche Diagnostic, Mannheim Germany). Of the bone turnover markers, osteocalcin and C-telopeptide were measured using the MODULAR ANALYTICS E170 (Roche Diagnostic, Mannheim Germany). BMD was determined at least one month after the fracture while patients were visiting the outpatient department. The BMD was assessed using a dual-energy x-ray absorptiometry (Delphi-W, Hologic, Waltham, MA, USA), which included the first to fourth lumbar spine segments and the proximal femur. However, this did not include the fractured lumbar spine or the ward triangle of the proximal femur. The representative value of BMD was taken from the lowest T-score of the proximal femur and the average of the two lowest scores of the lumbar spine.

Comparative analysis

We also compared the mean serum levels of $25(OH)D_3$, BMD and bone turnover markers between the two groups. In addition,

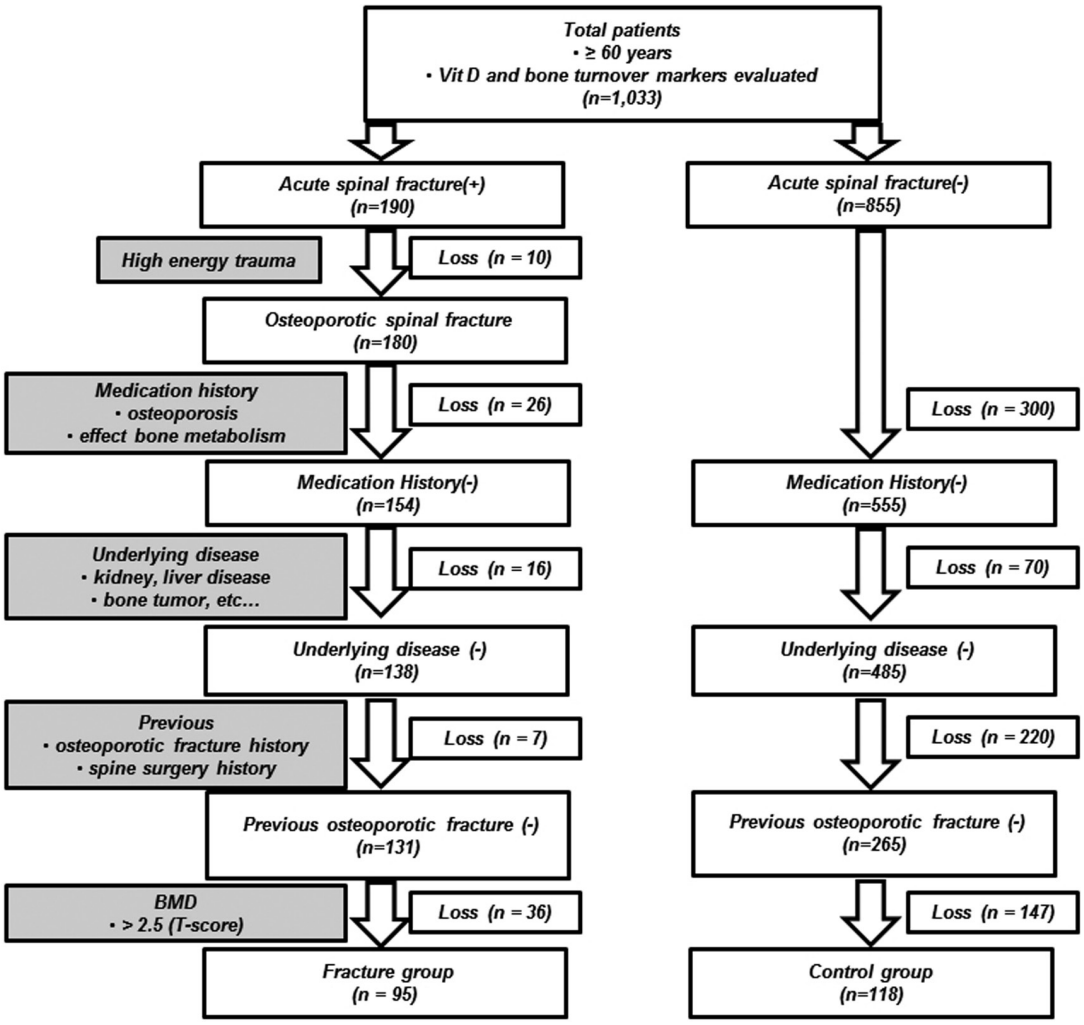

Fig. 1. Diagram of included patients uture study.

after the patients were divided into subgroups according to age, season and sex, we compared the vitamin D levels between the two groups.

Correlation analysis

In all participants, we analyzed the correlations between serum levels of 25(OH)D_3 and factor such as age, BMD and bone turnover markers.

Statistical analysis

We used Student's t-test to compare the age, sex, serum levels of 25(OH)D_3 bone turnover markers in each season and BMD. To identify the correlations between vitamin D, age, BMD, and bone turnover markers, we performed a Pearson correlation analysis. A p-value $< 0.05$was used to define significance. Statistical analysis was performed using SAS for Windows version 9.2 (SAS Institute Inc., Cary, NC).

Ethics statement

This study was approved by the institutional review board (IRB No. 2012-105). Informed consent was waived for this study since it is a case-control study and therefore presents no more than minimal risk to the subjects.

Conflicts of interest

The authors have no financial conflicts of interest.

Results

The mean age of the patients was 72.63±6.63 years (range,

60 to 97 years). Age did not show statistical significance between the two groups (p=0.14). Our clinical series of patients was composed of 30 males and 183 females. There is difference between other characteristic, but, statistically, there is no significance between the two groups (Table 1).

Comparative analysis we found that levels of serum 25(OH)D_3 was significantly lower in the fracture group than in the control group (Table 2). T-score was significantly lower in the fracture

Table 1. Demographic data

		Mean		p-value
		Fracture group (N=95)	Control group (N=118)	
Age (years)		73.43 ± 6.63	71.99 ± 7.26	0.14
Sex	Female (N/%)	83/87.4	100/84.7	0.58
Smoking (cigarette-years)		13.62 ± 23.80	12.46 ± 23.71	0.73
BMI (kg/m^2)		24.05 ± 3.47	24.91 ± 3.88	0.09

p-value <0.05.

Table 2. Mean serum 25(OH)D_3 (ng/ml) depending on age and sex

	Mean		p-value
	Fracture group	Control group	
Serum 25(OH)D_3 (ng/ml)			
Total	21.86 ± 17.18	28.03 ± 26.06	<0.05
According to age			
60-69 years (N=19, 46)	24.35 ± 22.25	29.33 ± 25.61	0.46
70-79 years (N=60, 59)	22.34 ± 16.27	28.73 ± 27.69	0.13
80+ years (N=16, 13)	17.11 ± 13.52	20.24 ± 19.62	0.62
Sex			
Male (N=12, 18)	24.48 ± 13.25	31.97 ± 26.95	0.08
Female (N=83, 100)	21.48 ± 17.71	27.32 ± 25.98	0.38

Normal range in author's hospital, Serum 25(OH)D_3 (ng/ml) : 30.1–100.0.

Table 3. Mean BMD* (T-score) and bone turnover markers (ng/ml)

	Mean		p-value
	Fracture group	Control group	
BMD* (T-score)			
Total	-3.83 ± 0.80	-3.23 ± 0.81	<0 .001
L-spine	-3.65 ± 0.95	-2.96 ± 1.21	<0.001
Lt. hip	-2.88 ± 0.94	-2.48 ± 0.83	0.002
Rt. hip	-2.87 ± 0.94	-2.49 ± 0.88	0.003
Bone turnover markers			
Osteocalcin (ng/ml)	16.72 ± 10.68	18.18 ± 10.89	0.33
C-telopeptide (ng/ml)	0.72 ± 0.42	0.50 ± 0.40	<0.001

BMD*: bone mineral density, Normal range in author's hospital, Osteocalcin (ng/ml) : men >50 years; 14-46/ post-menopause; 15-46/ pre-menopause; 11- 43, C-telopeptide (ng/ml) : men 50-70 years; 0.7/ men ≥ 70 years; 0.9/ post-menopause; 1.0/ pre-menopause; 0.1–0.6.

group .For the bone turnover markers, only serum C-telopeptide levels were significantly higher in the fracture group (Table 3). According to age and sex, serum 25(OH)D_3 levels were not significantly different in the two study groups. When seasonal variation was considered, vitamin D levels were significantly lower in the fracture group in autumn compared to the control group, but the differences were not statistically significant in spring, summer and winter.

Regardless of the presence of fracture, summer and autumn had higher levels of serum 25(OH)D_3 than spring and winter

Table 4. Mean serum 25(OH)D_3 (ng/ml) depending on seasonal variations

	Mean		p-value
	Fracture group	Control group	
Serum 25 (OH)D_3 (ng/ml)			
Spring (N=23, 29)	24.37±21.97	21.82±22.94	0.7
Summer (N=19, 30)	21.68±19.69	30.26±32.87	0.26
Autumn (N=29, 32)	21.51±13.61	31.83±19.81	0.02
Winter (N=24, 27)	20.22±13.96	28.27±28.40	0.23

p-value <0.05.

Table 5. Correlations between serum 25(OH)D_3 (ng/ml) levels and other variab

	Age	Osteocalcin	C-telopeptide	BMD	L-spine	Lt. hip	Rt. hip
r*	-0.145	0.068	-0.077	0.206	0.209	0.215	0.188
p-value	0.034	0.32	0.27	0.003	0.003	0.002	0.007

r*=correlation coefficient.

Table 6. Correlations between OSCF and Serum 25(OH)D_3 (ng/ml) leve

		OSCF	serum 25(OH)D_3
OSCF	r	1	-0.335
	p		0.013
	N	213	213
	r	-0.335	1
serum 25(OH)D_3	p	0.013	
	N	213	213

p-value <0.05, r*=correlation coefficient.

Table 7. Regression analysis between OSCF and serum 25(OH)D_3 (ng/ml) leve

		B	β	t	p	VIF
OSCF	serum 25(OH)D_3	-0.472	-0.329	-5.171	0.000	1.626
	BMI	-0.12	-0.091	-1.488	0.138	1.015
	Smoking	0.000	-0.009	-0.145	0.885	1.043
	Osteocalcin	-0.002	-0.071	-1.127	0.261	1.072
	C-telopeptide	0.016	0.343	4.505	0.000	1.585
	BMD	-0.169	-0.291	-4.585	0.000	1.100

p-value <0.05.

(Table 4).

Correlation analysis

We analyzed whether serum 25(OH)D_3 levels had a significant correlation with age, BMD, osteocalcin, and C-telopeptide in all patients. These levels had a weak negative correlation with age as well as a weak positive correlation with BMD. However, serum 25(OH)D_3 levels had no significant correlation with osteocalcin and C-telopeptide (Table 5). But, we found that the OSCF has correlation with serum 25(OH)D_3 levels using Pearson's correlation coefficient and regression analysis. (Table 6, 7)

Discussion

Our results showed that serum 25(OH)D_3 levels were significantly lower in the fracture group as compared with the non-fracture group. In previous studies, vitamin D deficiency is reportedly prevalent in patients with osteoporotic fracture. Moreover, it has also been reported that appropriate vitamin D supplement therapy lowers the prevalence of fracture.[13] Moniz et al.[17] found that 94% of patients with osteoporotic hip fracture had a vitamin D deficiency. In Korea, approximately 70% of patients who suffered an osteoporotic hip fracture were found to have a vitamin D deficiency.[18] In the current study, we analyzed the serum vitamin D levels in patients with OSCF; approximately 78% of them had a vitamin D deficiency. These results indicated that most of the fracture patients concurrently had a vitamin D deficiency. Despite also having a diagnosis of osteoporosis, vitamin D deficiency was seen in approximately 69% of the patients in the control group, confirming that the incidence of vitamin D deficiency was higher in the fracture group. Many studies have reported that vitamin D deficiency is associated with hip fracture or non-vertebral fracture, and its treatment affects both diseases. To date, however, almost no studies have examined the relationship between vitamin D deficiency and OSCF. We therefore conducted the current study to examine whether vitamin D deficiency was associated with the incidence of osteoporotic fracture in the fracture group as compared with the control group, in which patients experienced no osteoporotic fracture despite a diagnosis of osteoporosis.

In addition, vitamin D is involved in the protein synthesis and the growth of muscle cells, and it thereby maximizes muscle functions. The vitamin is also a part of the improvement of neuromuscular functions and the augmentation of reflex protective mechanisms. These activities contribute to increasing muscle strength, improving a person's sense of balance and lowering the risk of falling. Therefore, they have been reported to contribute to preventing the occurrence of bone fracture.[5,12,21] Presumably, patients with a vitamin D deficiency might be at increased risk of worsening osteoporosis and sustaining a fall injury. This risk increases the incidence of OSCF. It can therefore be inferred that serum 25(OH)D_3 levels were lower in the fracture group. But, in the current study, we analyzed the correlation between BMD and serum 25(OH)D_3 levels. We found that there was a weak positive correlation between them. Further large cohort study has to be performed for reveal the correlation between BMD and serum 25(OH)D_3 levels.

In the present study, we also compared serum 25(OH)D_3 levels between the seasons in both groups and found that they were significantly higher in summer and autumn as compared with winter and spring. Choi[22] performed an analysis of the correlations between serum 25(OH)D_3 levels, demographic data and lifestyle in Korean adults, reporting that serum 25(OH)D_3 levels were significantly higher in the summer and autumn than they were in the spring and winter. To explain this difference, these authors noted that vitamin D synthesis via the skin is inhibited because many Korean people wear long-sleeved clothing in spring and winter and predominantly stay indoors. It can therefore be inferred that the amount of sunlight exposure is a key factor for determining serum 25(OH)D_3 levels. During the summer, the altitude of the sun is relatively higher. The amount of UV-B absorbed from the ozone layer is the smallest, and the largest amount of UV-B rays reaches the earth surface.[23] Presumably, this variation might also cause the difference in serum 25(OH)D_3 levels between the seasons. We compared serum 25(OH)D_3 levels depending on the season between the fracture group and the control (non-fracture) group and found that they were significantly higher only in autumn in the control group. The differences in serum 25(OH)D_3 levels may not have reached statistical significance due to the small number of enrolled patients because we subdivided our clinical series of patients depending on the season. In addition, further large-scale studies will be warranted to compare serum 25(OH)D_3 levels depending on the season between the fracture group and the control group.

There was a weak negative correlation between serum $25(OH)D_3$ levels and age in the present study. But, according to Holick[23], with increased age, 7-dehydrocholesterol, which is normally present in the skin and converted to vitamin D in the presence of UV-B, would be decreasingly synthesized, and this drop would lower the synthesis of vitamin D.

The bone turnover markers; osteocalcin and C-telopeptide, are indicative of a patient's bone turnover rate. Patients are at increased risk of developing bone fracture when presented with a high bone turnover rate. [24] The assay of such bone turnover markers is a non-invasive test regimen, but is disadvantageous, however, due to a high degree of intrapersonal variation. The accuracy also can be decreased depending on such factors as circadian variation, daily variation, dietary intake or exercise.[25] Garnero et al.[26] conducted the EPIDOS study to identify the correlation between hip joint fracture and bone turnover markers using a prospective design in elderly patients; they found that osteocalcin had no significant correlation with the risk of developing hip fracture and C-telopeptide had a significant correlation with developing bone fracture irrespective of the severity of osteoporosis. Baseline osteoblastic markers before the fracture showed no significant difference in the patients with hip fracture as compared with their age-matched controls, and osteoclastic bone markers were significantly higher. Bjarnason et al.[27] also reported that there was no significant correlation between osteocalcin and vertebral osteoporotic fracture. Bauer et al.[28] also found that C-telopeptide correlated significantly with the occurrence of incidental vertebral fracture. Our results were also consistent with these reports. Osteocalcin had no significant correlation with fracture. But, C-telopeptide was higher in fracture group more than control group.

It has also been reported that bone turnover markers are additionally associated with various factors; such as age, sex, and intrinsic hormone, bone metabolism following the onset of bone fracture, intraday and interday variation, dietary intake and exercise, as well as vitamin D.[8,29,30] In the current study, we analyzed the correlation between vitamin D and bone turnover markers (osteocalcin and C-telopeptide) and found no significant correlation.

Our results were showed the difference in that we could not confirmed that there was a significant correlation between vitamin D deficiency and the occurrence of OSCF through a comparison of serum $25(OH)D_3$ levels between patients with OSCF and patients with osteoporosis without fracture, who served as the control group. But, we analyzed the correlations between vitamin D, season, age, BMD and bone turnover markers in patients with OSCF. Therefore, we have provided a necessity for estimating a causal relationship between vitamin D and the occurrence of bone fracture.

Our current study had the following two limitations

Firstly, we subdivided our clinical series of patients depending on age and season. It is therefore probable that our results might not have reached statistical significance because of the difference in the distribution of patients in the subgroups as well as the small number of enrolled patients. Seasonal variations of serum vitamin D level are different from papers, and it can be a confounding factor understanding results. In a future study, we will simplify factors affecting serum vitamin D level. Secondly, serum $25(OH)D_3$ levels are subject to change depending on the amount of solar irradiation, regional variations, dietary and living habits.[22, 23] We failed to consider the correlation between these factors. It is a way to solve this weakness of our paper using multivariate analysis in a f

Conclusion

Serum $25(OH)D_3$ levels were lower in patients with OSCF than they were in the control group, but, correlation is weak. We suggest the necessity for revealing the correlation. We suppose that maintaining appropriate serum $25(OH)D_3$ levels in patients with osteoporosis might be correlation with preventing the occurrence of OSCF. Nevertheless, further studies are warranted to examine whether vitamin D supplementation could lower the incidence of OSCF in elderly people with osteoporosis.

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Original Article | J Korean Soc Spine Surg. 2016 Mar;23(1):15-24. http://dx.doi.org/10.4184/jkss.2016.23.1.24

비타민 D 결핍과 저에너지 척추골절의 연관성

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연구계획: 후향적 연구
목적: 노인에서의 혈중 비타민 D의 농도와 골다공증성 척추 골절의 관계를 규명하고, 혈중 비타민 D의 농도와 다른 요인들과의 관계를 밝히고자 한다.
선행 연구문헌의 요약: 비타민 D는 골 조직에서의 칼슘 대사에 있어서 아주 중요한 역할을 담당하고 있으며 비타민 D가 부족할 경우 BMD의 감소 및 낙상, 골다공증성 골절의 위험이 증가한다.
대상 및 방법: 저자들은 60세 이상의 환자 중 의료 기록을 통하여 골다공증성 골절이 있는 95명의 환자 및 골절이 없는 골다공증 환자 118명을 분석하였다. 비타민 D와 골다공증성 골절의 관계뿐만 아니라 다양한 요인들과의 상관관계 및 회귀분석을 시행하였다.
결과: 혈중 비타민 D의 농도의 평균은 골다공증성 골절이 있는 그룹에서 골절이 없는 그룹에 비하여 낮았으며 상관 관계가 있는 것으로 나타났으며, 혈중 비타민 D의 농도의 평균은 가을에 가장 높고, 봄에 가장 낮은 것으로 나타났다. 또한 환자의 나이와 BMD와도 상관관계가 있음을 보여주었다.
결론: 노인에서 혈중 비타민 D가 낮은 경우는 골다공증성 골절의 위험인자가 될 수 있다.

색인 단어: 1,25 비타민 D, 척추 골절, 골다공증, 골밀도
약칭 제목: 비타민D 결핍과 척추골절

접수일: 2015년 8월 13일 **수정일:** 2015년 8월 18일 **게재확정일:** 2015년 11월 29일
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Journal of Korean Society of Spine Surgery. www.krspine.org. pISSN 2093-4378 eISSN 2093-4386