Gene Polymorphism Vitamin D receptor BsmI in Thalassemia Children in Al-Muthanna Province

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Abstract

Introduction: Vitamin D is crucial for calcium, phosphate homeostasis and mineralization of the skeleton, particularly through growth time developments. Vitamin D deficiency lead to rickets in children and osteoporosis in adult. The activity of vitamin D receptors (VDR) are responsible for the vitamin D so that the single nucleotide polymorphism was detected using BsmI. **Material and methods**: In this study, the vitamin D3 level were measured using enzyme-linked sorbent assay (ELISA) technique, followed by detection of the polymorphism VDR- BsmI gene using PCR and BsmI restriction enzyme assay (PCR-RFLP). **Results**: Vitamin D, alkaline phosphate and others biochemical were performed in 50 patients beta-thalassemia were divided into 25 males and same number females. The biochemical results demonstrated no significant difference $p<0.05$ between males and females according gender, whereas showed high significant serum calcium p value 0.048 according body mass index. We using RFLP-PCR technique to amplify VDR gene BsmI, DNA ladder molecular weight was 1000-2500 base pair and BsmI digestion showing predicted product in 823 to 175 pb in all lane. **Conclusions**: BsmI digestion was showing heterozygous mutant (Bb) and homozygous (BB). These result gene polymorphism VDR BsmI effected on vitamin D levels and related with bone diseases and process metabolism.

***Key words**: BsmI , VDR , thalassemia , gene , vitamin D , polymorphism.*

Introduction

Beta thalassemia syndrome are one of the most popular autosomal recessive hereditary defects diffuse global, with high dominance in the populations of the Mediterranean, Middle East, Central Asia, Indian subcontinent and Far East[1]. Thalassemia bone disease has increased as the major morbidity rate associated with thalassemia transfusion-dependent. Bone disease including of low bone mineral density (BMD), bone pain and fractures are private features of thalassemia[2]. The vitamin D receptor (VDR) gene is by far of the most widely investigated osteoporosis marker. The chosen polymorphism was a BsmI and FokI[3]. Few studies have described an associated between the BsmI polymorphism of VDR and thalassemia children[4] , and with skeletal and non-skeletal parameters in thalassemia major[5].The receptors of both VDR and calcitonin genes polymorphisms are linked with osteoporosis[6].The VDR polymorphism and decrease BMD has been linked also amongst patients with the *BB* VDR genotype[7].

In this contextual relationship, the fundamental interaction of the active form of vitamin D (1,25-dihydroxyvitamin D3) and its nuclear receptor VDR has been known as an important mediator of the innate immune response. The mechanism of action of VDR is by promoting the expression of multi antimicrobial peptides, consist of cathelicin and by the activation of autophagy of the infected cell, consequently limiting the intracellular growth of *Mycobacterium tuberculosis* in macrophage[8,9].

The human VDR gene four common SNPs that have been conducted an investigation extensively: ApaI (rs 7975232), BsmI G>A (rs 1544410), Taq T >C(rs 731236) and FokI T>C(rs2228570). The polymorphism BsmI and ApaI are both situated in intron 8 and TaqI is a wordless SNP in exon 9, and all are associated with in regulating the stability of the VDR mRNA[10,11] . The VDR FokI gene polymorphism causes a constitutional amendment. This SNP is a T/C transition at the translation initiation site of exon 2 at the 5′coding region of the gene. The change makes a new start codon (ATG to ACG), which

leads to the expression of a shorter VDR protein 424 amino acids, which has higher transcriptional action as compared to the full length VDR protein of 427 amino acids[12].

The aim of the current study conducts an investigation into the rate of occurrence of VDR gene polymorphism BsmI (rs1544410) in a groups of Iraqi populations. In thalassemia children patients inspect the relationship between VDR and the conservation bone health and metabolism.

Materials and Methods

Subject

The participants to the case study were enlisted with a future effect between September 2018 and September 2019 in the Samawa City (SC). A total of 50 patients. All entrant signed acquainted documentary approval previous to providing a blood sample and data privacy was protected according to the protocol Helsinki Declaration.

Parameters measurement

Five milliliters of blood outgoing under optimal status by venous blood from every child ,3ml on EDTA and 2ml on DNA extraction, thereafter VDR gene polymorphism whereas the other portion was centrifuged and sera were gotten and stocked under -20°C for measure of serum 25 hydroxy vitamin D3 with Enzyme Linked Immunosorbent Assay (ELISA). Serum vitamin level was assayed based on the manufacturer's directives. Currently accepted standards for diagnosed vitamin D values in thalassemia children are:

1- VD deficiency < 10 ng/ml

2- VD insufficiency 10-30 ng/ml

3- VD sufficiency 30-100 ng/ml[13].

Other laboratory criteria by utilizing Fujifilm clinical biochemistry (FUJIDRI-CHEM 4000i) inspections including: Alkaline phosphate(U/L), potassium(mmol/L),total protein(g/dL) and calicium(mmol/L), in addition computation body mass index(BMI) for all genders.

Genotyping

Genomic DNA was isolated by utilizing the phenol chloroform extraction method. Genotypes were uncovered by using PCR, followed by the BsmI restriction

fragment length polymorphism (rs 1544410) and carried out (PCR-RFLP). The BsmI upstream primer is 5′AAGACTACAAGTACCGCGTCAGTG-3′ and reverse downstream primer is 5′ AACCAGCGGGAAGAGGTCAAGGG-3′. The primers are in figure 1 A.823 pb fragment BsmI in the start codon of the VDR.DNA was extract by utilizing an axis column kit (Qiagen kit) polymerase chain reaction (PCR) amplification and enzymatic digestion with BsmI.

The BsmI genotypes were revealed by utilizing electrophoresis of the DNA samples 1.5% agarose gels and were named as followers: BB (not present restriction site); bb (not present restriction site); Bb (heterozygous of the restriction site). The PCR products for the BsmI polymorphism was 823pb and the restriction fragments were 175pb.

Statistical analysis

The data were analyzed by using SPSS version 22 for windows (SPSS, Chicago, IL, USA). The mean of data was predestined by one- way ANOVA and t-test. Moreover, frequency results were analyzed by pearson chi-square and Fisher exact test. The variations were indicated significant at $p>0.05$.

Results

This study was executed on 50 patients infected with thalassemia, 25 males (50%) and 25 females (50%) their ages extending between 1 to 12 years old. An allocation of the studied vitamin D3 according to the genders both were 15.6±3.9 in female and 15.5±4.2 in male that was less in females in table 1. P. value was 0.976, as well biochemical criteria were less in females except alkaline phosphate was higher registered 76±27.9 and 72.5±27 in males p-value was 0.650.

Table (1): Distribution of the studied parameters values according to the gender for patients with thalassemia:

Parameters	Gender		Reference Range	P value
	Female	Male		
Vitamin D3 (ng/mL)	15.6±3.9	15.5±4.2	<10Def. 10-30 Ins. 30-100 Suff.	0.976
ALK. Phosphate (U/L)	76±27.9	72.5±27	32-111	0.650
Potassium (mmol/L)	4.3±0.7	4.4±0.8	3.5-5.3	0.744
T. Protein (g/dL)	7.3±0.76	7.5±0.79	6.7-8.3	0.588
Calcium (mmol/L)	1.85±0.5	1.89±0.5	1.9-2.1	0.812

* represents a significant difference at $P \leq 0.05$. Data are expressed as Mean±SD.

Regarding gender groups (female and male), the studied parameters were

distributed and statistically analyzed. The results showed no significant difference $p>0.05$ for all studied parameters (Vitamin D3, ALK. Phosphate, Potassium, T. Protein and Calcium).

Table (2): Distribution of the studied parameters values according to the age for patients with thalassemia:

Parameters	Age Groups			P value
	1-4 Y	5-8 Y	9-12 Y	
Vitamin D3 (ng/mL)	15±4	15.8±4	15.7±4	0.848
ALK. Phosphate (U/L)	77.8±25.5	71.4±29.6	75.4±26.4	0.778
Potassium (mmol/L)	4.39±0.77	4.36±0.79	4.39±0.78	0.992
T. Protein (g/dL)	7.2±0.75	7.4±0.79	7.6±0.74	0.324
Calcium (mmol/L)	2±0.59	1.7±0.5	1.8±0.45	0.320

* represents a significant difference at $P \leq 0.05$. Data are expressed as Mean±SD.

Regarding age groups, the results revealed there are no significant differences $p>0.05$ among all the studied age groups for all studied parameters (Vitamin D3, ALK. Phosphate, Potassium, T. Protein and Calcium) in children with

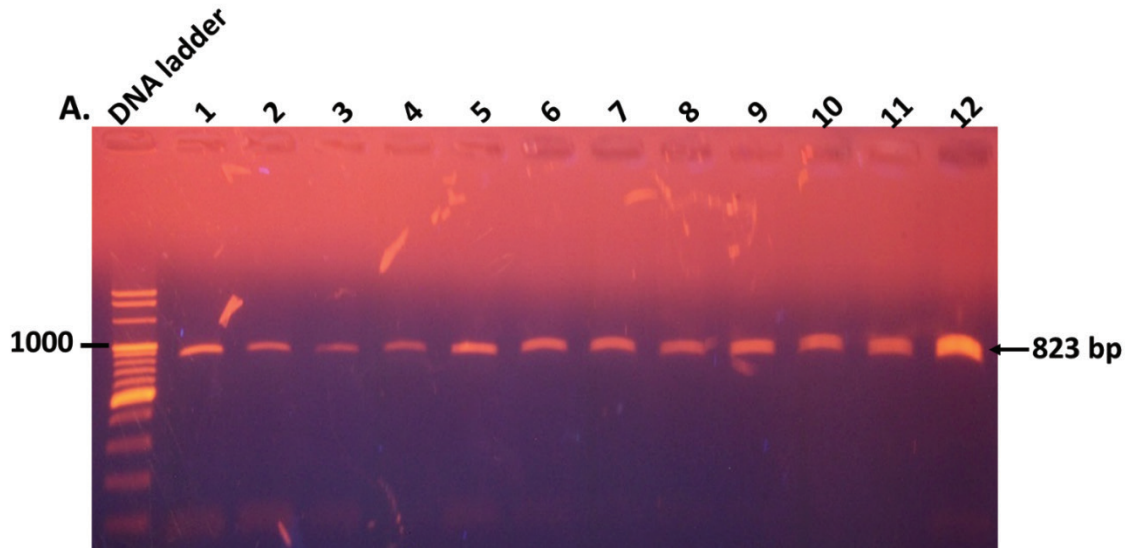
thalassemia.

Table (3): Distribution of the studied parameters values according to the BMI for patients with thalassemia:

Parameters	BMI Groups			P value
	<14.5	14.5-16.5	>16.5	
Vitamin D3 (ng/mL)	15±4.3	15.9±4.6	16.9±2.7	0.390
ALK. Phosphate (U/L)	72.5±26.8	87±33.2	72.6±26	0.478
Potassium (mmol/L)	4.3±0.7	4.6±0.9	4.4±0.6	0.550
T. Protein (g/dL)	7.41±0.7	7.46±0.8	7.54±0.8	0.893
Calcium (mmol/L)	1.8±0.47	2.3±0.58	1.6±0.53	0.048*

* represents a significant difference at $P \leq 0.05$. Data are expressed as Mean±SD.

Regarding BMI groups, the results Calcium revealed a strong significant differences $p<0.05$ among all the studied BMI groups, where p value is 0.048. in addition, the results of other parameters (Vitamin D3, ALK. Phosphate, Potassium and T. Protein) showed no significant differences $p>0.05$ among all the studied BMI groups in children with thalassemia.



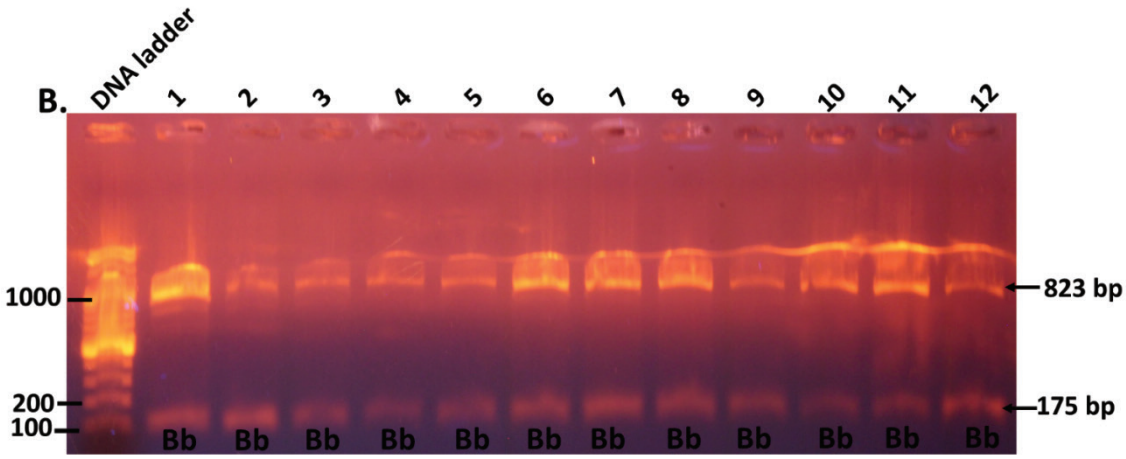


Figure 1: PCR-RFLP analysis of the *VDR* gene polymorphism, using *Bsm*I restriction enzyme. A. Agarose gel of the *VDR* gene amplification, showing predicted product of 823 bp. B. Agarose gel of *Bsm*I digestion, heterozygous mutant (Bb), showing predicted product of 823 bp and 175 bp for the all lane. DNA ladder: molecular weight: 1000–2500 bp.

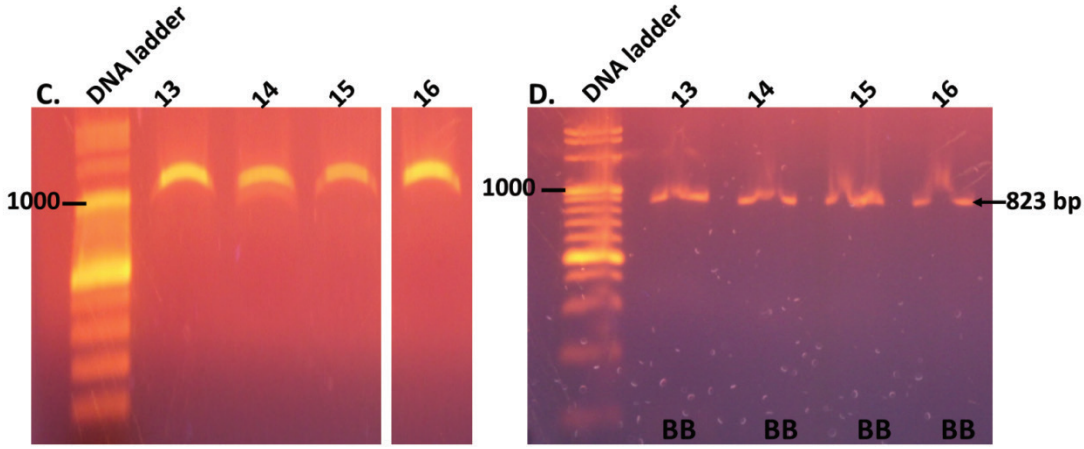


Figure 2: PCR-RFLP analysis of the *VDR* gene polymorphism, using *Bsm*I restriction enzyme. C. Agarose gel of the *VDR* gene amplification, showing predicted product of 823 bp. D. Agarose gel of *Bsm*I digestion, homozygous (BB), showing predicted product of 823 bp for the all lane. DNA ladder: molecular weight: 1000–2500 bp.

Discussion

Until now, there are four restriction fragment length polymorphism (Taq1, ApaI, FokI and BsmI) of VDR which have been studied related with thalassemia disease. Over the years, the interaction between VDR polymorphism and genetic diseases sensibility is still unclear. Several factors are responsible for the conflicting results, like racial variation, different genotyping methods, sample sizes, lifestyle features of people. The human VDR gene be composed of 11exons that jointly with related introns. Exon1A,1B, and 1C make up the 5'- noncoding region, and eight supplemental exons (2-9) encode VDR structural component[14] . BsmI situated in intron VIII. All of them were observed at the 3' end of the VDR in the during 1990s.In spite of, none of the effect the action of the VDR protein expression[15]. Our study demonstrated that a deficiency of hydroxylase vitamin D (25 OH D) is common in thalassemia children. Vitamin D mean level were no significantly at $P \leq 0.05$. Studies of serum vitamin D have shown harmonious with our results[16]. Prevalence of the vitamin D deficiency was found in children thalassemia patients. Level of serum vitamin D were low to normal, according with previous studied[17,18].The 25-OH-vitamin D to be had in these patients should be sufficient for a normal 1- hydroxylation in the kidney to make 1,25-OH-vitamin D. Serum 25-OH vitamin D concentrations are a lot of time higher (ng/ml) than those of the product of the

kidney enzyme, 1,25-OH-vitamin D (pg/ml).

In figure 2-C gene polymorphism BsmI in relation to thalassemia patients showed homozygous (BB) while in fig 1 showed heterozygous (Bb) and absent allele (bb) in this study that was conflicting with[19]. Actually it is not clear whether the BsmI polymorphism has an impact on the expression level or actin of the translated VDR protein[20] , but it is in strong related imbalance with the poly(A) microsatellite located in the 3 untranslated region[21] of the VDR gene, that evidence to effect VDR messenger RNA constancy and VDR translation activity[22] , demonstrated[23] observed a decrease VDR expression while, at the mRNA level[24] found an up- regulation. Likewise, to detect a clinical pertinent phenotype is prospect needful to contain in the analysis other genes implicated in the vitamin D metabolism as the linking the binding protein (GC) and the anabolism and catabolism enzymes[25,26]. Vitamin D has a significant role in multiple myeloma (MM) patients because of the mutual relations with calcium homeostasis bone metabolism[27] closely connected to calcium and phosphorus homeostasis.

Conclusion

Vitamin D is a steroid hormone which plays an important role in calcium homeostasis and skeletal metabolism. The vitamin D receptor (VDR) mediates the action of its linked and results in normal bone mineralization and reconstructing. Subsequently, the gene that encodes for the VDR is take into consideration a nominee gene for osteoporosis. This study revealed that the (BB) of the BsmI polymorphism was related with increased bone loss, whereas in (Bb) had decreased rates of bone loss.

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Conflict of Interests: No.

Ethical Clearance: Take from Thalassemia Centre by approval ethical committee.

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