



Serum Levels of Vitamin D in Patients with Multiple Myeloma

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Abstract

Aim: To investigate the serum levels of vitamin D in newly diagnosed patients with multiple myeloma.

Patients and methods: In this study we measured the serum levels of vitamin D in 37 patients (19 women, 18 men) at a median age of 68 years and a diagnosis of MM according to the International Myeloma Working Group (IMWG) criteria. The immunoassay tests used for the quantification of 25 (OH) – Vitamin D were original ELISA kits Immundiagnostic and the measurement was done before starting the treatment.

Results: Serum levels below the optimum (<30 ng/ml) were recorded in all 37 patients. The median value of vitamin D was 4.3±6.5 mg/ml, the maximum value measured was 24.7 mg/ml, which is below the lower limit of the reference value for deficiency.

Conclusions: In this study, we found extremely low serum vitamin D levels in most of the newly diagnosed MM patients.

Keywords

chemotherapy, multiple myeloma, radiotherapy, vitamin D

INTRODUCTION

Multiple myeloma (MM) is a malignant B-cell neoplasia characterized by a slow proliferation of malignant plasma cells in the bone marrow. MM has an incidence of about 10% of all hematologic malignancies and causes 2% of all deaths from malignancies.¹ In vitro studies have shown the effectiveness of vitamin D and its analogues on cell lines from MM patients. In vitro study on NCI-H929 multiple myeloma cell lines with vitamin D3 analogue (EB1089) found that EB1089 inhibit cell growth by inducing cell cycle arrest in G1 phase as a dose-dependent process. In addition, EB1089 was also found to induce cell apoptosis by upregulating the Bcl-2 protein without altering the Bax

protein. The fact that the expression of p53, p21, and Bax was not altered by EB1089 indicates that EB1089-induced apoptosis is p53-dependent. Most likely, EB1089 leads to apoptosis by activating the p38 mitogen-activated protein kinase (p38MAP kinase) and suppressing the extracellular signal-regulating kinase (ERK) with subsequent activation and without alteration of the caspase cascade (caspase3).² Another study found that EB1089 had a synergistic effect with dexamethasone in inducing apoptosis and that IL-6, LIF OSM and anti-gp130MoAb could not prevent the apoptotic effect of this combination. EB1089 also downregulated the gp80 expression on the myeloma cells which led to a decreased production of soluble IL-6Ra.³ The mechanism of action is mediated by binding to the vitamin D re-

ceptor (VDR).⁴ VDR has a high affinity for 1,25(OH)₂D₃.⁵ Data on the significance of the vitamin D polymorphism for the MM origin and also for the MM outcome are divergent. In a study involving 75 patients with MM and a control group of 150 healthy individuals, the significance of 3 VDR gene polymorphisms (Apal, Bsm1, and FokI) was examined by PCR-RFLP method. There was no significant association between Apal and Bsm1 and the risk of MM. However, a significant link between the FokI polymorphism and the risk of developing MM has been demonstrated. A significant correlation between the f genotype variants with creatinine levels, albumin levels and stage III in the Durie & Salmon staging system has also been found.⁶ Vitamin D deficiency is a health problem with pandemic proportions. Globally, 1 in 7 people (14%) are believed to have insufficiency or deficiency of vitamin D.⁷ Despite the lack of strong consensus on the experimental levels of vitamin D, experts consider the lower limit of 30 ng/mL to be an adequate level of vitamin D (Table 1).⁸

Table 1. Diagnostic levels of vitamin D recommended by American Endocrine Society

25(OH) level (ng/ml)	25(OH) level (nmol/L)	Laboratory diagnosis
<10	<25	Severe deficiency
<20	<50	Deficiency
20-30	50-75	Insufficiency
30-100	75-250	Normal in sunny countries
>100	>250	Excess
>150	>325	Intoxication

The suggested limit of 25(OH)D for optimal skeletal health is that level of 25(OH)D which minimizes PTH to a minimum and maximizes calcium absorption to a maximum.^{9,10} Vitamin D treatment recommendations depend on many factors such as age group, body weight, ethnicity (skin type) as well as latitude of residence. In addition, these recommendations do not apply to people suffering from a particular disease as well as having a vitamin D deficiency.¹¹ For patients with laboratory confirmed vitamin D deficiency, a serum vitamin D level lower than 20 ng/mL (50 nmol/L) treatment with vitamin D is required. In patients with vitamin D deficiency, the therapeutic dose (dependent on age and body weight) should be given according to available regional or national treatment recommendations, with duration of treatment of 1 to 3 months.^{8,12} There are many studies looking for a link between vitamin D deficiency and its importance as a risk factor for the occurrence of neoplastic diseases, as well as its impact on disease outcome. The aim of the present study was to investigate vitamin D levels in newly diagnosed patients with MM.

PATIENTS AND METHODS

Patients

The study included 37 patients (19 women, 18 men) with a median age of 68 years and a diagnosis of MM according to the criteria of the International Myeloma Working Group (IMWG) (Table 2). The patients were diagnosed at the clinic of Clinical Hematology at St George University Hospital in Plovdiv (MU-Plovdiv) from December 2014 to February 2016. The study was approved by the Ethics Committee of MU-Plovdiv and was conducted in accordance with the Helsinki declaration. All patients participating in the study signed informed consent. Patients' characteristics are presented in Table 2.

Table 2. Patients' characteristics

Patients (n=32)		
Gender	W	51.4%
	M	48.6%
Mean age	68 years (38-86)	
Clinical stage Durie & Salmon	IA	10.8%
	IB	0%
Clinical stage ISS	IIA	13.5%
	IIB	5.4%
	IIIA	18.9%
	IIIB	51.4%
Clinical stage ISS	I	13.5%
	II	13.5%
	III	70.0%

Statistical analysis

Methods of descriptive statistics and methods of statistical inferences and conclusions were used to conduct the study. Data analysis was carried out using the IBM SPSS, version 25.0 and MS Excel 2017, which allowed rapid and accurate preparation of the results for further analysis. The perceived critical level of significance in testing the null hypothesis H₀ is $\alpha=0.05$ (Z criterion=1.96), with a 95% guarantee probability.

Measurement of vitamin D

Serum tests for vitamin D were run at the first hospitalization of patients before starting treatment. Serum vitamin D levels were measured at the Central Clinical Laboratory at St George University Hospital in Plovdiv. An immunoenzyme method was used to quantify 25 (OH)-vitamin D level, using original ELISA kits Immundiagnostic, Austria, Catalog No. K 2109.

RESULTS

The youngest patient was 38 years old and the oldest patient – 86 years (Fig. 1).

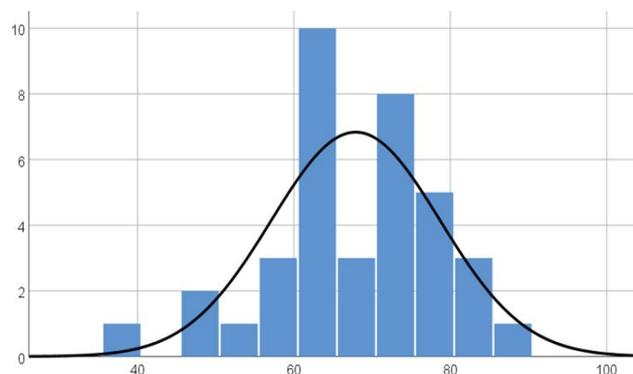


Figure 1. Distribution of patients by age.

No statistically significant difference was found between serum vitamin D levels in men (6.2 ± 5.2 mg/ml) and women (6.7 ± 6.1 mg/ml) ($H=0.004$, $p=0.952$). There was also no significant difference in age groups ($H=7.9$, $p=0.094$). Only the mean values of the patients in the 69-78-year group (4.1 ± 3.4 mg/ml) were significantly lower than those in the 59-68 age group (9.4 ± 7.2 mg/ml). According to Durie & Salmon staging system, 51.4% of patients were in stage IIIB ($n=19$) (Fig. 2), and using ISS staging system 73.0% of patients were in stage III ($n=27$) (Fig. 3). No statistically

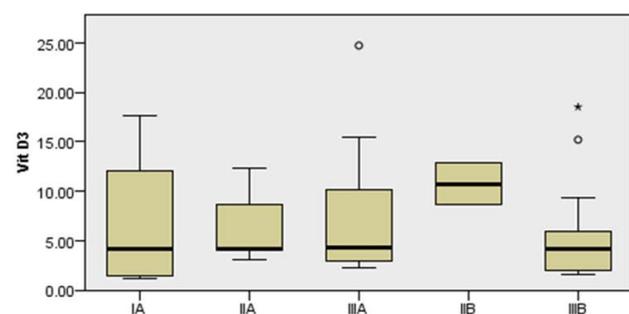


Figure 2. Serum vitamin D levels according to Durie & Salmon staging system.

significant difference was found between the mean values of serum vitamin D levels and the clinical stages of the disease, as determined by the stage system of Durie & Salmon ($H=3.46$, $p=0.484$), as well as the ISS staging system ($H=2.22$, $p=0.329$).

A complete blood count (CBC) was done in all patients. CBC count was performed with the following results: mean hemoglobin (Hgb) 92.5 ± 19.5 g/l, mean leukocyte count (WBC) 6.6 ± 4.8 G/l, and mean platelet count (PLT) 222.1 ± 118.2 G/l. In all 37 patients with newly diagnosed MM, serum levels of vitamin D below optimal (<30 ng/ml) were detected, 1 patient had vitamin D insufficiency, serum levels between 20-30 ng/ml, while the remaining 36 patients had vitamin D deficiency (vitamin D level below 20 ng/ml). Severe vitamin D deficiency (vitamin D level <10 ng/ml) was reported in 81% of patients. The results are presented in Fig. 4.

Radiological bone changes were found in 64.9% ($n=24$) of patients, respectively 35.1% ($n=13$) of patients had a normal radiographic images. From the patients with radiological changes, according to the Merlini scale the bone changes with multiple osteolytic foci and/or pathological fractures (grade 2) were 45.8% ($n=11$) patients, followed by those with multiple pathological fractures and/or destruction of the skeletal segment (grade 3) 33.3% ($n=8$). The lowest was the percentage of patients with single osteolytic lesions and/or osteoporosis, 20.8% (Fig. 5). No statistically significant difference was found between vitamin D levels and myeloma bone disease evaluated using Merlini scale.

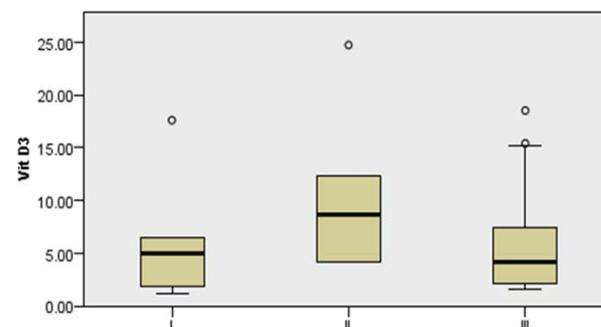


Figure 3. Serum vitamin D levels according to ISS staging system.

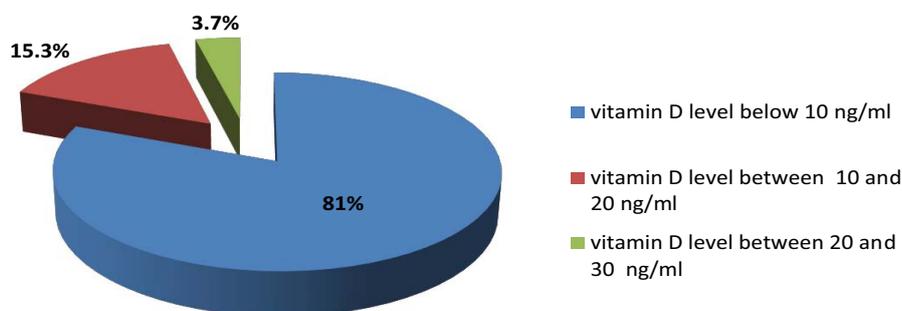


Figure 4. Distribution of patients by vitamin D level.

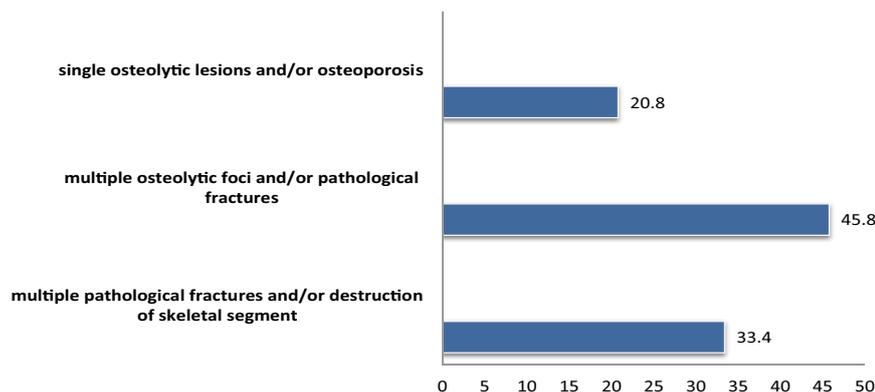


Figure 5. Radiological bone changes according to Merlini scale (n=24).

Also in patients with MM, we did not find any significant association between vitamin D level with hemoglobin level (Spearman’s $\rho=0.10$, $p=0.541$), leukocyte count (Spearman’s $\rho=0.23$, $p=0.178$) as well as platelet counts (Spearman’s $\rho=0.15$, $p=0.363$). There was also no significant association with the ESR level (Spearman’s $\rho=0.14$, $p=0.412$), CRP (Spearman’s $\rho=-0.05$, $p=0.781$), LDH (Spearman’s $\rho=-0.28$, $p=0.100$) and the level at $\beta 2$ mg (Spearman’s $\rho=-0.17$, $p=0.318$).

Induction treatment was initiated in all 37 patients, with the highest rates being those receiving CyBORDEX therapy (59.5%; n=22). The remaining patients received chemotherapy regimens PAD, CVAD and endoxan/dexamethasone. Radiotherapy was performed in all patients with bone disease and symptomatic fractures. No statistically significant differences were found between the mean vitamin D levels and response to treatment ($H=4.9$, $p=0.177$) (Fig. 6).

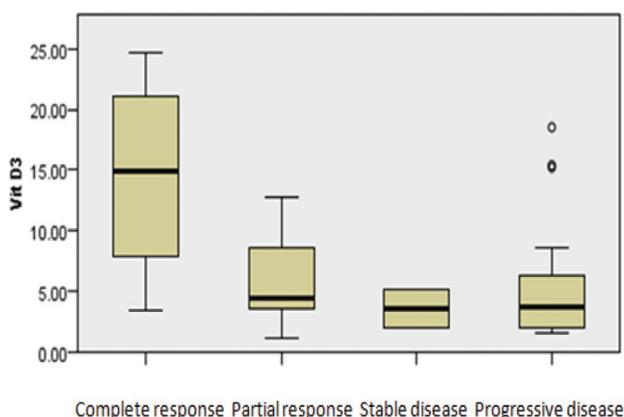


Figure 6. Serum vitamin D levels and first-line treatment response.

DISCUSSION

Multiple myeloma is a plasma cell neoplasia with an increasing frequency. The proliferation of plasma monoclonal cells in bone marrow leads to overproduction of a monoclonal protein (M protein), bone destruction, and suppres-

sion of normal hematopoiesis. Nowadays, there is a lot of data regarding the effect of vitamin D on cellular processes of differentiation, proliferation, angiogenesis, and apoptosis.¹³ Low serum vitamin D levels are associated with a higher risk of NHLs, including MM.¹⁴ In a study including 148 patients with newly diagnosed MM, vitamin D deficiency was reported in 24% of patients (vitamin D level of 20 ng/ml). Vitamin D deficiency was found in 16% of patients in stage I, 20% in stage II, and in 37% of patients in stage III according ISS staging system. Patients with vitamin D levels had elevated C-reactive protein levels as well as high serum creatinine levels. In our study, no statistically significant difference was found between the level of vitamin D and the clinical stage according to both staging systems as well as the levels of C-reactive protein and creatinine. No correlation was found between vitamin D levels and myeloma bone disease, which was also confirmed in our study.¹⁵ In another retrospective study involving 83 patients (mean cohort age 66.3 years) with MM diagnosed between December 2007 and December 2014 in deficiency of vitamin D (vitamin D level below 10 ng/ml) was found in 32.5% of patients, 54.1% had insufficient vitamin D level (10-30 ng/ml) and 13.3% of patients had an optimal vitamin D level (above 30 ng/ml). Also, this study found no correlation between the stage of the disease and the level of vitamin D as well as myeloma bone disease. A correlation was found between vitamin D levels and plasma cell percentage. In patients with vitamin D deficiency, the average percentage of plasma cells in the bone marrow was 44.8%, in patients with insufficiency 20.6%, and in patients with the optimal level, the percentage of plasma cells in the bone marrow was on average 13.3%.¹⁶ Our study found extremely low serum vitamin D levels in the majority of newly diagnosed patients with MM.

Is vitamin D a potentially modifiable risk and therapeutic factor in MM?

CONCLUSIONS

In this study, we found extremely low serum vitamin D levels in most of the newly diagnosed MM patients.

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Уровни витамина D в сыворотке крови у пациентов с множественной миеломой

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Резюме

Цель: Исследовать уровни витамина D в сыворотке крови у впервые выявленных случаев пациентов с множественной миеломой (ММ).

Пациенты и методы: В этом исследовании мы измерили уровень витамина D в сыворотке у 37 пациентов (19 женщин, 18 мужчин), средний возраст которых составил 68 лет, которым был поставлен диагноз ММ в соответствии с критериями Международной рабочей группы по миеломе (International Myeloma Working Group (IMWG)). Иммуноанализы, использованные для количественного определения 25 (ОН) – витамина D, представляли собой оригинальные наборы для иммунодиагностики ELISA, и измерение проводили до начала лечения.

Результаты: Уровни сыворотки ниже оптимального (<30 ng/ml) были установлены у 37 пациентов. Среднее значение витамина D составило 4.3 ± 6.5 mg/ml, максимальное измеренное значение – 24.7 mg/ml, что ниже нижнего предела референтного значения недостаточности.

Заключение: В этом исследовании мы обнаружили чрезвычайно низкий уровень витамина D в сыворотке крови у большинства пациентов с впервые диагностированной ММ.

Ключевые слова

химиотерапия, множественная миелома, лучевая терапия, витамин D
