DOI Number: 10.5958/0974-1283.2019.00207.X

Gene Polymorphism Vitamin D receptor FokI in Thalassemia Children in AL-Muthanna Province

Mohammed Qasim Waheeb[1], Hanaa Ali Aziz[1], Yasir Adil Jabbar Alabdali[1]

[1]Department of Biology, College of Science, Al Muthanna University, AL-Muthanna, Iraq

Abstract

Background and Objective: Vitamin D receptor is considered genetic variants that related with vitamin D status. Our study was recorded polymorphism in vitamin D receptor (VDR) FokI in beta thalassemia children. In this study has been shown polymorphism VDR FokI dominant (FF) ,hybrid(Ff).VDR is includes a beginning codon polymorphism (BCP) that consist of three codon above the course of a second beginning site (ATG).The BCP can be located by the restriction enzyme Fok I,which allele(f)references first of the restriction site ATG is presents, whereas the allele(F) references its missing . Materials and Methods: In this study vitamin D3 levels were evaluated by Enzyme Linked Immunosorbent Assay(Elisa).FokI gene polymorphism were analyzed by using polymerase chain reaction- restriction fragment length polymorphism assay (PCR-RFLP) were estimated in 50 participants children beta thalassemia were distributed to 25 male and 25 female. Results: Patients had significant decrease vitamin D and serum calcium p=0.084 and p=0.751 respectively, alkaline phosphate was recorded p=0.665, potassium= 0.278 and total protein p=0.521. in the male study 84% had VD insufficiency and 16% deficiency ,female study 96%had VD insufficiency and 4% deficiency . Conclusion: Vitamin D3 was higher in female more than male and recorded in age category 9-12 years old .VDR FokI gene polymorphism effect VD status ,genotype FF,Ff appeared in our study and absence genotype ff.

Keywords *: Vitamin D receptor, Gene, polymorphism , FokI ,beta- thalassemia.*

Introduction

Thalassemia disease is a common hereditary disorder has been observed in Mediterranean countries[1] that caused increasing anemia and production erythropoietin .As a result widening of bone marrow may share osteoporosis[2] define is a disease infects bones ,which leads by a decrease bone mineral density(BMD) and a retrogradation in bone tissue structure, with a resultant raised bone frailty and become prone to breakage[3] .BMD is associated with vitamin D receptor (VDR) alleles located on the neighboring area of the 3'- end area[4] add to the 5'- beginning codon area[5]. The nucleotide sequence of VDR gene consists of two potential translation inception(ATG or start) site[6].

A hormone steroid, Vitamin D (VD) is fateful for health skeleton and mineral metabolism. It is play role on osteoblasts and osteoclasts and reaction with other tissues and contribute in keeping a balance state between bone rotation and bone growth[7] as well as the essential agent for normal calicium and phosphate balance[8]. VD deficiency is an increasing extent specific with thalassemia patient, furthermore to the definitive evidence role of VD in the conservation of various organ systems. Biological mechanisms of action VD status need more attention to focus on this particular group of patients[9].

VDR represents as a nuclear transcription factor that regulate manufacturing of protiens contributed in bone mineral homeostasis and cell reproduction[10]. The VDR of gene exists within chromosome 12(q12-q14) and consists of 11 exons that stretch75-100 kb. The 5′ non coding end of the gene contains zones of exons 1a,1b and 1c[11] . The translated VDR protein is encoded with exons 2 to 9. Exons 7 to 9 play a crucial role in linking of VDR to its bind VD[12] ,many single nucleotide polymorphisms(SNP) that could likely amend the expression and energization of VDR that have been most a lot of studies[13].

FokI T > C (restriction site RS 10735810) is the most common SNP studied of this gene and shared in the treatment of many diseases[14,15]. The FokI polymorphism is transmission at the translation beginning site of exon 2 in the 5′ coding zone in the VDR of gene.A lot of VDR mutations and cancellations have been specified in patients with a variety of diseases .Mostly often the genetical distortions produce in a VDR that is incapable to link to 1,25-OH-D[16] . Many polymorphisms have been announced. The most expansively studied VDR polymorphisms consist of FokI,BsmI,TaqI and ApaI[17].

The aim of the present study investigated the frequency of VDR gene polymorphism FokI (rs 10735810) in a cohort of Iraqi populations .In thalassemia children patients analyzed the relationship between VDR and the keeping bone health and metabolism.

Materials and Method

The present study was performed with take whole blood on fifty patients thalassemia pediatric patients with thalassemia disease. They were 25 male and 25 females and was age average raging from 1to 12 years old from period May 2018 to October 2018. The inclusion criteria VD ,alkaline phosphatase, potassium, total protein , calcium and body mass index and exclusion others criteria because not linked in the objective study. All patients were inducted from Thalassemia Unit that located in Feminine and Children hospital in AL-Muthanna Province to take blood dose . Data privacy was protected according to the protocol Helsinki Declaration and was agreed by the medical ethics committee .Written acquainted approval was collected from patients and their parents for each participant.

Parameters measurement

Five milliliters of blood withdrawn under optimal condition by venous blood from every child, 3ml on EDTA and 2ml on DNA extraction ,subsequently VDR gene

polymorphism while the other part was centrifuged with speed five minutes and sera were obtained and stored under -20°C for assay of serum 25 hydroxy vitamin D3 with Enzyme Linked Immunosorbent Assay (ELISA) type direct. Serum vitamin level was measured according to the manufacturer's directives .Presently accepted standards for diagnosed VD values in thalassemia children are:

1- VD deficiency < 10 ng/ml

2- VD insufficiency 10-30 ng/ml

[18]. 3- VD sufficiency 30-100 ng/ml

Others laboratory parameters by using Fujifilm clinical biochemistry(FUJI DRI-CHEM 4000i) investigations , in addition measurement body mass index(BMI) for both genders.

Genotyping

Genomic DNA was isolated by using the phenol chloroform extraction method .Genotypes were revealed by using polymerase chain reaction restriction fragment length polymorphism (rs 10735810) and performed (PCR-RFLP). PCR reaction was performed in 50 µl including Master Mix, 0.5 µM forward, reverse primers, 1-2 µl DNA template and nuclease free water. PCR programme for Phusion normally.The FokI upstream primer is 5′ AGCTGGCCCTGGCACTGACTCTGCTCT-3′and reverse downstream primer is 5′ ATGGAAACACCTTGCTTCTTCTCCCTC-3 ′.The primer are shown in figure 1 A.273 kb fragment FokI in the start codon of the VDR .DNA was extract by using a pivot column kit (Qiagen kit) catalogue number of the kit Cat No./ID: 28104 polymerase chain reaction (PCR) amplification and enzymatic digestion with FokI.

The FokI genotypes were detected by using electrophoresis of the DNA samples in 1.5% agarose gels and were named as followes : FF (not present restriction site) ; ff (present restriction site) ; Ff (heterozygous of the restriction site). The PCR products for the FokI polymorphism was 273pb and the restriction fragments were 198pb and 75pb. All participants were genotyping for FokI gene polymorphism through the implementation of DNA extraction from circumferentially blood white cells whole blood with a genomic DNA extraction kit .

Statistical analysis

The data were analyzed by using (SPSS) version 22 for windows (SPSS, Chicago, IL,USA).The mean of the data was evaluated by one- way ANOVA and t-test. Furthermore, frequency results were analyzed by pearson chi-square and Fishers exact test. Differences were considered statistically significant at $p>0.05$.

Results

This study was carried out on 50 patients infected

with thalassemia , 25 males(50%) and 25 females(50%) their ages ranging between 1 to 12 years old

Distribution of the studied vitamin D3 according to the genders both were 16.5±3.7 in female and 14.5±3.9 in male that was higher in female in table 1.p value was 0.084, likewise biochemical parameters were higher in female except calcium was higher in male recorded 1.92±0.56 and female was the least 1.87±0.5.p value was 0.751.

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)	16.5±3.7	14.5±3.9	<10 Def. 10-30 Ins. 30-100 Suff.	0.084
ALK. Phosphate (U/L)	68.6±26.5	65±31.9	32-111	0.665
Potassium (mmol/L)	4.49±0.7	4.25±0.7	3.5-5.3	0.278
T. Protein (g/dL)	7.52±0.75	7.38±0.69	6.7-8.3	0.521
Calcium (mmol/L)	1.87±0.5	1.92±0.56	1.9-2.1	0.751

* 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).

In table 2 distribution biochemical analysis acoording to the age in thalassemia patients VD was higher in ages 9-12 years old and p value was0.605,while biochemical parameters were values variable.

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)	15.3±3.2	15.2±4.2	16.8±5	0.605
ALK. Phosphate (U/L)	65.6±30	73.5±28	53.7±21	0.251
Potassium (mmol/L)	4.7±0.7	3.9±0.5	4.2±0.7	0.002*
T. Protein (g/dL)	7.38±0.5	7.35±0.8	7.91±0.6	0.142
Calcium (mmol/L)	2±0.4	1.8±0.5	1.6±0.5	0.142

* 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, T. Protein and Calcium) in children with thalassemia, except of Potassium that showed a significant difference p<0.05 (p value is 0.002).

In table 3 distribution parameters according to body mass index BMI , VD was the higher in <14.5 as well in total protein, while BMI 14.5-16.5 was higher in alkaline phosphate, potassium and calcium 68±32 ,4.7±0.78 and 1.9±0.55 respectively.

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

Parameters	BMI Groups			P value
	<14.5	14.5-16.5	>16.5	
Vitamin D3 (ng/mL)	15.9±3.7	15.5±2.9	14±5.4	0.511
ALK. Phosphate (U/L)	66.9±27	68±32	65±29	0.979
Potassium (mmol/L)	4.2±0.73	4.7±0.78	4.3±0.88	0.276
T. Protein (g/dL)	7.5±0.8	7.2±0.4	7.3±0.6	0.531
Calcium (mmol/L)	1.8±0.56	1.9±0.55	1.9±0.4	0.863

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

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

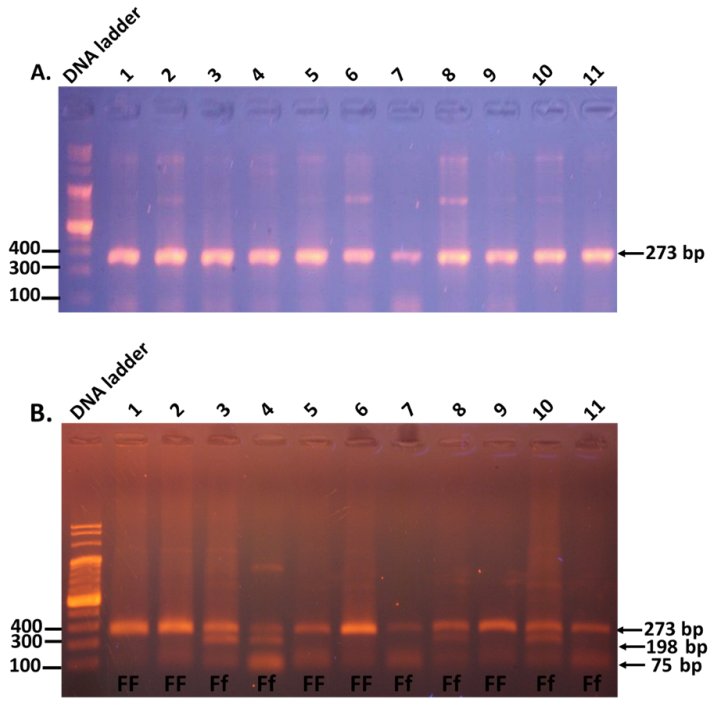

Figure (1): PCR-RFLP analysis of the *VDR* gene polymorphism, using *Fok*I restriction enzyme. A. Agarose gel of the *VDR* gene amplification, showing predicted product of 273 bp. B. Agarose gel of *Fok*I digestion, homozygous wild type (FF), showing predicted product of 273 bp for lane (1,2,5,6 and 9) and heterozygous mutant (Ff), showing predicted product of 273 bp, 198 bp and 75 bp for lane (3,4,7,8,10 and 11). DNA ladder: molecular weight: 1000–2500 bp.

Discussion

The permanence of patients with thalassemia major has gradually improved with advances in therapy ; however although bone diseases stay continual complications .Sufficient revolving levels of vitamin D are fundamental for optimal skeletal health and reducing breaking risk[19] .Most of the biological activities of vitamin D are mediated by an intracellular receptor VDR in that many single nucleotide gene polymorphism have been specified.Vitamin D deficiency is growingly specified among thalassemia patients[20]. Consequently, in an effort to increase our understanding of the interaction between vitamin D status and the genetic polymorphisms of one parameter of the VDR(FokI) in case study male and female carried out on a cohort of Iraqi beta thalassemia patients.

Table(4) Genotype FokI Frequency of patients according to the gender:

FokI gene	Male(no)	Percentage%	Female(no)	Percentage%	Total(no)	Percentage%
FF	11	44	10	0.4	21	42
Ff	14	56	15	0.6	29	58
ff	0	0	0	0	0	0

In this study genotype FokI Frequency was revealed 42% of the patients were homozygous for F allele , 58% were heterozygous for Ff allele and absence for f allele in both genders, these results in table 4 was appeared contradictory with [8,25. In table (1) Low serum calcium levels with rise level of serum alkaline phosphate was noted in our study that compatible with the researcher[21] have informed hypocalcemia as a late complication of iron overload in beta thalassemia. The researcher[22] revealed VD deficiency, osteomalacia and rickets in thalassemia patients as a result of immersed 25 hydroxylation of vitamin D because of iron overload and following liver disorder.Other mechanism leading to deranged calcium , phosphate and VD homeostasis comprise reduced intake,weaken absorption and decreased synthesis of vitamin D[23].

Vitamin D influences on bone mineralization directly by the genomic mechanism through VDR and indirectly via its energizing of intestinal calcium and phosphorus absorption[24]. VD deficiency in thalassemia patients is caused by decreased intake, lower sun exposure , imperfect skin synthesis linked with jaundice or defective 25 hydroxylation of VD in the liver because hepatic siderosis[19]. In the current study the results in table 1 showed no significant differences in the serum vitamin D levels .These fundings were not sudden that compatible with[25].

Conclusions

FokI genotypic Ff of VDR can be considered as a risk factor as whom patients suffering from bone disease and thus have amounts appropriate vitamin D and calcium that contribution in bones developing and raise bone mass which leads to prevent osteoporosis in thalassemia patients. VDR genotype and biochemical levels of calcium and VD to detect out who is more influence to osteoporosis.

Acknowledgement: The authors wish to thank the management of thalassemia centre in AL-Children Teaching Hospita in AL-Muthanna for presenting facilities in accomplishing this work.

Conflict of Interest: Nil.

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

Funding: Self funding.

References

[1] Gaudio A, Morabito N, Xourafa A, Macri I, Meo A, Morgante S, Trifiletti A, Lasco A, Frisina N. Bisphosphonates in the treatment of thalassemia-associated osteoporosis. Journal of endocrinological investigation. 2008 Feb 1;31(2):181-4.

[2] Mahachoklertwattana P, Pootrakul P, Chuansumrit

A, Choubtum L, Sriphrapradang A, Sirisriro R, Rajatanavin R. Association between bone mineral density and erythropoiesis in Thai children and adolescents with thalassemia syndromes. Journal of bone and mineral metabolism. 2006 Mar 1;24(2):146-52.

[3] Peck WA. Consensus development conference: diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med. 1993;94(6):646-50.

[4] Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Nature. 1994 Jan;367(6460):284.

[5] Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal mexican-American women. Journal of Bone and Mineral Research. 1996 Dec;11(12):1850-5.

[6] Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J, O'Malley BW. Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proceedings of the National Academy of Sciences. 1988 May 1;85(10):3294-8.

[7] Hou YC, Wu CC, Liao MT, Shyu JF, Hung CF, Yen TH, Lu CL, Lu KC. Role of nutritional vitamin D in osteoporosis treatment. Clinica Chimica Acta. 2018 May 18.

[8] Elhoseiny SM, Morgan DS, Rabie AM, Bishay ST. Vitamin D receptor (VDR) gene polymorphisms (FokI, BsmI) and their relation to vitamin D status in pediatrics βeta thalassemia major. Indian Journal of Hematology and Blood Transfusion. 2016 Jun 1;32(2):228-38.

[9] Dimitriadou M, Christoforidis A, Fidani L, Economou M, Vlachaki E, Athanassiou-Metaxa M, Katzos G. A 2-year prospective densitometric study on the influence of Fok-I gene polymorphism in young patients with thalassaemia major. Osteoporosis International. 2016 Feb 1;27(2):781-8.

[10] Pollock N, Dijkstra P, Chakraverty R, Hamilton B. Low 25 (OH) vitamin D concentrations in international UK track and field athletes. South African Journal of Sports Medicine. 2012;24(2).

[11] Al-Eisa AA, Haider MZ. Vitamin D receptor gene TaqI and ApaI polymorphisms and steroid responsiveness in childhood idiopathic nephrotic syndrome. International journal of nephrology and renovascular disease. 2016;9:187.

[12] Zhou H, Xu C, Gu M. Vitamin D receptor (VDR) gene polymorphisms and Graves' disease: a meta-analysis. Clinical endocrinology. 2009 Jun;70(6):938-45.

[13] Uitterlinden AG, Fang Y, van Meurs JB, Pols HA, van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004 Sep 1;338(2):143-56.

[14] Shaker O, Nassar Y, Ayoub S, Elrazki M, Zahra A. Impact of FokI (rs10735810) and BsmI (rs1544410) on Treatment of Chronic HCV Patients With Genotype 4. Journal of clinical laboratory analysis. 2016 Nov;30(6):1021-7.

[15] Meamar, R., Javadirad, S.M., Chitsaz, N., Ghahfarokhi, M.A., Kazemi, M. and Ostadsharif, M. Vitamin D receptor gene variants in Parkinson's disease patients. *Egyptian Journal of Medical Human Genetics*. 2017;18(3), pp.225-230.

[16] Malloy PJ, Pike JW, Feldman D. The vitamin D receptor and the syndrome of hereditary 1, 25-dihydroxyvitamin D-resistant rickets. Endocrine reviews. 1999 Apr 1;20(2):156-88.

[17] Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factor alleles to normal physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin. Proceedings of the National Academy of Sciences. 1992 Aug 1;89(15):6665-9.

[18] Bowden SA, Robinson RF, Carr R, Mahan JD. Prevalence of vitamin D deficiency and insufficiency in children with osteopenia or osteoporosis referred to a pediatric metabolic bone clinic. Pediatrics. 2008 Jun 1;121(6):e1585-90.

[19] Soliman A, De Sanctis V, Yassin M. Vitamin D status in thalassemia major: an update. Mediterranean journal of hematology and infectious diseases. 2013;5(1).

[20] Dimitriadou M, Christoforidis A, Fidani L, Economou M, Perifanis V, Tsatra I, Katzos G, Athanassiou-Metaxa M. Fok-I gene

polymorphism of vitamin D receptor in patients with beta-thalassemia major and its effect on vitamin D status. Hematology. 2011 Jan 1;16(1):54-8.

[21] Tantawy AA, El MK, Moustafa T, Elsedfy HH. Bone mineral density and calcium metabolism in adolescents with beta-thalassemia major. Pediatric endocrinology reviews: PER. 2008 Oct;6:132-5.

[22] Chatterton BE, Thomas CM, Schultz CG. Liver density measured by DEXA correlates with serum ferritin in patients with β-thalassemia major. Journal of Clinical Densitometry. 2003 Sep 1;6(3):283-8.

[23] Pollak RD, Rachmilewitz E, Blumenfeld A, Idelson M, Goldfarb AW. Bone mineral metabolism in adults with β-thalassaemia major and intermedia. British journal of haematology. 2000 Dec;111(3):902-7.

[24] Bikle DD. Vitamin D and bone. Current osteoporosis reports. 2012 Jun 1;10(2):151-9.

[25] Abbassy HA, Elwafa RA, Omar OM. Bone mineral density and vitamin D receptor genetic variants in egyptian children with beta thalassemia major on vitamin D supplementation. Mediterranean journal of hematology and infectious diseases. 2019;11(1).