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The Prognostic Value of Vitamin D Insufficiency & Vitamin D Receptor Gene Polymorphism in Adult Acute Myeloid Leukemia Patients

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Abstract

Background: Vitamin D regulates many aspects of cellular growth and differentiation in normal and cancer cells. There is growing evidence for both serum vitamin D level and VDR gene polymorphism as prognostic factors in hematologic malignancies. **Aim of this work:** Evaluation of vitamin D serum level and VDR FOKI polymorphism as prognostic factors in adult AML patients. **Patients & Methods:** Eighty subjects were included in this study, 50 adult patients with newly diagnosed AML and 30 apparently healthy controls matched for age and sex. Venous blood samples were withdrawn from all subjects for measurement of serum 25(OH) vitamin D using competitive photo chemiluminescence and molecular detection of VDR (FOKI) polymorphism, which was done by RFLP PCR. All patients received the standard induction chemotherapy regimen 3 & 7. **Results:** The rate of vitamin D insufficiency was significantly higher in AML patients compared to controls (58% vs 16%, p = 0.03). The mutant FOKI genotype (FF & Ff) was found in 52 % of patients compared to 23 % of controls (p = 0.02). Patient with sufficient vitamin D level showed a significantly higher complete response rate compared to those with insufficient level (90% vs 44%, p = 0.02), while none of the other clinical features showed significant relation. Patients with wild type FOKI polymorphism (FF) were more likely to have favorable cytogenetics, while patient with mutant FOKI polymorphism were more likely to have poor cytogenetics (p = 0.03). The CR rate was highest in the wild type FF group (87.5%) followed by the heterozygous Ff group (50%), while none of the patients in the homozygous ff group achieved CR (p = 0.04). **Conclusion:** VDR FOKI polymorphism and serum vitamin D level showed a significant impact on the treatment outcome of adult AML patients suggesting their potential

role as prognostic factors in these patients. Longer follow up will be needed to study the impact on overall and disease free survival.

Keywords

Vitamin D, VDR, FOKI, Polymorphism

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous disorder characterized by clonal expansion of myeloid progenitors in the bone marrow and peripheral blood [1]. In spite of the advancement in AML treatment options, the prognosis is variable, where number of patients die from intensive chemotherapy complications, resistance or relapse to treatment [2]. Several factors as gene mutations, chromosomal translocations and polymorphisms are involved in the pathogenesis of leukemia [3].

Vitamin D (VD) is a steroid hormone produced in the skin that can regulate many aspects of cellular growth and differentiation through a nuclear transcription factor and exerts its action through specific intracellular receptors that present in normal and cancer cells. Modification of vitamin D receptor (VDR) can be a clinical approach in multiple cancers like leukemias [4].

Most patients with newly diagnosed (AML) show VD deficiency and low levels of VD are significantly associated with poor disease outcome, while higher VD levels are related with a better outcome [5].

Among the VDR polymorphisms, the FokI single nucleotide polymorphism which is the translation start site in exon 2 is the only one that results in a VDR protein with a different structure. There is growing evidence for a relationship between VDR gene polymorphism and the prognosis of hematologic malignancies [6].

A higher level of VD in serum of AML patients is associated with a good response to treatment, longer survival and better prognosis conversely low VD levels can be considered as a risk factor that should be appreciated [5].

2. Subjects and Methods

This study was conducted in Clinical Pathology and Medical Oncology Departments, Faculty of Medicine, Zagazig University Hospitals during the period from March 2017 to March 2018. Informed consent was obtained from all individuals. Eighty subjects were included in this study, they were classified into two groups, Control group 30 individuals (19 males 63.3% & 11 females 36.7%), their ages ranged from 19 - 60 years with median age 46.3 years. Patient group 50 individuals (28 males 56% & 22 females 44%) with median age 34 years ranging from 18 - 60.

Inclusion criteria included the following:

a) Age > 18 years old

b) Diagnosed as de novo AML.

Exclusion criteria included the following:

a) Age > 60 years old

b) Promyelocytic leukemia (M3)

c) Other malignancy.

Routine laboratory investigations were done to both groups including CBC, liver & kidney functions and LDH. Specific laboratory investigations to AML patients including: Bone marrow aspiration and examination using Leishman and cytochemical stained smears, Immunophenotyping by flow cytometry using Becton Dickenson FacsCalibar device, Cytogenetic analysis, where karyotyping was performed by G banding technique using image analyser Imstar (Paris, France).

Serum 25(OH) vitamin D measurement:

Using competitive photo chemiluminescence on the Elecsys and Cobasanalyzers (Roche diagnostic). The analyzer automatically calculates the analyte concentration of each sample (ng/mL). Vitamin D deficiency was defined as a 25(OH)D level of less than or equal 20 ng/mL.

Molecular detection of VDR (FOKI) polymorphism included the following steps:

Sample collection:

5 (mL) of venous blood were drawn and poured into Ethylene diamine tetraacetic acid (EDTA) blood container.

DNA extraction and molecular analysis:

QIAamp DNA blood mini kit (QIAGEN) was used and Genomic DNA was extracted from samples. 40 µl of the reaction mixture was prepared for each sample containing 6 µl of genomic DNA, 1 µl of each forward primer (5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3') and reverse primer (5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'), 10 µl master mix (MyTaq Red Mix) and 22 µl sterile distilled water.

By using Thermal Cycler DNA samples were amplified (Gene Amp). PCR was done (Perkin Elmer, USA, system 2400) with cycling conditions: Denaturation at 95˚C for 1 min, 35 cycles, each consisted of 95˚C for 15 seconds, 61˚C for 15 seconds & 72˚C for 10 seconds. One final cycle of extension at 72˚C for 7 minutes.

Amplified products were digested by using FOKI restriction enzyme to study Vitamin D receptor gene FOKI polymorphism. The digested products were analyzed by electrophoresis on 2% agarose gel containing ethidium bromide and were visualized by ultraviolet light transillumination. The amplified 265-bp fragment after digestion with FOKI restriction enzyme gave rise to:

a) Undigested 265-bp fragment indicated the presence of the F allele;

b) Appearance of 196-bp and 69-bp fragments indicated the presence of the f allele.

The wild variant FF resulted in one fragment at 265-bp, homozygous variant ff resulted in two fragments at 196-bp and 69-bp while the heterozygous variant Ff resulted in 3 fragments at 265,196 and 69-bp (**Figure 1**).

Treatment plan:

Patients were treated by an induction regimen 3&7 regimen consisting of continuous infusion cytarabine (100 mg/m^2) daily for 7 consecutive days combined with 3 days of doxorubicin (45 mg/m^2). Written informed consent was taken from all patients and study protocols were approved by the institutional review board at the faculty of medicine, Zagazig University.

Statistical Analysis:

The statistical package for social science (SPSS version 16) was used for analysis of the data. For descriptive statistics, the Mean ± SD and Median (range) were used. Comparison between variables was done using the T-test and Chi-square test "and their equivalents" for quantitative and qualitative variables, consequently. The level of significance was fixed at 5%, where p-value < 0.05 is considered significant.

3. Results

The present study included two groups: Control group (30 normal individuals) and patient group (50 de novo adult AML patients), matched for age and sex. Patients were diagnosed and treated at the Clinical Pathology and Medical Oncology Departments, Faculty of Medicine, Zagazig University Hospitals. The results were statistically analyzed and came to the following:

This study included 50 newly diagnosed AML patients of which 56 % were males and 44 % were females and the median age was 34 years ranging from 18 - 60. The median serum vitamin D level was significantly higher in controls compared to AML patients (29.4 vs 15.6, p = 0.01). Vitamin D insufficiency was found in 58 % of AML patients compared to 16 % of control group (p = 0.03). The FOKI wild genotype FF was found in 76 % of controls compared to 48 % of patients and the heterozygous genotype Ff was found in 44 % of patients compared to 23.3% of controls, while the homozygous genotype ff was only found in patients (8%) (Table 1).

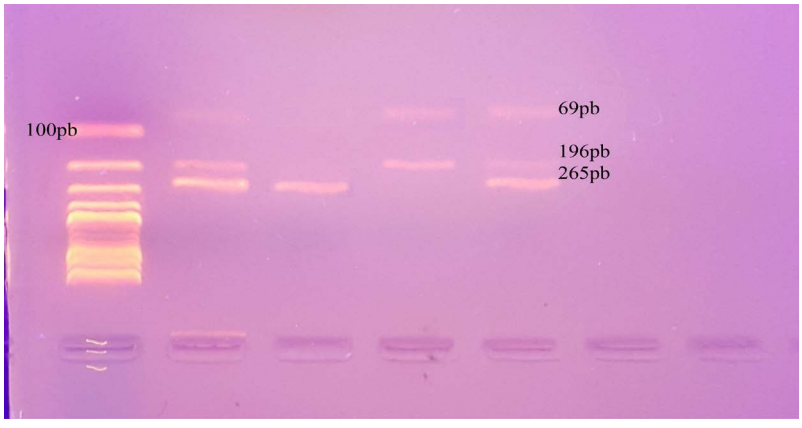

Figure 1. Gel electrophoresis of PCR-RFLP technique of amplified FOKI genotypes. Lane 1 and 4: Represent the heterozygous Ff genotype showing 3 fragments of 265, 196 and 69 bp; Lane 2: Represent the wild type FF showing 1 fragment of 265 bp; Lane 3: Represent the homozygous ff genotype showing 2 fragments of 196 and 69 bp.

Table 1. Serum vitamin D levels and FOKI polymorphism in controls and AML patients.

Variable	Control group (No = 30)		Patient group (No = 50)		P
Vitamin D (ng/mL)					
Median	29.4		15.6		**0.01**
Range	6.3 - 41.5		5.4 - 38.7		
State of vit. D					
Sufficient	25	83.4%	21	42%	**0.03**
Insufficient	5	16.6%	29	58%	
FOKI Genotype					
FF	23	76.7%	24	48%	
Ff	7	23.3%	22	44%	**0.02**
ff	0	0	4	8%	
FOKI Alleles					
F	53	88.3%	70	70%	**0.01**
f	7	11.7%	30	30%	

Clinical characteristics of the AML patients in relation to vitamin D sufficiency are presented in (Table 2). The patients were divided into two groups based on the level of vitamin D (insufficient < 20 ng/mL and sufficient ≥ 20 ng/mL). No significant difference was found between patients with normal and low vitamin D levels regarding age, sex, BMI, bone marrow blasts, peripheral blood blasts, hemoglobin, total leukocytic count and platelet count. Moreover the FAB subtype and cytogenetic risk groups showed no significant relation to vitamin D level. However, patients with sufficient vitamin D level had a significantly higher complete response rate compared to patient with insufficient vitamin D level (90% vs 44%, p = 0.02).

Clinical characteristics of the AML patients in relation to VDR FOKI polymorphism are presented in (Table 3). The patients were divided into three groups (wild type FF, heterozygous Ff and homozygous ff). The complete response rate was highest in the wild type FF group (87.5%) followed by the heterozygous Ff group (50%) and lowest in the homozygous ff group (0%) (p = 0.04). Seventy-five percent of patients with homozygous ff genotype had poor cytogenetics followed by 63.6% for patients with heterozygous Ff genotype compared to only 12.5% for patients with wild FF genotype (p = 0.03). There was no significant difference between the three groups regarding age, sex, BMI, bone marrow blasts, peripheral blood blasts, hemoglobin, total leukocytic count, platelet count or FAB subtype.

4. Discussions

Vitamin D is a prohormone that is acquired mainly from the diet or through dermal synthesis following exposure to sunlight; it is enzymatically activated in

Table 2. Clinical characteristics of AML patients in relation to vitamin D serum level

	Vit. D Insufficient (<20 ng/mL) No = 29	Vit. D Sufficient (≥20 ng/mL) No = 21	P
Age (median, range)	32 (18 - 58)	37 (19 - 60)	0.3
Gender			
Malen (%)	17 (58.6)	11 (52.4)	0.5
Femalen (%)	12 (41.4)	10 (47.6)	
BMI (kg/m^2)	22.4 (19.1 - 30.7)	24.3 (17.4 - 31.5)	0.5
Bone marrow blasts (%)	60 (25 - 96)	63 (23 - 97)	0.7
Peripheral blood blasts (%)	30 (0 - 55)	32 (0 - 53)	0.6
Hb (gm/dL)	10 (7 - 13)	9 (6 - 14)	0.8
PLTs ($\times 10^3$/uL)	50 (10 - 220)	55 (8 - 300)	0.7
TLC ($\times 10^3$/uL)	18 (1 - 33)	12 (0.8 - 40)	0.4
FAB subtype			
M1	4 (13.8)	3 (14.3)	
M2	11 (37.9)	6 (28.6)	0.5
M4	6 (20.7)	8 (38.1)	
M5	8 (27.6)	4 (19)	
Cytogenetic risk group			
Favorable n (%)	9 (31)	8 (38.1)	0.09
Intermediate n (%)	7 (24.1)	6 (28.6)	
Poor n (%)	13 (44.8)	7 (33.3)	
CR rate n (%)	13 (44.8)	19 (90.5)	**0.02**

the liver and kidneys. The active form of the vitamin (1.25(OH)2D) freely passes into the cells through the membranes and binds with the nuclear vitamin D receptor (VDR) to form a heterodimer complex with retinoid X receptor (RXR), which then binds to vitamin D3-responsive elements in the promoter region of the gene and modulates target gene transcription [7].

In our study, we observed a statistically significant lower vitamin D level in AML patients compared with healthy controls. Similar to previous studies done by Bobilev *et al.* [8] and Elkerdany *et al.* [9], who declared that vitamin D play an important role in regulation of cellular differentiation, proliferation together with apoptosis and angiogenesis. Moreover, Munker *et al.* [10] reported that vitamin D is a very potent inhibitor of CD34 leukemic blasts, while preserving the activity of normal human CD34+ hematopoietic progenitor cells. In agreement with these results, Lappe *et al.* [11] have shown that serum 25(OH)D concentration was a significant independent predictor of the risk of cancer and supplemental calcium and vitamin D are associated with a reduced risk of cancer.

Moreover, Thomas *et al.* [12] observed a significant association between circulatory 25(OH)D and malignant cell burden. They observed that lower levels of circulatory 25(OH)D appeared to be related to a progressive stage of the disease and poor response to therapy and to the aggressiveness of the disease. Thus, it is a potential marker of prognosis in AML patients.

Table 3. Clinical characteristics of AML patients in relation to VDR FOKI polymorphism.

	VDR FOKI Polymorphism			P
	FF n = 24	**Ff n = 22**	**ff n = 4**	
Age(median, range)	31 (18 - 60)	36 (18 - 59)	33 (20 - 54)	0.4
Gender				
Male n (%)	13 (54.2)	12 (54.5)	3 (75)	0.6
Female n (%)	11 (45.8)	10 (45.5)	1 (25)	
BMI (kg/m^2)	22.5 (17.4 - 31.2)	25.3 (19.1 - 31.5)	24.8 (19.5 - 30.7)	0.7
Bone marrow blasts (%)	63 (23 - 90)	58 (25 - 96)	75 (60 - 97)	0.6
Peripheral blood blasts (%)	33 (0 - 45)	29 (0 - 50)	35 (20 - 55)	0.8
Hb (gm/dL)	9	10	8	0.5
PLTs ($\times 10^3$/uL)	52	58	48	0.6
TLC ($\times 10^3$/uL)	8	12	16	0.6
FAB subtype				
M1	4 (16.7)	2 (9.1)	1 (25%)	
M2	7 (29.2)	9 (40.9)	1 (25%)	0.7
M4	8 (33.3)	6 (27.3)	0	
M5	5 (20.8)	5 (22.7)	2 (50%)	
Cytogenetic risk group				
Favorable n (%)	14 (58.3)	2 (9.1)	0 (0)	0.03
Intermediate n (%)	7 (29.2)	6 (27.3)	1 (25)	
Poor n (%)	3 (12.5)	14 (63.6)	3 (75)	
CR rate n (%)	21 (87.5)	11 (50)	0 (0)	0.04

In the current study, there was no significant difference between the median values of BMI in both groups. Elkerdany *et al.* [9] did not find any relation between the two groups as regard BMI. However, Vashi *et al.* [13] stated that after adjusting the age, every 1 kg/m^2 increase in BMI was significantly associated with 0.42 ng/mL decline in serum 25(OH) vitamin D. Moreover, Wortsman [14] explained this finding by the fact that higher BMI or obesity has been found to be associated with considerably lower circulatory concentrations of 25(OH)D because of its deposition in body fat compartments.

In this study, wild genotype (FF) was significantly lower in AML group than controls (48% vs 76.7%), while heterozygous genotype (Ff) was significantly higher in the AML group (44%) than controls (23.3%). Our results go in line with those of Ibrahim *et al.* [15] who reported that wild FF genotype was significantly lower in AML group than controls, while heterozygous genotype Ff was significantly higher in AML group than controls. Esfahani & Ghoreishi [6] stated that wild FF genotype was lower in controls than the AML group, while heterozygous genotype Ff is higher in controls than AML group.

Moreover, in our study the mutant f allele is significantly higher in AML group than controls (8% vs 0%). This was close to that reported by Ibrahim *et al.* [15] who stated that the frequency of the f allele was 14% in the AML group and

8% in the control group.

Esfahani & Ghoreishi [6] studied the association of VDR polymorphisms (c) with AML involving 133 patients and 300 controls and found a significant association between VDR TaqI polymorphism and AML in which TaqI polymorphism was associated with complete remission in AML patients. Accordingly, any genetic variations of VDR may elucidate the association of vitamin D levels, its metabolism and VDR polymorphism with development of AML and various cancers [16].

This great discrepancy between different studies could be attributed to the fact that the addressed problem is a genetic disease with a mutation that ultimately may have a different prevalence in different populations. Other possible explanations for this difference include different distribution of FAB subtypes of AML patients and mutation detection methods.

After induction therapy, our study demonstrated a statistically significant difference between the VD sufficient and insufficient groups as regard complete remission (CR) rate being 90.5% vs 44.8% respectively. The same finding was reported by Elkerdany *et al.* [9] who found a significant association between vitamin D level and CR rate. On the other hand, Lee *et al.* [17] stated that the level of vitamin D was not associated with the probability of attaining CR. Discrepancies in CR achievement may be related to the differences in patient population analyzed with respect to their size, age and racial difference.

In the current study, the AML group showed no association between VDR FOKI genotypes and FAB classification. However, it revealed a significant association between FOKI genotypes and cytogenetic risk categories. Homozygous genotype ff and heterozygous Ff genotypes showed adverse cytogenetics (75% and 63.6% respectively), while FF genotype was mostly of favorable cytogenetics (58.3%).

Vit D deficiency has been reported to predispose individuals to increased risk of developing of cancers. Consequently epidemiological and experimental evidence support a role for Vit D in cancer prevention and treatment in many types of cancers [18]. A significant inverse relationship was found between 25(OH)D levels and length of hospital stay [19].

Vitamin D level seems to add prognostic information in AML patients. However, its predictive value is still unclear. We can hypothesize that not only the prevalence of VD deficiency is high in hematologic malignancies, but it reduces the response of these patients to treatment. It is recommended to conduct clinical trials to evaluate the effect of VD supplementation on the therapeutic outcomes of these patients [20]

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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