



Original Research Article

Evaluation of Vitamin D binding protein (DBP) gene polymorphism in Vitamin D deficient patients attending a tertiary care hospital—a pilot study

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ABSTRACT

Introduction : Polymorphism in Vitamin D binding protein (DBP) has been implicated as one of the causes for Vitamin D deficiency. However there is paucity of data regarding the effect of genetic polymorphism in DBP in Vitamin D deficient patients in our population. This pilot study was undertaken to analyze the common genetic polymorphism in vitamin DBP in these population and its effect on vitamin D supplementation.

Materials and Methods : 80 vitamin D deficient subjects were selected by convenient sampling. Genetic analysis for DBP (GC) gene polymorphism (rs7041 + rs4588) was carried out in all these individuals after informed consent and correlated with the vitamin D levels post supplementation.

Results : Six combinations of genotype were obtained (rs7041 + rs4588): TT+CA, TG+CC, TT+CA, TT+CC, TT+AA, GG+CC. A third of all individuals (33%) were found to have the TG+CA genotype, followed by about 26 % of individuals having the GG+CC genotype. TT+CA group was found to have 13% individuals and a tenth of all individuals belonged to each of the groups with TG+CC and TT+AA genotypes. Least proportion of individuals was found to have the TT+CA genotype (6%). There was no significant difference in the vitamin D levels with individual polymorphism (p value <0.01). However the combined genotype had an effect, with homozygotes for both such as TT-CC, TT-AA showing least response and heterozygotes such TG-CA and CG-CC showing better response

Discussion and Conclusion: In this study, the individual SNP (rs7041 and rs4588) did not seem to significantly influence the response to vitamin D supplementation. However the combined genotype seemed to influence the proportion of patients showing improvement after supplementation. The homozygotes for both such as TT-CC, TT-AA showing least response and heterozygotes such TG-CA and CG-CC showing better response.

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1. Introduction

Vitamin D deficiency is in pandemic proportion now. To prevent or combat this deficiency/insufficiency, vitamin D supplementation is an easy, effective and cost-effective strategy. However, in response to a given dose of vitamin D supplementation, the increase in 25(OH)D concentration has been reported to differ between individuals.¹⁻⁵ Because

of the wide inter-individual variation,⁶ the one-size-fits-all approach does not work with vitamin D supplementation, and it is imperative that clinicians take those factors affecting the response to vitamin D supplements into account and individualize their strategy. Response to vitamin D supplementation can be explained by several environmental and demographic factors. Recently, Zittermann et al^{7,8} published a systematic review concerning the importance of body weight for the dose-response relationship with circulating 25(OH)D. The authors demonstrated that 34.5%

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of variation in circulating 25(OH)D was explained by body weight, followed by type of supplement (D2 or D3) (9.8%), age (3.7%), calcium intake (2.4%) and basal 25(OH)D concentrations (1.9%), leaving approximately 50% of the variations to unknown factors. Some of the factors evaluated are the role of genetic factors such as vitamin D receptor (VDR) vitamin D binding protein (DBP) genotypes etc. The relationship between DBP genotype and levels of vitamin D in circulation has been examined in several studies and some studies have shown that the Vitamin D levels vary with specific DBP genotypes.

In response to a given dose of vitamin D supplement, the increase in 25(OH)D concentration has been reported to differ between individuals. However all these studies have been carried out in African and Americans. Since ethnicity is known to affect gene polymorphism, how these genetic variations affect vitamin D levels in our population needs to be explored. Although vitamin D binding protein (DBP) levels have been implicated as one of the causes, there is paucity of data regarding the effect of genetic polymorphism in DBP on the response to vitamin D supplementation in our population. Hence this study was undertaken to test the hypothesis that if polymorphism in DBP gene affects circulating concentration of vitamin D then these polymorphisms may also influence the response to supplementation in vitamin D deficient individuals. The main aim of the study was to evaluate the frequency distribution of DBP polymorphism in patients whose vitamin D levels do not improve on vitamin D supplementation and to compare the same with subjects whose vitamin D levels show improvement after supplementation. If the study shows an association with DBP polymorphism, it would further help explain the differences in response to vitamin D supplementation in our population.

2. Materials and Methods

This study was conducted at St. John's Medical College Hospital. Subjects were selected from those attending endocrine OPD by convenient sampling. 80 Subjects who were found to be vitamin D deficient (less than 20ng /mL) during their checkup and were put on standard supplementation with oral vitamin D were recruited after informed consent. At the end of treatment, Vitamin D was estimated as part of standard follow up. Subjects who are found to be defaulters with medication on follow up were excluded from the study. The subjects were then grouped as follows: Group1 (n=20): Subjects whose vitamin D levels continued to be less than 30 ng /mL after treatment and Group 2(n=60): Subjects whose vitamin D levels are >30mg/dL after treatment. Vitamin D (25 hydroxy) estimation was carried out on fully automated Chemiluminescence method on Centaur from Siemens. DBP was estimated by ELISA method. Genetic analysis for

DBP polymorphism was carried out in both these groups as follows.

DNA extraction: DNA was extracted from leukocytes by using the standard Phenol chloroform methods. Purity of DNA was checked by running the DNA samples on agarosegel and using optical density measurements on nanodrop. Single nucleotide polymorphism in GC gene are identified from NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP>). The loci selected for this study include: rs7041 and rs 4588. The polymorphism rs 7041 results in T to G transversion (Asp to Glu) and rs4588 results in C To A transversion (Thr to lys)

The relevant segment of the DNA was amplified by using Polymerase chain reaction (PCR)The digested samples were run on agarose gel. After staining with ethidium bromide the bands were visualized under the ultraviolet (UV) transilluminator. They were then sent for DNA sequencing to identify the polymorphism.

3. Results

This study was conducted on 80 vitamin D deficient subjects who were on vitamin D supplementation. Based on Vitamin D levels after treatment the subjects were grouped into two groups of subjects, group one whose vitamin D levels continue to be less than 30 ng /mL after treatment (20/30 : 62.5%) and Group 2, subjects whose vitamin D levels are >30mg/dL after treatment. (12/30: 37.5%). Table 2 shows the frequency distribution of the two polymorphism in the two groups of patients. Vitamin D deficient adults were genotyped for two non-synonymous single nucleotide polymorphisms (SNPs), D432E (rs 7041) and T436K (rs 4588) in the VDBP (GC) gene. Six combinations of genotype were obtained (rs7041 + rs4588): TT+CA, TG+CC, TT+CA, TT+CC, TT+AA, GG+CC. A third of all individuals (33%) were found to have the TG+CA genotype, followed by about 26 % of individuals having the GG+CC genotype. TT+CA group was found to have 13% individuals and a tenth of all individuals belonged to each of the groups with TG+CC and TT+AA genotypes. Least proportion of individuals was found to have the TT+CA genotype (6%). As mentioned in Table 3, there was no significant effect of individual SNP on the response to vitamin D supplementation. However the analysis of effect of combined Genotype on vitamin D response in the study groups the homozygotes for both such as TT-CC, TT-AA showing least response and heterozygotes such TG-CA and CG-CC showing better response as shown.Figures 1 and 2

4. Discussion

The relationship between vitamin D receptor (VDR) and vitamin D binding protein (DBP) genotype and levels of 25(OH)D in circulation has been examined in several studies,^{9–11} though very few studies have examined the

Table 1: Conditions for identifying of the selected GC polymorphism

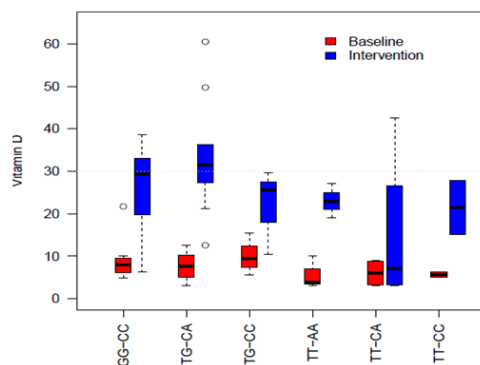
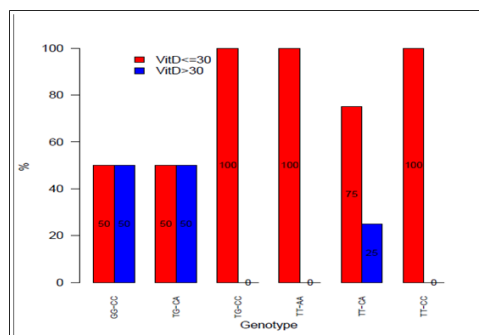
rs no	Primers for PCR amplification (5' – 3')	Annealing temp(°C)	Restriction enzyme
rs7041 and rs 4588	F:GGAGGTGAGTTTATGGAACAGC R: GGCATTAAGCTGGTATGAGGTC	66.3	Hae111

Table 2: Frequency distribution of individual SNP in the study groups

Genotype	Vitamin D<30 ng/mL	Vitamin D>30 ng/mL
rs7041		
TT	8(40%)	1(10%)
GG	5(25%)	4(40%)
TG	7(35%)	5(50%)
rs 4558		
CC	9 (45%)	4(40%)
AA	3(15%)	0(0%)
CA	8(40%)	6(60%)

Table 3: Effect of Individual polymorphism on Vitamin D response

SNP	Genotype	vitamin D response Mean difference (SD)	P value
rs.7041	GG	16.96 (12.32)	
	TT	13.27 (11.79)	
	T/G	22.63 (14.41)	0.263
Rs 4558	AA	17.39 (5.95)	
	CC	15.57 (10.56)	
	C/A	21.04 (16.49)	0.579

**Fig. 1:** Effect of combined Genotype on vitamin D response in the study groups**Fig. 2:** Effect of combined Genotype on vitamin D response in the study groups

effect of DBP genotype on 25(OH)D response to vitamin D supplementation.^{12,13} In an open-label randomized intervention trial, Fu et al. examined the contribution of DBP D432E and T436K SNPs to variation in 25(OH)D response to either 600 IU/day or 4000 IU/day vitamin D for one year¹³. The presence of 436 K allele was associated with lower 25(OH)D concentrations at baseline. However, the percentage increase in 25(OH)D concentration from baseline in both groups was in opposite directions; those with KK genotype had the largest increase followed by TK and then TT genotypes. In a multiple linear regression model, dose and 436 K, but not 432 E contributed significantly to overall variance, 22% ($p < 0.0001$) and 8.5% ($p < 0.001$), respectively. It should be noted that baseline 25(OH)D levels were not included in this model. The observed pattern could be due to the lower baseline 25(OH)D concentrations in carriers of 436 K allele. Furthermore, the impact of DBP genotype on response to vitamin D supplementation appears to be partly vitamin D-type specific. Serum-25(OH)D response to supplementation with vitamin D was examined in 39 healthy adults given 400 IU/day vitamin D3 or vitamin D2. The percentage increase in total 25(OH)D and 25(OH)D3 following supplementation with vitamin D3, but not with vitamin D2, was significantly affected by rs4588 genotype. Compared to CA and AA alleles, participants homozygous for GC2 allele (CC) had a significantly larger increase in 25(OH)D and 25(OH)D3

In our study, the individual SNP (rs7041 and rs4588) did not seem to significantly influence the response to vitamin D supplementation. However the combined genotype seemed to influence the proportion of patients showing improvement after supplementation. The homozygotes for both such as TT-CC, TT-AA showing least response and heterozygotes such TG-CA and CG-CC showing better response. Although the exact reasons for this not clear, DBP SNPs such as rs7041 and 4588 generate functionally different proteins due to glycosylations. This may affect the affinity of the protein to vitamin D and also alter the half life and affect the levels of vitamin D in the blood. This may be one of the reasons responsible for the varied response to vitamin D supplementation in different individuals. However this needs to be compared with the general population who are vitamin D sufficient to draw association between the presence of this polymorphism and vitamin D deficiency

4.1. Limitation

The Small sample size and lack of comparison of the polymorphism in healthy vitamin D sufficient individuals are some of the limitations of the study. The study also did not rule out malabsorption syndrome in these patients.

5. Conclusion

In this study, the individual SNP (rs7041 and rs4588) did not seem to significantly influence the response to vitamin D supplementation. However the combined genotype seemed to influence the proportion of patients showing improvement after supplementation. The homozygotes for both such as TT-CC, TT-AA showing least response and heterozygotes such TG-CA and CG-CC showing better response.

5.1. Acknowledgement

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6. Source of funding

None.

7. Conflict of interest

None.

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