



Vitamin D receptor gene polymorphism in Egyptian pediatric acute lymphoblastic leukemia correlation with BMD



Maha Tantawy^a, Mahmoud Amer^c, Tarek Raafat^b, Nayera Hamdy^b

^a Children Cancer Hospital Egypt, Cairo, Egypt

^b National Cancer Institute, Cairo University, Cairo, Egypt

^c Faculty of Science, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:

Received 6 November 2015

Revised 21 March 2016

Accepted 29 March 2016

Available online 30 March 2016

Keywords:

Acute lymphoblastic leukemia

Osteoporosis

Vitamin D receptor

Polymorphism

Bone mineral density

ABSTRACT

Introduction: We studied the frequencies of the 3' and 5'-end vitamin D receptor (VDR) gene polymorphisms and their correlation with bone mineral density (BMD) in Egyptian pediatric acute lymphoblastic leukemia (ALL) patients receiving calcium and vitamin D supplements. The purpose of this study is to find out the relation between VDR polymorphism and the response to vitamin D intake in pediatric ALL cases who receive corticosteroid therapy which predispose to osteoporosis. This study might shed the light on some genetic variants that are effect the response of individuals to vitamin D therapy.

Methods: Forty newly diagnosed pediatric ALL cases were studied. Three SNPs at the 3'-end of the VDR gene (BsmI rs1544410, ApaI rs739837 and TaqI rs731236) and two SNPs at the 5'-end (Cdx-2 rs11568820 and GATA rs4516035) were analyzed by Allelic discrimination assay. Of those twenty-six cases with initial BMD data available were further analyzed with regards to the effect of various VDR genotypes/haplotypes on BMD.

Results: The genotype frequencies at 3'-end of VDR gene were, TaqI TT 23%, Tt 54% and tt 23%, BsmI bb 19.2%, Bb 65.4% and BB 15.4% and ApaI AA 12%, Aa 27% and aa 61%. The frequencies at the 5'-end were Cdx-2 GG 34.5%, GA 54% and AA 11.5% and GATA AA 8%, AG 50% and GG 42%. Eight and four possible haplotypes were observed at the 3' and 5'-ends of the VDR gene respectively. The Tt genotype was significantly correlated with high BMD as compared to other TaqI genotypes ($P = 0.0420$). There was a trend towards higher BMD with the genotype Bb as compared to other BsmI genotypes. No statistical significance was found between the other VDR genotypes or haplotypes studied and BMD.

Conclusions: This is the first report on VDR gene polymorphisms in Egyptian pediatric ALL patients. The Tt genotype was associated with increased BMD. Our study showed marked genetic heterogeneity in VDR gene in Egyptian pediatric ALL patients.

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E-mail address: Maha.tantawy@yahoo.com (M. Tantawy).

1. Introduction

One quarter of all cases of pediatric malignancies is acute lymphoblastic leukemia (ALL) (Pui, 1997). Corticosteroids and methotrexate (MTX) are used in the treatment of ALL and affect bone metabolism. MTX causes increase resorption and inhibition of bone formation. Corticosteroids cause a decrease in osteoblastic activity and have direct effect on bone matrix. In addition, children undergoing cancer therapy may have limited physical activity and suffer from malnutrition during their illness, further influencing the achievement of potential peak bone mass (Mandel et al., 2004).

The treatment of ALL is one of the most complex treatment plans used in any type of cancer. It is divided into three phases, starting with the *induction phase*, followed by the *consolidation phase* (or intensification) and finally *continuation phase* according to total therapy study XV for newly diagnosed patients with acute lymphoblastic leukemia:

- Induction phase (6–7 weeks) consists of a combination of several medications, most often a steroid (*dexamethasone* or *prednisone*).
- Consolidation phase (8 weeks) uses most often a steroid (*dexamethasone* or *prednisone*), *vincristine*, an *anthracycline* (*daunorubicin*, *epirubicin* or *idarubicin*) and *asparaginase*. Other medications may be used, such as *cytarabine*, *cytoxan*, *methotrexate* and *rituximab* but is more variable in its schedule and depends on the particular subtype of ALL.
- Continuation phase (120 weeks/female–146 weeks/male) consists of 6-MP, *methotrexate*, *vincristine*, and *prednisone*, given over a period of 2 to 3 years. Studies found no benefit to increasing this time, outcomes become worse if the maintenance period was shorter. Continuation therapy is not given for Burkitt's lymphoma leukemia, as this subtype has a high cure rate with induction and consolidation therapy alone (Gökbuget and Hoelzer, 2009).

Osteoporosis is considered a multifactorial disease resulting from interactions between genetic and environmental factors. It is characterized by the increased risk of fragility fractures and reduced bone mineral density (BMD). The genetic factors play key functions (50–85%) in the pathogenesis of osteoporosis. Vitamin-D receptor (VDR) gene has been considered as an important candidate gene in the modification of BMD and the development of osteoporosis (Ralston, 2002). The VDR gene is located on chromosome 12q13 (100 kb) (Nejentsev et al., 2004). Variation in DNA sequence of the coding and non-coding region by a single nucleotide polymorphism (SNP) may influence the stability, quantity and activity of the VDR protein and rate of VDR mRNA formation. Over 470 SNPs have been reported in the human VDR gene (Ogunkolade et al., 2002).

The 3' un-translated region of the VDR gene includes TaqI, BsmI and ApaI gene polymorphisms (Morrison et al., 1992). TaqI is located in exon 9 (Morrison et al., 1994), BsmI is located between exon VIII and the three non-coding regions. There is a strong linkage disequilibrium between the BsmI and TaqI polymorphism such that the B allele (mutant) is usually associated with the t allele (mutant) and the BB genotype is almost equivalent to the tt genotype (Arai et al., 1997). The ApaI is located in intron 8 at the 3'-end of the VDR gene. BsmI and ApaI are considered as silent single nucleotide polymorphisms (SNPs). These polymorphisms don't change the amino acid sequence of the encoded protein, however, they may affect gene expression through regulation of mRNA stability (Kostner et al., 2009). Cdx-2 and GATA are common variants in the promoter region around exons 1e and 1a and are often analyzed as a haplotype block. Both of them are located at the 5'-end of the VDR gene (Fang et al., 2005). The aim of the present work was to detect the frequency of the VDR polymorphism and their association with BMD in Egyptian pediatric acute lymphoblastic leukemia patients.

Table 1

Vitamin D receptor (VDR) genotype frequencies in 40 Egyptian pediatric ALL cases.

VDR genotype frequencies (%) n = 40a			
BsmI	BB (5/40)12.5%	Bb (23/40)57.5%	Bb (12/40)30%
ApaI	AA (7/40)17.5%	Aa (11/40)27.5%	Aa (22/40)55%
TaqI	TT (16/40)40%	Tt (17/40)42.5%	Tt (7/40)17.5%
Cdx-2	GG (17/40)42.5%	GA (19/40)47.5%	AA (4/40)10%
GATA	AA (5/40)12.5%	AG (16/40)40%	GG (19/40)47.5%

^a (BsmI; b = wild, B = mutant) (ApaI; A = wild, a = mutant) (TaqI; T = wild, t = mutant) (Cdx-; G = wild, A = mutant) (GATA; A = wild, G = mutant).

2. Patients and methods

2.1. Patient selection

The study group consisted of forty patients with newly diagnosed ALL. They included 28 (70%) males and 12 (30%) females, their age ranged from 4 to 18 years. All cases were recruited from the clinical pathology and radiology departments of the children's cancer hospital, Cairo, Egypt. Patients' guardians were approached and accepted their participation in the study and gave their written consent. All patients were treated according to total XV protocol and received calcium & vitamin D supplements. According to the presence of initial BMD, twenty-six of these cases were further analyzed with regards to the effect of various genotypes and haplotypes on BMD.

2.2. Measurement of bone mineral density (BMD)

Dual energy X-ray absorptiometry (DXA) provided estimates of BMD of the lumbar spine. All DXA results were expressed as age matched and sex-matched SDS, Special pediatric software was used for children who weighed less than 30 kg.

2.3. VDR gene polymorphism

Genomic DNA was extracted by using salting out technique (Miller et al., 1988). Three SNPs at the 3'-end of the VDR gene (BsmI rs1544410, ApaI rs739837 and TaqI rs731236) and two SNPs at the 5'-end (Cdx-2 rs11568820 and GATA rs4516035) were analyzed using Allelic Discrimination Assay.

Real Time PCR was performed using 12.5 µl TaqMan® Universal PCR Master Mix, No AmpErase UNG (2×), 1.25 µl SNP Genotyping Assay mix (TaqMan probes) (20×), 10.25 µl Dnase Free Water and 1 µl DNA (20 ng), to bring the final reaction volume to 25 µl. According to the manufacturer's instructions and the information in the Applied

Table 2

Vitamin D receptor (VDR) haplotype frequencies at the 3'-end and 5'-end in 40 Egyptian pediatric ALL cases.

VDR haplotype frequencies			
BsmI/ApaI/TaqI	Number	Cdx-2/GATA	Number
baT	26	GA	23
BaT	7		
bAT	19	GG	31
BAT	9		
bAt	7	AG	23
bat	19		
BaT	20	AA	12
Bat	23		

Table 3

The frequencies and the correction between Z score of BMD before and during treatment for various genotypes of VDR SNPs.

Genotype frequencies (%)	BMD before treatment	BMD during treatment	P value
BsmI			
bb 19.2%	-0.780 ± 0.8167	-0.860 ± 1.0991	0.9150
Bb 65.4%	-1.082 ± 1.4732	-0.859 ± 1.2278	0.2970
Bb 15.4%	-1.100 ± 0.6164	-1.175 ± 0.8221	0.8110
Apal			
AA 12%	-0.800 ± 0.9539	-0.567 ± 0.8737	0.2220
Aa 27%	-0.900 ± 1.5524	-0.743 ± 1.1326	0.7630
Aa 61%	-1.125 ± 1.2168	-1.044 ± 1.1905	0.7260
TaqI			
TT 23%	-0.667 ± 0.9136	-1.233 ± 0.9812	0.2060
Tt 54%	-1.107 ± 1.4809	-0.557 ± 1.1850	0.0420*
tt 23%	-1.200 ± 1.0178	-1.400 ± 0.9423	0.4230
Cdx-2			
GG 34.5%	-1.100 ± 0.8000	-1.056 ± 1.0026	0.8560
GA 54%	-1.186 ± 1.4930	-1.007 ± 1.1552	0.4710
AA 11.5%	-0.067 ± 0.9074	0.000 ± 1.2490	0.9600
GATA			
AA 8%	-1.150 ± 0.2121	-0.250 ± 0.7778	0.2660
AG 50%	-1.092 ± 1.5207	-1.008 ± 1.1146	0.7630
GG 42%	-0.927 ± 1.0451	-0.909 ± 1.2169	0.9520

* The difference was significant, $P < 0.05$.

Biosystems manual, the thermal conditions were as follows (initial denaturation at 95 °C for 10 min, 40 cycles of 92 °C for 15 s (denaturation) and 60 °C for 1 min (annealing/extension) using 7500 Applied Biosystems fast Real-Time PCR system.

2.4. Statistical analysis

Descriptive statistics for both measurements in each gene was done using mean and standard deviation. A repeated measures ANOVA model was done to check if there was statistical significant difference between the genotype-time interactions. The difference between means of both groups in each genotype was done using paired t-test. P-values cut off for significance was set at 0.05 for all tests. All statistical procedures have been done using SPSS v 20.

3. Results

The genotype frequencies of the 3' and 5' VDR SNPs are shown in Table 1. We observed 8 possible haplotypes at the 3'-end and 4 possible haplotypes at the 5'-end. The haplotype frequencies for the three SNPs at the 3'-end and the 5'-end of the VDR gene are shown in Table 2. According to presence of initial BMD before treatment twenty-six cases 17 (65%) males, 9 (35%) females were analyzed further for the effect of various genotypes and haplotypes on the BMD after vitamin D administration.

The TaqI genotype distribution in this group of patients were TT (23%), Tt (54%) and tt (23%) (Table 3). The Tt genotype was found to be significantly associated with increased BMD as compared to the other two genotypes ($P = 0.0420$) (Fig. 1 & Table 3). The correction between Z score of BMD before and during treatment for BsmI, Apal, Cdx-2 and GATA, various genotypes did not reveal any statistical significance (Table 3). No significant association was found between BMD before and during treatment and haplotype distribution for the three SNPs at the 3'-end of the VDR gene (Table 4) and for the two SNPs at the 5'-end (Table 5).

4. Discussion

We analyzed VDR gene polymorphisms in 40 Egyptian pediatric ALL cases.

The TaqI genotype frequencies were TT 23%, Tt 54% and tt 23%. Bhanushali et al., 2009 reported that in 143 healthy Indian individuals, the TaqI genotype frequencies were TT 49%, Tt 43%, tt 8%. In a Japanese Population based Osteoporosis Study (JPOS), 1434 women were analyzed. TaqI genotype frequencies were 78.7%, 19.6% and 1.7% for TT, Tt and tt respectively (Morita et al., 2004). In 78 unrelated normal Syrian individuals, the TaqI genotype distribution was 36%, 58% and 6% for TT, Tt and tt, respectively (Haddad, 2014). The prevalence of the three BsmI genotypes in our patients was BB 19.2%, Bb 65.4% and bb

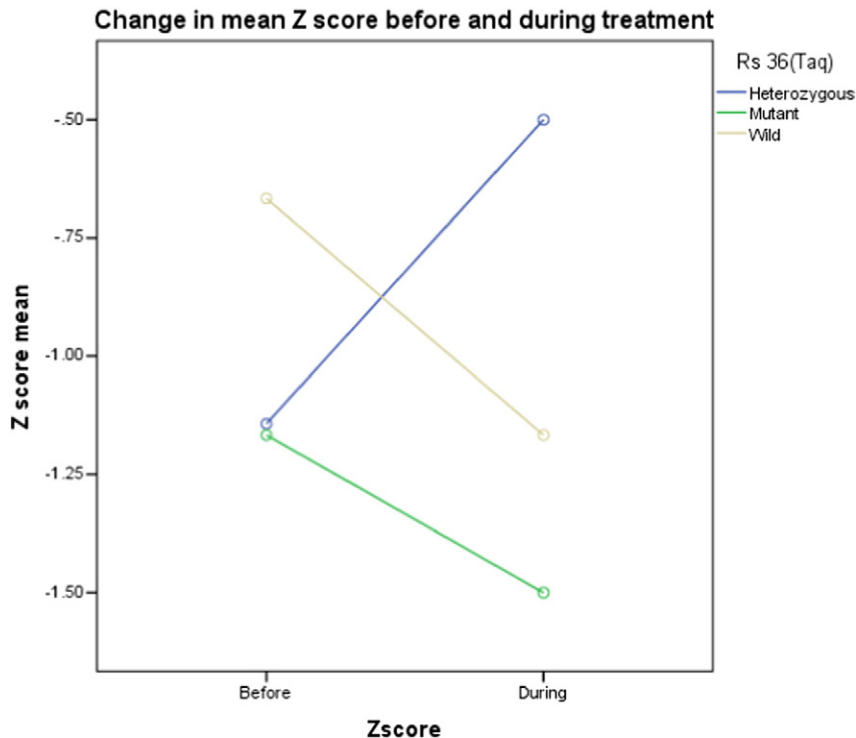


Fig. 1. The correction between Z score of BMD for TaqI genotypes before and during treatment. (—) heterozygous genotype Tt, (—) mutant genotype tt, (—) wild genotype TT, Rs36 TaqI (reference 731236). X axis >> Z score mean, Y axis >> before and during treatment.

Table 4

The correction between Z score of BMD before and during treatment with haplotype distributions for three SNPs at the 3'-end of the VDR gene (BsmI, ApaI and TaqI).

Haplotype (BsmI, ApaI, and TaqI)	Number	BMD before treatment	BMD during treatment	P value
bat	16	-1.1438 ± 1.47376	-0.7188 ± 1.23786	0.088
baT	17	-1.0059 ± 1.40467	-0.7941 ± 1.20751	0.452
bAt	6	-1.1500 ± 1.53851	-0.6333 ± 1.19944	0.261
bAT	10	-0.8700 ± 1.34582	-0.6900 ± 1.01593	0.612
Bat	19	-1.1368 ± 1.36878	-0.9053 ± 1.11129	0.223
BaT	14	-1.1357 ± 1.48459	-0.8071 ± 1.20030	0.188
BAt	6	-1.1500 ± 1.53851	-0.6333 ± 1.19944	0.261
BAT	7	-0.9429 ± 1.50760	-0.4857 ± 1.16251	0.239

15.4%. In a study including 198 Korean girls diagnosed with adolescent Idiopathic scoliosis, the distribution frequency of BsmI was BB 25.3%, Bb 51.5%, bb 23.2% (Suh et al., 2010). P. Marozik et al. (2013) reported BB 22.2%, Bb 57.4% and bb 20.4% in the Belarusian women. In the present study, the prevalence of the three ApaI genotypes were AA 12%, Aa 27% and aa 61%. In a Japanese Population based Osteoporosis Study (JPOS), 1434 women were analyzed, ApaI genotype frequencies were AA 8.9%, Aa 41.0% and aa 50.1% (Morita et al., 2004). In 78 unrelated normal Syrian healthy population, the ApaI genotype distribution was 42%, 47% and 10% for AA, Aa and aa respectively (Haddad, 2014). In the Belarusian women with osteoporosis, P. Marozik et al., 2013 found that the genetic frequencies of ApaI polymorphism were 42.6%, 44.4% and 13% for AA, Aa and aa respectively. This marked variability in the genotype frequencies can be explained in part by the ethnic differences and also by the various numbers of the cases analyzed in each report. In our work, the patients with Tt genotype had a significantly higher BMD in response to vitamin D supplements than patients with TT, tt genotypes. On the other hand no statistical significance was found between BsmI and ApaI genotypes and BMD. However the Bb genotype showed a trend towards an increase in BMD that didn't reach a statistical significance. Analyzing a large number of cases would clarify such correlation. To the best of our knowledge this is the first report on the correlation between the VDR 3'-end genotypes with BMD in Egyptian pediatric ALL. In the present work, the prevalence of the three Cdx-2 genotypes in our patients was GG 34.5%, GA 54% and AA 11.5%. In a study including 198 Korean girls diagnosed with adolescent Idiopathic scoliosis the frequency distribution of Cdx-2 were GG 68.6%, GA 24.7%, and AA 6.5% (Suh et al., 2010). The prevalence of the three GATA genotypes in our patients was AA 8%, AG 50% and GG 42%. To the best of our knowledge, no other reports were found analyzing the GATA genotypes. In our study, no statistical significance was detected between the various genotypes of Cdx-2 and GATA polymorphisms and BMD in Egyptian pediatric ALL patients.

In our patients eight possible haplotypes at VDR 3'-end were found. There was no association between any of these haplotypes and BMD. In a study by Te Winkel et al. (2010) he investigated the effect of treatment of acute lymphoblastic leukemia on BMD in sixty-nine Dutch children and reported 4 haplotypes at the 3'-end (baT, BAt, bAT and BAT). De Jongh et al. (2011) studied a large population-based cohort of older Dutch individuals (923 men & women) and reported 3 haplotypes at VDR 3'-end (baT, BAt, bAT). Our patients showed 4 extra haplotypes than those reported by Te Winkel et al., 2010 (bAt, bat, BaT, Bat) and 5

Table 5

The correction between Z score of BMD before and during treatment with haplotype distributions for two SNPs at the 5'-end of the VDR gene (Cdx-2 and GATA).

Haplotype (Cdx, GATA)	Number	BMD before treatment	BMD during treatment	P value
AA	10	-1.2400 ± 1.68470	-1.0000 ± 1.22927	0.496
AG	17	-0.9882 ± 1.45167	-0.8294 ± 1.19831	0.556
GA	15	-1.1000 ± 1.40915	-0.9067 ± 1.08593	0.454
GG	21	-1.1524 ± 1.30561	-1.1000 ± 1.08351	0.769

more haplotypes compared to the study of De Jongh et al., 2011 denoting the marked genetic variability of the Egyptians compared to Dutch population. In agreement with our results Te Winkel et al. and De Jongh et al. (2011) didn't find any association between VDR3'-end haplotypes and BMD. In our patients, we detected 4 possible haplotypes at 5'-end. Te Winkel et al. (2010) reported 3 haplotypes (GA), (GG) and (AG). De Jongh et al., 2011 reported 3 haplotypes GG, GA, AA. In our work, no statistical significant association was found between any of these haplotypes and BMD. On the contrary, Te Winkel et al. (2010) reported a lower BMD associated with haplotype (AG) and De Jongh et al. (2011) reported an association between (GG) haplotype and increased mortality risk, which was partly explained by the occurrence of osteoporotic fracture. This discrepancy could be partially explained by the variability in the number of cases tested.

In conclusion, we investigated the association between VDR gene polymorphism and BMD in Egyptian patients with acute lymphoblastic leukemia. To the best of our knowledge this is the first report studying such association. Our study revealed a positive correlation between the heterozygous genotype of the TaqI gene and BMD and a trend towards increase BMD with Bb genotype. No statistical significance was found between the other VDR genotypes or haplotypes studied and BMD. Further studies on large number of cases are recommended to confirm these finding. In our patients more haplotypes were detected at the 3'-end of the VDR gene compared to those reported in other populations denoting the marked genetic heterogeneity in the Egyptian pediatric ALL patients.

Conflict of interest statement

Maha Tantawy, Mahmoud Amer, Tarek Raafat, and Nayera Hamdy declare that they have no conflict of interest.

Research involving human participants and/or animals

The research involving human participants.

Informed consent

The study was performed according to the Egyptian ministry of health and population (EMHP) guideline for the use of human subject's materials and institution review board IRB ethics committee approval (IRB 8/2013).

Acknowledgments

Funding was supported by the Children Cancer Hospital – Cairo-Egypt.

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