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CLINICAL STUDY

A possible connection between circulating 25-hydroxy-vitamin D and molecular response in chronic myeloid leukemia

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ABSTRACT
OBJECTIVE: In this study we aimed to evaluate whether there is a link between circulating 25-OH-D levels and molecular response in chronic myeloid leukemia (CML).
MATERIAL AND METHOD: A total of 61 patients with CML (31 women, 30 men) were recruited in this cross-sectional study.
RESULTS: Binary logistic regression analysis demonstrated that increased vitamin D levels were independently associated with molecular response in subjects with CML.
CONCLUSION: Our results indicated for the first time in the literature that severe deficiency of vitamin D was independently associated with molecular unresponsiveness in subjects with CML. 25-OH-D may be contributing to molecular response in the patients *(Tab. 3, Ref. 24)*. Text in PDF *www.elis.sk.*
KEY WORDS: chronic myeloid leukemia, vitamin D deficiency, molecular response.

Introduction

Vitamin D deficiency is a common problem all over the world. Approximately 25–50 % of patients examined in routine clinical practice have vitamin D levels below the optimal range, and it is estimated that about 1 billion people have vitamin D insufficiency worldwide (1–3). Vitamin D is produced by skin exposure to sunlight (ie, ultraviolet B radiation) and it is also obtained from dietary sources including supplementation. Serum levels of 25-hydroxyvitamin D (25[OH]D) reflect whole-body vitamin D stores and are used to assess individual vitamin D adequacy or insufficiency. A low vitamin D level can be diagnosed using a blood test called 25-hydroxy vitamin D (25OHD). Although there is no formal definition of vitamin D deficiency, some groups use the following values in adults (3).

- A normal level of vitamin D is defined as a 25OHD concentration above 30 ng/mL (75 nmol/L).
- Vitamin D insufficiency is defined as a 25OHD concentration of 20 to 30 ng/mL (50 to 75 nmol/L).
- Vitamin D deficiency is defined as a 25OHD level below 20 ng/mL (50 nmol/L).

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25 (OH)D is converted to 1,25-dihydroxyvitamin D (1,25[OH]2D), the physiologically active form of vitamin D, via the action of 1-hydroxylase primarily in the kidneys. Once formed, 1,25 (OH)2D exerts its biologic effects by binding to the vitamin D nuclear transcription factor receptor, which regulates the expression of nearly 200 genes (4). Vitamin D has a central role in maintaining serum calcium and skeletal homeostasis, as well as several other cellular effects, including regulation of differentiation, proliferation, apoptosis, metastatic potential, and angiogenesis (5). Growing evidence suggest that there is a connection between vitamin D and cardiometabolic diseases and cancer. Several reports published recently suggest that low serum 25 (OH)D levels may be associated with increased incidence of colorectal (6, 7), breast (8, 9) and other cancer types (10). A population-based, double-blind, randomized placebo-controlled trial found that women who increased their daily vitamin D intake by 1100 IU reduced their risk of cancer by 60–77 %, in consistency with the results above (11).

Identification of Vitamin D Receptors (VDRs) in almost all immune system cells primarily in antigen-presenting cells such as active T and B lymphocytes, active macrophages and dendritic cells as well as their detection in many types of tissues, attracted attention of the researchers to the role of vitamin D in immune regulation. Although low levels of 25-OH-D were associated with various malignancies, the causal effect of vitamin D deficiency on the development of malignancy remains unknown. The evidence for the relation between vitamin D levels and the risk of solid tumor development is gradually increasing, but the risk of hematological malignancy development is still unknown.

Although many investigations were conducted in recent years on the effects of vitamin D levels on the prognosis of malignant diseases, there is not enough data yet in the literature concerning

CML (chronic myelocytic leukemia) patients. It was aimed in this study to evaluate vitamin D levels and calcium bone metabolism in CML patients, and to assess the relationship between vitamin D levels and prognosis.

Material and methods

Subjects

In this cross-sectional study, 61 CML patients (31 women, 30 men) were recruited from hematology clinics of Katip Celebi University Ataturk Training and Research Hospital between June 2014 and September 2014. History of receiving vitamin D/Ca supplements and presence of other malignancies were exclusion criteria. The study was approved by the human research ethics committee of the hospital and informed consent was obtained from all participants. Vitamin D status was defined as; severe deficiency ≤ 10 ng/ml; deficiency 10–20 ng/ml; insufficiency 20–30 ng/ml; sufficiency ≥ 30 ng/ml (3). Treatment responses were evaluated according to the ELN recommendations (12, 13).

Bcr-abl

RQ-PCR method was used for qualitative and quantitative analysis of BCR-ABL (b3a2/b2a2) fusion transcripts. RNA isolation from peripheral blood was performed with QIAamp RNA Blood Mini (Qiagen Inc. Valencia, CA.) Kit. RNA purity was analyzed by spectrophotometry in the absorbance range of 260–280 nm. RT kit (Qiagen, USA) kits was used for cDNA synthesis. BCR-ABL1 Mbcr IS-MMR Kit (Qiagen, USA) was used for RQ-PCR assays. PCR amplification and product analysis assay were performed on RG-6000 real-time instrument (Corbett Research-Australia). PCR conditions were performed according to the manufacturer's recommendations. Software Rotor Gene Q series 1.7 was used for qualitative and quantitative analysis.

Vitamin D measurements

25-hydroxy vitamin D blood samples of the subjects were collected into standard vacuum tubes containing separation gel, allowed for half an hour for the formation of clot, and then centrifuged for 10 minutes at 3500 rpm to separate serum samples. All serum samples were stored frozen at –80 °C until the day of analysis. In all samples, 25-OH vitamin D3 levels were determined quantitatively using electrochemiluminescence immunoassay method by Cobas brand E601 model modular autoanalyser (Hoffman-La Roche, Grenzacherstrasse 124 CH-4070 Basel, Switzerland). The results were reported in ng/ml.

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences Software, version 18.0 (SPSS Inc., Chicago, USA). The patients were divided into two groups according to their vitamin D levels (group 1 ≤ 10 ng/ml, group 2 > 10 ng/ml) and these two groups were compared with respect to clinical and laboratory parameters using Mann-Whitney U test. The difference in gender distribution between the groups was analyzed by Chi-square analysis.

To evaluate the effects of vitamin D levels on molecular response, we used binary logistic regression analysis. In our model, molecular response was defined as a dependent variable and the subjects were categorized according to their vitamin D levels as ≤ 10 ng/ml and >10 ng/ml. In order to clarify the effect of potential confounders such as Sokal score, gender and type of treatment, we added these variables to the model consecutively. Confidence interval (CI) for all reported values was 95 %. A two-sided p value of < 0.05 was considered statistically significant.

Results

A total of 61 patients (31 women, 20 men) were included in the study. The mean age of the subjects was 54.5 years and the mean follow-up period was 5.65 years. Molecular response was determined in 36 of 61 patients included in the study. As for treatment, Imatinib was used in 46 patients, Dasatinib in 7, Nilotinib in 7 and Hydroxyurea in 1 patient. Patients' data with respect to demographic characteristics and laboratory values are shown in Table 1.

As for vitamin D levels; 37 (60.7 %) patients had ≤ 10 ng/ml, 19 (31.1 %) patients had 10–20 ng/ml and 5 (8.2 %) patients had 20–30 ng/ml. None of the subjects in our study population had sufficient levels of Vitamin D. In our study, patients were divided

Tab. 1. Demgraphic and laboratory characteristics of the subjects (n = 61).

Variables	Mean±SD
Age (years)	54.39±15.21
Follow-up (years)	5.65±3.22
Gender (F/M)	31/30
WBC (103/μL)	7100±3200
Hgb (mg/dl)	12.38±1.61
PLT (103/μL)	232.000±77.000
Sokal score	0.97±0.18
Creatinine (mg/dl)	0.89±0.28
ALT (U/L)	21.11±19.31
LDH (U/L)	216.31±55.04
PTH (pg/dl)	98.12±54.24
25-OH-D	10.00±5.49
≤10 ng/ml, n (%)	37 (60.7)
10–20 ng/ml, n (%)	19 (31.1)
20–30 ng/ml, n (%)	5 (8.2)
Ca (mg/ml)	9.10±0.44
P (mg/ml)	3.02±0.72
Albumin (mg/ml)	4.20±0.26
24-hour urine Ca extraction (mg)	99.09±67.05
Co-morbidities	
Type 2 diabetes, n (%)	12 (19.6)
Hyperlipidaemia, n (%)	14 (22.9)
Hypertension, n (%)	15 (24.5)
Coronary artery disease, n (%)	8 (13.1)
Treatment	
Imatinib, n (%)	46 (75.4)
Dasatinib, n (%)	7 (11.5)
Nilotinib, n (%)	7 (11.5)
Hydroxyurea, n (%)	1 (1.6)

Tab. 2. Comparison of the demographic and laboratory characteristics of the subjects according to vitamin D levels (≤ 10 ng/ml and higher vitamin D levels).

Variables	≤10 ng/ml (n=37)	>10 ng/ml (n=24)	p[a]
Age (years)	60.00 (49.50–66.00)	49.50 (36.75–64.75)	0.109
Gender (F/M)	19/18	12/12	0.918
Disease duration (years)	6.33 (3.00–8.00)	5.50 (3.29–7.00)	0.500
Sokal score	1.03 (0.91–1.10)	0.90 (0.72–1.14)	0.246
WBC 10^3/μL	6660 (5085–7900)	6235 (5090–7922)	0.685
Hbg (ml/dl)	12.50 (11.05–12.90)	13.25 (12.02–13.80)	0.016*
PLT 10^3/μL	231 (175–287)	212 (170–254)	0.447
PTH (pg/mL)	120.00 (58.00–144.00)	64.00 (44.00–98.00)	0.007*
Albumin (mg/dl)	4.20 (4.05–4.30)	4.30 (4.10–4.40)	0.133
Ca (mg/dl)	9.20 (8.95–9.40)	9.25 (8.65–9.40)	0.767
P (mg/dl)	3.10 (2.65–3.60)	2.95 (2.30–3.62)	0.375
24-hour urine Ca (mg)	70.00 (40.00–135.00)	100.00 (60.00–160.00)	0.239
Creatinine (mg/dl)	0.80 (0.70–1.04)	0.83 (0.76–0.97)	0.399
ALT (U/L)	17.00 (10.00–20.50)	17.00 (14.00–26.75)	0.155
Molecular response, n (%)	18 (48.6)	18 (59.0)	0.041*

Tab. 3. Logistic regression analysis for molecular response across ≤ 10 ng/ml or higher vitamin D levels.

	OR	95 % CI	p
Model 1	3.167	1.026–9.770	0.024*
Model 2	2.341	1.126–4.867	0.032*
Model 3	2.338	1.122–4.871	0.034*
Model 4	2.336	1.103–4.947	0.037*

OR – odds ratio, CI – confidence interval. A p value of < 0.05 was considered significant (*).

into two groups according to vitamin D levels: < 10 ng/ml and > 10 ng/ml. There was no significant difference between the two groups in terms of age, gender, disease duration, Ca-P levels, and creatinine levels. Patients with lower vitamin D levels had lower levels of hemoglobin and PTH. The distribution of demographic and laboratory levels of the patients with respect to vitamin D levels is shown in Table 2. Molecular response was determined in 18 of the 37 patients (48.6 %) with low levels of vitamin D while 18 of the 24 patients (59.0 %) with high levels of vitamin D and the difference was statistically significant (p = 0.041).

Discussion

In the present study, we demonstrated for the first time that lower circulating 25-OH-D levels were independently associated with molecular unresponsiveness in subjects with chronic myeloid leukemia, after adjusting for potential confounders.

Epidemiological studies showed that reduced vitamin D levels may be related with increased cancer incidence and mortality (14, 15). This hypothesis has been further supported by studies showing that activated vitamin D receptor induced differentiation and apoptosis whereas inhibited proliferation, invasion and angiogenesis (16). Previous studies have also shown an inhibitory effect of vitamin D on megakaryocyte proliferation and collagen synthesis in the bone marrow (22, 23). It was thought that Vitamin D activity was associated with the reduction of the bone marrow collagen content, and on the opposite side, its deficiency was associated with abnormal accumulation of collagen in the bone marrow.

In a recent study by Campiotti et al, CML patients treated with tyrosine kinase inhibitors were followed-up and vitamin D levels were found to be higher in patients with complete molecular response with respect to those with major molecular responses. In addition, side effects of tyrosine kinase inhibitor treatment were found to be more common in patients with lower levels of vitamin D (24). That study supported the relation between vitamin D levels and treatment response as well as treatment-related side effects.

In a study searching for any correlation between vitamin D levels and prognosis in myeloproliferative diseases and MDS, vitamin D levels showed no relation with prognosis in polycythemia vera, myelofibrosis and MDS, in contrast to what was observed in CML (18). In another study, however, vitamin D therapy was shown to improve prognosis in MDS (19). Shanafelt et al followed the process from diagnosis to treatment prospectively in CLL patients, and determined that treatment procedures had to be initiated earlier in patients with vitamin D deficiency (17).

A pooled analysis of 10 studies found that higher levels of recreational sun exposure, which would be anticipated to increase vitamin D levels, was associated with a lower risk for non-Hodgkin lymphoma (21). Also, a meta-analysis showed a statistically significant protective effect of sunlight/UVR exposure on non-Hodgkin lymphoma (20).

There are some limitations in the current study. The population size was relatively small and the cross-sectional study design cannot prove causality. In such a pioneering study on this topic, our findings are promising, but this association should be verified in a larger population using a prospective-cohort design. In brief, our results indicate for the first time that decreased circulating 25-OH-D levels were associated with molecular unresponsiveness in patients with CML. 25-OH-D may contribute to molecular response in CML patients. To our knowledge, there is no data in the literature on the relationship between vitamin D and molecular response in patients with CML.

As a result; there are studies showing that vitamin D levels are associated with prognosis in various hematological malignancies. In this study too, vitamin D levels were determined to affect the molecular response in CML. We suggest that vitamin D levels should be measured in patients with hematological malignancy and vitamin D replacement therapy should be performed in patients with vitamin D deficiency.

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