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Association of *Vitamin D Binding Protein BP rs2282679 Gene* Polymorphism and Serum Levels of Vitamin D in Patients with Vitiligo

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Abstract

For vitamin D to have an impact, the vitamin D receptor (VDR) must be expressed and activated in the nucleus. The VDR has several known genetic variants. Biological impacts can be caused by changes in DNA sequences known as "polymorphisms" that are common in the population. Vitiligo is a disease that causes loss of skin color in patches, and it is a chronic (long-lasting) autoimmune disorder loss of color. The skin turns a milky white color because the skin cells that create pigment, called melanocytes, are attacked, and destroyed. For comparison, the study included 80 samples total; 40 of them came from vitiligo sufferers (male and female) and 40 from healthy individuals. Under aseptic conditions, five milliliters of blood were collected by puncturing a vein with disposable syringes. The 2 ml of each sample was placed in an EDTA tube and kept at -20°C until the polymerase chain reaction amplification and detection of the vitamin D binding protein BP (rs2282679) gene (ARMS-PCR) method could be performed to avoid repetitive thawing and freezing. The remaining 3 ml were moved to a sterile gel tube, left to clot at room temperature, and then spun at 2500 rpm for 10 minutes to prevent the Vitamin D ELISA Kit (Mabtech USA) from undergoing repeated thawing and freezing. The serum that had been isolated was placed in Eppendorf tubes and quickly refrigerated at -20°C until it could be used again. With p=0.644 for gender and p=0.813 for age, the results did not show any significant differences between the groups. The current study showed (37.5%) of vitiligo patients have a positive family history, 25(OH) D level decreased in inpatients compared to control, and showed that the most abundant gene is AA in vitiligo patients compared to controls, and this enhanced decreased result of 25(OH) D. Also suggested an association between vitamin D binding protein BP rs2282679 polymorphism and the levels of vitamin D in vitiligo patient.

Keywords: Vitiligo, polymorphism, vitamin D, genotype, cholecalciferol

INTRODUCTION

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Skin pigmentation caused by the death of melanin cells in the cutaneous epidermis is a hallmark of vitiligo, an inherited pigmentary condition. Based on surveys conducted across large populations globally, the prevalence of this disease has been estimated to be 1–2%. Both sexes are equally affected by this disease, and they do not discriminate based on age or race [1]. Vitiligo prevalence estimates vary greatly, ranging from 0.004% to 2.28% globally [2]. Vitamin D is a substance found in food, and the body can also be manufactured. It is considered a hormone and a type of fat-soluble vitamin (A, D, E, and K). It absorbs and preserves calcium and phosphorus, which are considered important and necessary elements in

building bones [3]. Studies have shown that it reduces bone cancer infection and inflammation. Vitamin D formation occurs through the skin with the formation of cholecalciferol (vitamin D3), which occurs by exposing the skin to ultraviolet rays. The liver converts cholecalciferol to calcidiol, and vitamin D is considered its basic form [4]. Subsequently, the kidneys convert calcidiol into calcitdiol, and vitamin D is considered in its active form, which ultimately binds to endogenous D receptors [4, 5]. Its main function is to maintain normal levels of calcium and phosphorus in the blood by increasing the effectiveness of the small intestine in absorbing these two minerals from food [6]. It also regulates parathyroid hormone levels and enhances renal calcium absorption. Calcitriol interacts with vitamin D receptors in the small intestine, thereby stimulating the absorption of calcium and phosphorus [7]. Calcitriol also binds to the vitamin D receptor in bone cells to stimulate (RANK) receptor activator, which in turn interacts with (NF-κB) in immature cells, and thus they become mature bone cells resorbing, as they work to remove calcium and phosphorus from the bones and thus maintain their levels in the bloodstream [8]. A circulating protein called vitamin D-binding protein is produced by the liver and encoded by the group-specific component (GC) gene. It binds to and carries 25(OH)D throughout the bloodstream, which affects the bioavailability of active 25(OH)D [9]. With almost 200 single-nucleotide polymorphisms (SNPs), the VDR gene on chromosome 12q13.11 is particularly unique. *rs2228570*, *rs1544410*, and *rs731236* are among the most studied. Possible variants of the VDR protein with different transcriptional capacities could arise from the *rs2228570* polymorphism, which is positioned at the start codon of exon 2. These variants can be long (allele-f) or short (allele-F) variants. This gene's 3'-untranslated region (3'-UTR) contains *rs1544410* (in intron 8) and *rs731236* (in exon 9). Although changes in the VDR locus or nearby genes may affect the expression and function of proteins, the clinical significance of these differences remains unclear [10]. In this study, we investigated how vitamin D serum levels in vitiligo patients correlate with the *rs2282679* polymorphism in the vitamin D-binding protein BP (shown in Appendix).

MATERIALS AND METHODS

Between March and August 2023, 40 patients (14 men and 26 females), ranging in age from 25 to 40 years were investigated. A control group of 40 individuals (16 men and twenty-four women, was also included in the study. These patients were clinically deemed healthy and had no history of systemic diseases. Individuals who had malignant tumors, kidney illness, high blood pressure, diabetes, infectious disease, positive HCV antibodies, or any other rheumatologic condition were not included in the study. Five milliliters of blood were drawn aseptically by inserting a disposable syringe into a vein. For the ARMS-PCR method, which involves amplification and detection of the VDBP *(rs2282679) gene* by polymerase chain reaction, 2 ml of each sample was placed into an EDTA tube and kept at -20°C until needed. For the Vitamin D ELISA Kit (Mabtech USA) test, which requires the sample to be frozen and thawed multiple times, the remaining 3 ml was transferred to a sterile gel tube, allowed to clot at room temperature, and spun at 2500 rpm for 10 min. The isolated serum was placed in Eppendorf tubes and quickly refrigerated at -20°C until further use. The study design is illustrated in Figure 1. The research adhered to the ethical rules of the Al-Diwaniyah Teaching Hospital, and all patients consented verbally.

RESULTS

Characteristics of the Study Population

The present study enrolled 40 vitiligo patients and 40 control subjects. The demographic characteristics of the patients and controls are shown in Table 1. According to age, the mean age of patients with vitiligo was 33.23 ± 8.80 years old and that of control subjects was 32.32 ± 6.34 years old and there was no significant difference between both groups (P = 0.813). Regarding gender, overall, 30 (37.5%) male and 50 (62.5%) female were included. Patients with vitiligo included 14 (35.0 %) cases were male gender and 26 (65.0 %) cases were female, while control subjects included 16 (40.0 %) cases were male gender and 24 (60.0%) cases were female and there was significant difference in the frequency distribution of patients and control subjects according to sex (P = 0.644). Regarding BMI, the present results show a highly significant increase of BMI in patients with vitiligo in comparison with healthy control, 28.21 ± 3.21 versus 21.34 ± 1.39 respectively, ($p < 0.001$).

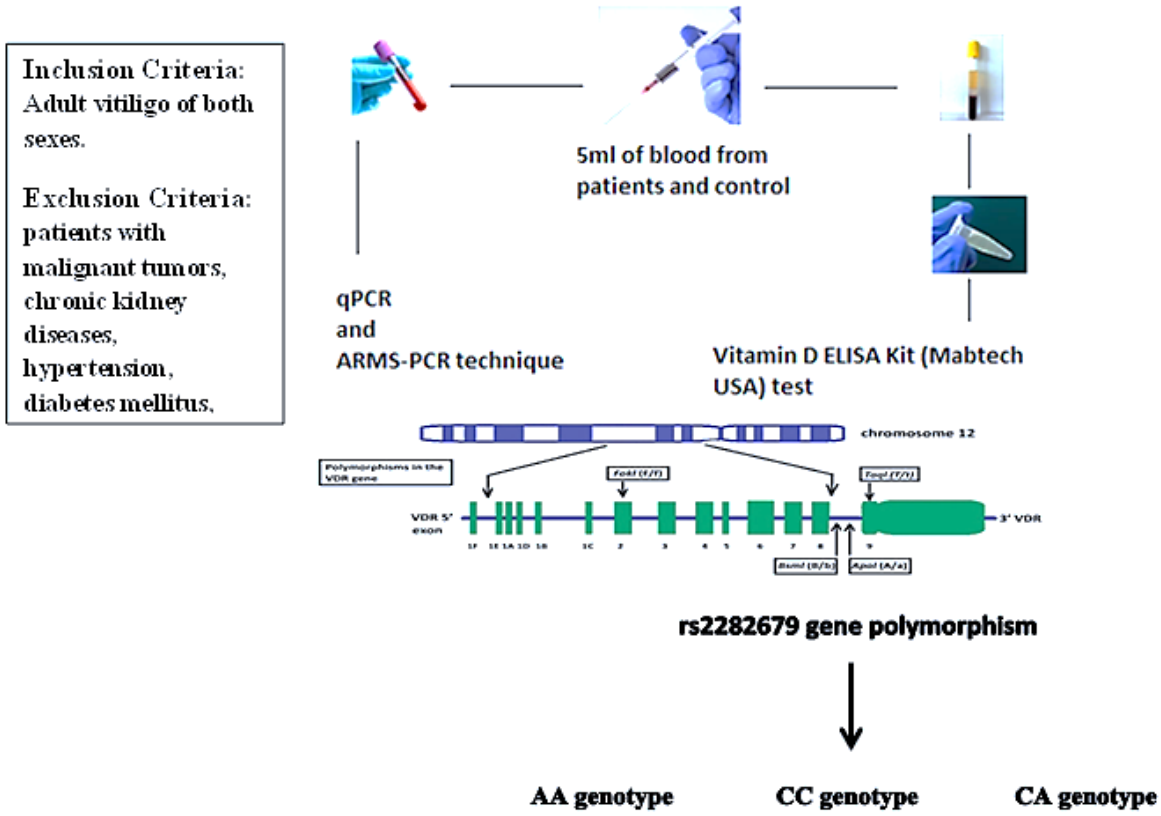

Figure 1. Study design of this study.

Table 1. Characteristics of patients with Vitiligo and healthy subjects.

Characteristics	Vitiligo patient (n=40)	Healthy (n=40)	*P*
Age (years)	33.23 ± 8.80	32.32 ± 6.34	0.813
Gender			
Male	14 (35.0 %)	16 (40.0 %)	0.644
Female	26 (65.0 %)	24 (60.0%)	
BMI	28.21 ± 3.21	21.34 ± 1.39	< 0.001
25(OH) D level (ng/mL)	14.45 ± 4.19	38.11 ± 8.88	< 0.001

Family History of Hashimoto Thyroiditis

Family history is an important contributory factor to vitiligo. This study showed that 15 (37.5%) of vitiligo patients have a positive family history, and 25 (62.5%) of vitiligo patients have a negative family history Figure 2.

Measurements of Vitamin D Parameters

Vitamin D concentrations in patients with vitiligo were significantly lower than in healthy control subjects (14.45 ± 4.19 ng/mL and 38.11 ± 8.88 ng/mL, respectively, P< 0.001), Figure 3.

Detection of Genes Polymorphisms

Vitamin D-binding proteins and their distribution BP rs2282679 gene polymorphism were discovered using ARMS-PCR technology. At this locus, there are three genotypes, AA, AC, and CC. The dominant homozygote genotype yielded an amplified product of 205 bp, indicating that only the C allele was present. The mutant homozygote genotype showed an exclusive A-allele amplification at a product size of 205 kb. The A-allele was amplified at 205 bp and the C allele at 125 bp in the heterozygous genotype,

as shown in Figure 4, Table 2. The Hardy-Weinberg equilibrium was maintained in the genotype distribution across all study groups.

Compared to controls, the frequency of AA genotypes was much higher in vitiligo patients (P=0.005). Furthermore, at rs2282679, vitiligo patients were more likely to possess the A-allele of DBP compared to healthy individuals (P=0.001) (Table 3). After a thorough comparison between DBP rs2282679 genotypes and vitamin D levels, the current results showed that patients with the mutant AA genotype had higher mean serum vitamin D hormone levels than the other groups, but this difference was not statistically significant (P= 0.231) (Table 4).

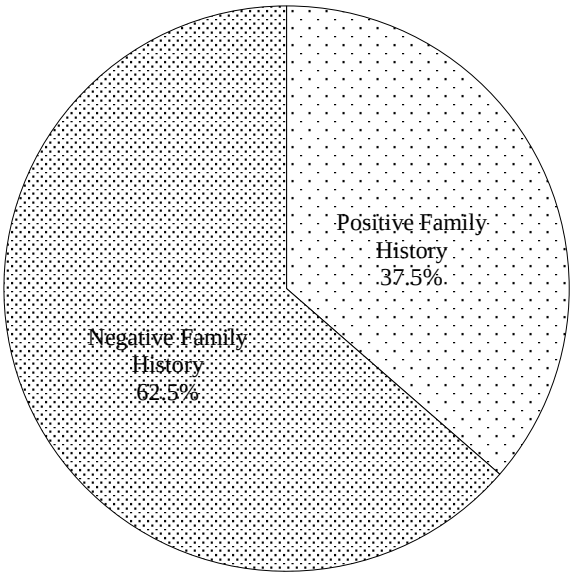

Figure 2. Distribution of patients with Vitiligo according to family history.

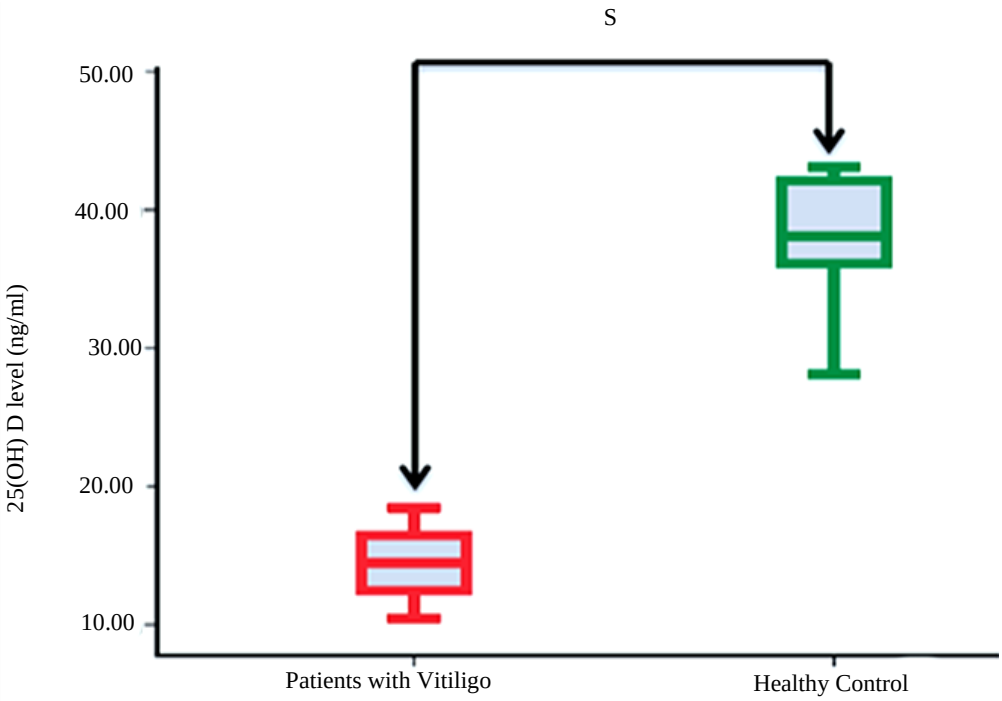

Figure 3. Serum level of Vitamin D in patients with Vitiligo and healthy control subjects.
S: statistically significant P < 0.05.

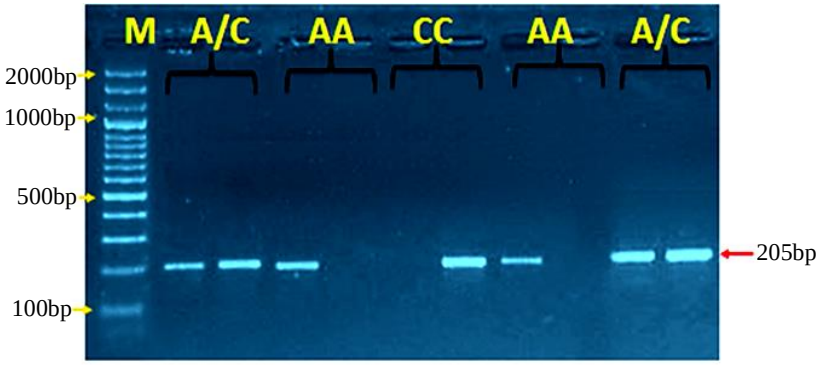

Figure 4. Genomic study of the vitamin D-binding protein (VDBP) (rs2282679) gene polymorphism using ARMS-PCR product analysis on an agarose gel electrophoresis. M stands for marker, which ranges from 2000 to 100 bases. Homozygotes of the wild type (AA) were only seen in the A-allele, those of the mutant type (CC) were only seen in the C allele, and those of the (A/C) heterozygote were seen in both the A and C alleles. The presence of either the A or C allele was detected at a product size of 205 kb.

Table 2. The ARMS-PCR Primers with their sequence and amplicon size.

Primer	**Sequence (5'-3')**	**Product size**
Wild type Forward Primer	TCTGTCTCTTAATTATCTCACGA	205 bp
Mutant Forward Primer	TCTGTCTCTTAATTATCTCACGC	
Common Reverse Primer	CACAGCCTCAGTTCCTATGT	

Table 3. *DBP rs2282679* POLY genotype frequency in patients and healthy control.

Mode	***DBP rs2282679***	**Patients *n* = 40**	**control *n* = 40**	***P***	**OR**	**95% CI**
Co-dominant	AA	12 (30.0%)	3 (7.5 %)	0.005	6.22	1.53–29.9
	C/A	10 (25.0%)	9 (22.5 %)	0.317	1.72	0.58–5.07
	CC	18 (45.0 %)	28 (70.0 %)	*Reference*		
Dominant	AA+ C/A	22 (55.0 %)	12 (30.0 %)	0.023 ¥ S	*Reference*	
	CC	18 (45.0 %)	28 (70.0 %)		0.350	0.139–0.88
Recessive	AA	12 (30.0%)	3 (7.5 %)	0.009 ¥ S	5.28	1.36–20.5
	C/A +CC	28 (70.0%)	37 (92.5%)		*Reference*	
Alleles	A	34 (42.5%)	15 (18.8%)	0.001 ¥ S	3.2	1.56-5.65
	C	46 (57.5%)	65 (81.2%)		*Reference*	

Table 4. The association between ARMS-PCR finding and vitamin D levels in patients with vitiligo.

Hormones	**ARMS-PCR finding**			
	***CC genotype* n =18**	***CA genotype* n = 10**	***AA genotype* n =12**	***P value***
Vitamin D levels				
Mean ± SD	**16.34 ± 4.32**	**15.42 ± 4.28**	**11.89 ± 4.16**	**0.231 † NS**
Range	12.31–24.14	9.41–23.18	8.0–22.48	

***SD**: standard division;*

DISCUSSION

There was no statistically significant difference in age or gender between the control group and the vitiligo patients, according to the results. Due to the matched nature of the groups, the study also failed to detect any statistically significant differences in terms of sex or age. There was no statistically

significant difference between the sexes and age groups compared with Abdelmawla et al. [11]. Variables differed between the healthy group (18 females and 12 males) and the vitiligo group (20 females and 10 males). The patient age groups were as follows: 20–40 (37.7 ± 11.6) and 20–38 (36.5 ± 11.4) years. Among those who had vitiligo, 37.5% had a favorable family history according to the study. Even though 62.5% had never had the condition, the vitiligo group was not significantly different from the healthy group. Vitiligo in the family tree. D's immunomodulatory actions are unclear; however, our knowledge is expanding. There is a dearth of evidence to support the concept that vitamin D helps prevent autoimmune illness in humans, despite in vitro and animal evidence to the contrary [12]. Multiple sclerosis, inflammatory bowel disease, type I diabetes mellitus, rheumatoid arthritis, and multiple sclerosis are among autoimmune illnesses that have been linked to reduced vitamin D levels compared to healthy controls, according to a substantial body of research. However, these studies did not been able to definitively establish a causal relationship between the two. The effects of 1,25(OH)2D3 on dendritic cell maturation and differentiation, T cell proliferation, and Th1 cytokine secretion have been demonstrated in multiple studies. In addition, 1,25(OH)2D3 has the potential to boost IL-10 secretion and the number of regulatory T cells [13]. Despite numerous studies attempting to explain this, the significance of vitamin D in vitiligo is still not understood. According to Koizumi et al. [14], vitamin D can suppress the production of proinflammatory and proapoptotic cytokines such as IL-6, IL-8, TNF-α, and TNF-γ. Through the production of sphingosine-1-phosphate, Sauer et al. [15] showed that 1,25(OH)2D3 inhibited the death of human melanocytes. Efforts have also been made to treat vitiligo with topical vitamin D. One study suggested that calcipotriol can activate keratinocytes and melanocytes, which in turn stimulates melanin synthesis [16].

Vitamin D concentrations were found to be considerably lower in vitiligo patients compared to healthy control subjects in the current study (14.45 ± 4.19 ng/mL vs. 38.11 ± 8.88 ng/mL, respectively, P<). Upala *et al*, [17] found a significant association between low 25(OH)D levels and vitiligo, which is consistent with these results. Kim et al. [18] found no statistically significant difference in the mean serum levels of 25(OH)D between 172 vitiligo patients and the normal Korean population; however, the current data contradict this. In addition, I disagree with Khurrum et al. [19], who stated that the vitamin D levels of the control subjects and vitiligo sufferers were the same.

This study revealed that CC was the most common genotype in the control group (70% of cases), whereas AA was the most common genotype in the vitiligo group (30% of cases). These differences were statistically significant.

This study also showed that the degree of vitiligo was higher in AA genotype patients than in CC and CA genotype patients, but the difference was not statistically significant. A Romanian study showed that homozygous mutant CC version APa1 was significantly associated with vitiligo susceptibility, which is inconsistent with our results, which may be because this study used a different allele [20, 21]. In comparison with the results of this study, the homozygous mutant AA was significantly associated with vitiligo susceptibility.

CONCLUSIONS

25(OH)D, which is derived from both food and skin, is the most reliable measure of vitamin D intake. Multiple studies have linked autoimmune illnesses, such as vitiligo, to low vitamin D3 levels and shown that this nutrient has potent immunosuppressive effects. As a result, vitamin D may influence both innate and adaptive immune responses via stem cells, macrophages, and T- and B-lymphocyte receptors. As vitamin D stimulates melanin synthesis, the incidence of vitiligo may increase. The study confirmed that polymorphisms of the VDR gene were associated with vitiligo in Iraqi patients, as allelic variation in the VDR gene or other genes associated with an imbalance of this gene may lead to the development of vitiligo.

This supports our findings, which showed a lower level of 25(OH)D level in the serum of patients compared to the healthy group, and which can be linked genetically, as the study found that the AA

gene was the most abundant compared to CC and CA, and for the most prominent allele, A (42.5%) was inpatient group with statistically significant differences. In conclusion, the increase in the AA gene in the serum of patients compared to healthy people is associated with a decrease in 25(OH)D levels, which in turn causes a deficiency of melanin in the skin, which is the pigment that gives the skin its natural color. This leads to the development of vitiligo, which leads to the appearance of white spots on the skin. In the end, we conclude that the higher the percentage of the AA genotype in the patient's serum, the lower the level of vitamin D, meaning that the relationship between them is inverse.

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Conflict of Interest

The authors declare no conflict of interest.

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APPENDIX

Abbreviation	*Full name*
BP	Binding Protein
VDR	vitamin D receptor
DNA	deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
ARMS	Amplification-refractory mutation system
PCR	Polymerase chain reaction
ELISA	Enzyme-linked immunosorbent assay
RANK	Receptor activator of nuclear factor kB
NFkB	Nuclear factor kappa B
SNPs	Single-nucleotide polymorphisms

