

ORIGINAL PAPER

Evaluation of 1,25-dihydroxyvitamin D3 pathway in patients with chronic urticaria

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Summary

Background: Previous studies showed the role of vitamin D (Vit D) on the progression of chronic urticaria. To the best of our knowledge, there are no other results regarding the contribution of single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) and vitamin D binding protein (VDBP) genes in chronic urticaria (CU).

Aim: In the present study, we investigated the Vit pathway and the association between VDR and VDBP gene polymorphisms and CU risk in Iranian population.

Methods: All participating individuals in the present study were evaluated for serum Vit D and VDBP concentration VDR rs1544410 and rs2228570 and VDBP rs7041 using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The associations of studied analytes and three SNPs with clinical and laboratory outcomes were investigated in CU patients.

Results: Patients with CU showed lower Vit D compared to controls (19.26 ± 1.26 vs. 31.72 ± 7.14 ng/ml, P -value = 0.006). There was a significant correlation between Vit D levels and urticaria activity score. Serum VDBP was significantly higher in CU patients than controls (1317.3 ± 183.71 vs. 395.77 ± 12.96 µg/ml, P -value < 0.0001) and had a positive correlation to progression of CU. The A allele of this polymorphism might be a potential risk factor for progression of CU [odds ratio 4.3434, 95% confidence interval (1.7331–10.8852), Z -statistic = 3.133, P -value = 0.0017].

Conclusion: In summary, this study demonstrated that change in Vit D pathway in the level of gene or protein may be a risk factor for progression of CU.

Introduction

Chronic urticaria (CU) is identified as the recurring urticarial episodes that are lasting for at least 6 weeks.¹ The most important etiological factors for progress of CU are foods, drugs,

aeroallergens, infections, contact allergens and autoantibodies to the IgE.^{2,3} It has been revealed that 1,25-dihydroxycholecalciferol or 1,25-dihydroxyvitamin D3 (Vit D) has potential immunomodulatory activity and can determine the allergic disease

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severity⁴ by impact on modulation of several immune cell system especially mast cells.^{5,6} Previous studies showed the possible role of Vit D in various chronic diseases such as infectious diseases,⁷ malignancies,⁸ autoimmune diseases and allergic disease,^{9,10} including atopic dermatitis¹¹ and asthma.¹² The causal rule of Vit D insufficiency in allergic diseases has been previously shown by several studies.^{9–11} The direct association between Vit D insufficiency and allergic disorders might be discussed by this fact that Vit D is essential for proliferation, survival, differentiation and function of immune systems cells.¹³ Previous studies emphasized the protective role of Vit D on development of allergic symptoms. Especially in the case of chronic urticarial (CU), it was revealed that serum Vit D significantly decreased in patients with urticaria compared to healthy subjects.^{14,15} Significant decline in serum Vit D was also newly reported in patients with acute urticaria.¹⁶ In a meta-analysis study on 547 urticaria cases and 517 controls, Wang *et al.* demonstrated that patients with urticaria had lower level of Vit D compared with healthy controls. They also showed that Vit D deficiency (not Vit D insufficiency) may be considered as potential risk factor for progression of urticaria [odds ratio (OR) 4.09, confidence interval (95% CI) 1.32–12.73].¹⁷ In addition, previous studies also showed the potential role of Vit D supplementation on the improvement of allergic symptoms in CU patients with low serum Vit D.^{6,18,19}

However, there are some literature with conflicting results with regards to the association between Vit D and allergic diseases.^{20–22} Vit D insufficiency could play a causal rule in progression of allergic disease based on the studied population and the age of subjects. Besides, these contradictory data could be explained by variations in the proteins involved in the Vit D metabolism and function pathway. Vitamin D binding protein (VDBP) and vitamin D receptor (VDR) are two important proteins need for correct performance of Vit D. VDBP is the main Vit D carrier protein in the circulation and increased binding form of Vit D with VDBP can cause lower bioavailability of this vitamin in target organs.^{23,24} In contrast, low levels of VDBP, even in the absent of sufficient Vit D, may reverse adverse effects of low Vit D.²⁵ There are three different allele of VDBP in the serum that highly diverse in concentration and affinity to Vit D and distinguish by genetic polymorphism in the gene encoding VDBP named as group-specific component (GC).²⁵ Among all GC gene single nucleotide polymorphisms (SNPs), polymorphism in exon 11 (g57904T>G; rs7041, p.Asp432Glu) is one of the most attractive genetic variation and has been reported to be associated with greater change in VDBP concentration.²⁶ It has been shown that the frequencies of T or G allele of rs7041 highly change by race. Previous studies showed that the frequency of TT genotype in Asian people is higher than Caucasian people, so the concentration of VDBP is lower and as a result the bioavailability of Vit D is higher in Asian populations.^{26,27} The other main protein involve in Vit D pathway is VDR which form a heterodimers with retinoid X receptor (RXR) and together with Vit D interact with DNA to initiate epigenetic modification of chromatin and transcription of specific genes.^{28,29} VDR is one of the nuclear receptor superfamily members located in the chromosome 12q13 region. The human VDR gene is located on chromosome 12 and contains 8 exons that produce a 427 amino acid polypeptide.³⁰ Biological activity of Vit D is applied by interaction with VDR and polymorphism in VDR gene may result in alteration in VDR function and Vit D action.³¹ With regards to VDR gene polymorphism, rs1544410 and rs2228570 are frequently studied SNPs in association with allergic diseases and highly altered the VDR.³²

The concurrent association between VDBP and VDR with progression of allergic disorders especially CU is not understood well. Besides, the associations between VDR and VDBP polymorphisms with allergic disorders are much controverted³¹ and to the best of our knowledge, there is no other study with regard to the association between VDR and VDBP polymorphisms with CU. Hence, in the present study we aimed to compare the serum Vit D and VDBP concentrations in patients with CU and healthy subjects. Besides, the present study attempts to assess the potential role of VDR rs1544410 and rs2228570 and VDBP rs7041 SNPs on risk of disease and clinical outcomes in a Kurdish population with CU.

Material and methods

Study population

This case-control study was conducted during March 2016 to April 2017. A total of 110 patients were diagnosed with CU, according to the European Academy of Allergology and Clinical Immunology (EAACI) and also the World Allergy Organization (WAO).^{33,34} All studied subjects were admitted to the Kurdistan Asthma and Allergy Clinic (Sanandaj, Iran). Among them, 40 individuals were males and 70 were females (average age was 32.75 ± 8.82 and 35.95 ± 10.39 years, respectively). The study also involved 110 subjects (41 males and 69 females) as control group with an average age of 31.19 ± 7.32 years, without a known history of CU at least for the past 3 years. All patients showed urticarial symptoms at least twice a week for more than 6 weeks. The inclusion criteria were presence of urticaria symptoms for at least 6 weeks, score 10 for weekly 5-day score, IgE between 30 and 700 IU/ml, body weight from 25 to 100 kg. The main causes of CU including drug, food, infection, neoplastic or systemic disorders and patients with exercise-related urticaria, physical urticaria, urticarial vasculitis and angioedema were used as the exclusion criteria in the study. The patients received antihistamines therapy and also those who treated with systemic steroids or immunosuppressive medicine during 3 months before beginning the sampling were excluded from the study. The patients with a history of other co-morbidities including kidney or liver diseases, hypertension, Duhring's disease, mast cell tumors, diabetes and calcium disorders were also excluded. A questionnaire form contains history, the clinical findings and exacerbating factors was completed for each patients. Evaluation of disease severity was calculated by Urticaria Activity Score (UAS) and autoimmune nature of urticaria was carried out using Autologous Serum Skin Test (ASST). All individuals were from Kurdistan, a province in western Iran with a population consisting of Kurds. Written informed consent was obtained from all patients and the study was approved by the ethics committee of Kurdistan University of Medical Sciences.

Measurement of total serum IgE, Vit D and VDBP concentration

Venous blood samples were obtained and immediately centrifuged, and then serum was separated and frozen at -20°C until assayed. Serum levels of Vit D and VDBP were measured with specific enzyme-linked immunosorbent (ELISA) assay from Monobind Inc. (Lake Forest, CA 92630, USA) and (BioVendor, Czechia), respectively. The concentration of serum IgE, Vit D and VDBP were expressed as IU/ml, ng/ml and $\mu\text{g/ml}$, respectively.

Table 1. Primer sequence and reaction condition

SNPs	Primer sequence	Annealing temperature (°C)	Restriction enzyme	Product size (bp)
VDR rs1544410	F 5>3: GGAGACACAGATAAGGAAATAC R 5>3: CCGCAAGAAACCTCAAATAACA	60	FspI	GG: 175 + 73 GA: 248 + 175+73 AA: 248
VDR rs2228570	F 5>3: GCACTGACTCTGGCTCTGAC R 5>3: ACCCTCCTGCTCCTGTGGCT	60	BseGI	TT: 282 + 59 TC: 341 + 282+59 CC: 341
VDBP rs7041	F 5>3: CAAGTCTTATCACCATCCTG R 5>3: GCCAAGTTACAATAACAC	55	BsuRI	TT: 809 TG: 809 + 577+232 GG: 577 + 232

Table 2. Clinical and laboratory characteristics of patient group and control group

Characteristics	Studied groups		P-value
	Patients (n = 110)	Controls (n = 110)	
Age (years), (mean ± SEM)	34.78 ± 1.057	31.19 ± 0.756	>0.05
Gender (male/female)	40/70	41/69	
ASST (% positive)	46.6		
UAS7 (mean ± SEM)	27.33 ± 1.29		
Serum total IgE levels (IU/ml), (mean ± SEM)	104.28 ± 7.75	64.47 ± 4.03	<0.05
Serum Vit D levels (ng/ml), (mean ± SEM)	19.26 ± 1.26	31.72 ± 7.14	0.006
Serum VDBP levels (µg/ml), (mean ± SEM)	1317.3 ± 183.71	395.77 ± 12.96	<0.0001
Serum total Ca levels (mg/dl), (mean ± SEM)	9.01 ± 0.12	9.65 ± 0.25	>0.05

PCR-RFLP analysis: DNA extracted from whole blood samples according to our previous studies.^{35–38} In brief, after incubation of blood samples with lysis buffer DNA selectively precipitated. The insoluble DNA has washed and desalted by wash buffer, and it was stored at –20°C pending simultaneous analysis. The VDR rs1544410 and rs2228570 and VDBP rs7041 SNPs were identified using PCR restriction fragment length polymorphism. The PCR reactions were performed in a final volume of 25 µl using PCR Master Mix kit (CinnaGen Inc, Tehran, Iran), 10 pmol of each primer with final concentration of 400 nM and 100 ng DNA. The primers that were used for PCR reactions are shown in Table 1. The PCR conditions was: 5 min at 95°C (initial denaturation), followed by 35 cycles of 94°C for 30 s (denaturation) and annealing for 30 s and 72°C for 30 s using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). In each PCR run, samples with no DNA template were used as negative controls. Amplified DNA fragments were cut by restriction enzymes purchased from Fermentas (Fermentas, Thermo Fisher Scientific, Inc., Waltham, USA) according to manufacture instruction. The genotypes were identified by electrophoresis of DNA fragments generated after digestion. Table 1 shows the enzymes were used for RFLP and the interpretation of RFLP results.

Statistical analysis

Statistical analyses were performed using SPSS 16 (SPSS Inc., Chicago, USA). The chi-squared test was employed to evaluate whether the alleles or genotype frequencies differ between cases and controls. For 2×2 contingency tables, the OR and its 95% CI were calculated to determine if the association was statistically significant at nominal 5% level. Since holding the

normality assumption is crucial when testing hypothesis, the non-parametric one Sample Kolmogorov-Smirnov test was applied to determine this assumption. Results were presented as mean ± SEM if normality assumption met. Comparison of the possible differences between studied groups were done by the independent samples T test or Mann-Whitney statistical tests. One Way ANOVA followed by the post hoc, Tukey and Dunnett tests were used to analyse mean differences between more than two groups. If normality assumption was violated, the Kruskal-Wallis test was used instead. The association between two variables was calculated using the Spearman correlation coefficient. In all performed hypothesis tests, a P-value <0.05 was considered as statistically significant.

Results

Demographic and clinical characteristics

The demographic and laboratory parameters of both patients and control group enrolled in this study were summarized in Table 2. The demographic data included age and gender, and the clinical and laboratory parameters included ASST, UAS, Vit D, VDBP, total IgE and Ca concentration. ASST results were positive in 46.6% of the patients with CU. Mean ± SEM for UAS score in patients was 27.33 ± 1.29. Serum total IgE level (104.28 ± 7.75 vs. 64.47 ± 4.03 IU/ml, P-value <0.05) was also higher in CU patients compared to controls. Serum total IgE had an indirect significant correlation with ASST and UAS (Spearman's $\rho = 0.27$, P-value = 0.083). In addition, ASST had a significant direct correlation with UAS (Spearman's $\rho = 0.735$, P-value <0.0001) and an indirect correlation with Ca (Spearman's $\rho = -0.373$, P-value <0.0001). There were not any significant differences between

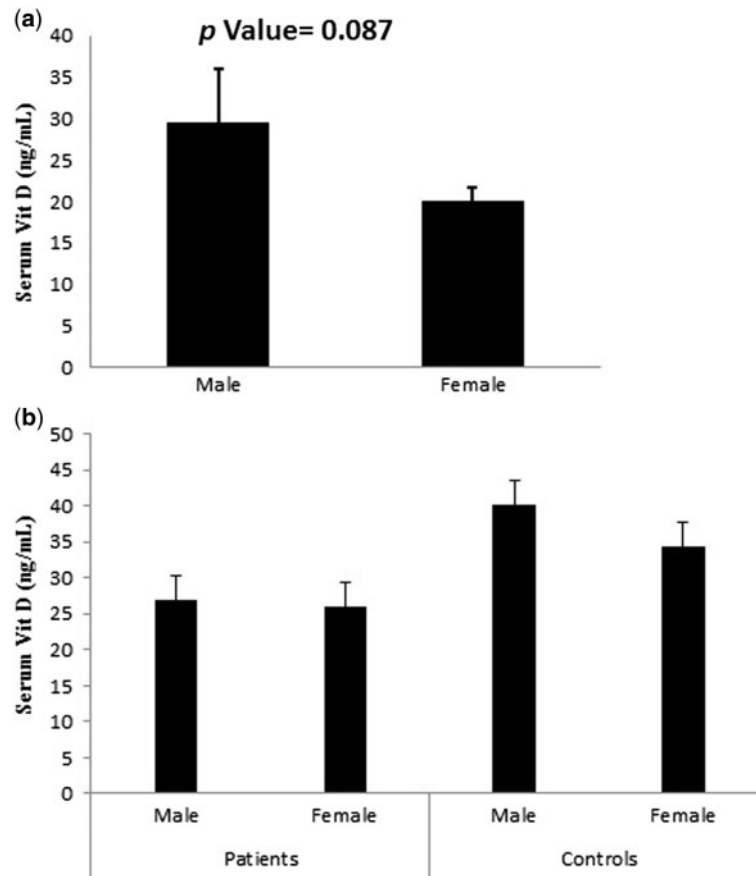


Figure 1. Serum Vit D in males and females. (a) Serum Vit D levels (in ng/ml unit) are indicated as a block value was in a higher concentration (29.53 ± 6.42 ng/ml) for the male group and decreasing concentration to 20.00 ± 1.66 ng/ml for females with a non-significant P -value = 0.087 for their difference. (b) According to the plot, serum Vit D has no significant differences (P -value = 0.340) between males (26.99 ± 6.9 ng/ml) and females (26.08 ± 3.83 ng/ml) in CU patients. The difference between Vit D concentration in controls for males (40.17 ± 17.01 ng/ml) and females (34.04 ± 10.38 ng/ml) was not also significant (P -value = 0.555). Serum Vit D in female subjects has also no significant difference, but there was a significant difference between male subjects in patient and control groups and also male subjects in controls with females in patient group (P -value < 0.05).

male and female subjects for age, ASST, UAS, total IgE and Ca concentration (data not shown).

Serum Vit D and VDBP

Vit D was significantly lower in CU patients compared to controls. Mean $\pm$ SEM serum Vit D was 19.26 ± 1.26 ng/ml for subjects vs. 31.72 ± 7.14 ng/ml for controls (P -value = 0.006) (Table 2). Vit D deficiency or insufficiency was seen in 58.02% of the subjects with CU and 48.89% of the healthy controls. Decreased level of serum Vit D was significantly associated with increased susceptibility to CU (Spearman's $\rho = -0.197$, P -value = 0.027). There was not any significant difference in Vit D levels between male and female patients (P -value = 0.087) (Figure 1a). Serum Vit D was not significantly different between case and controls for female subjects, but males with CU had lower Vit D (29.52 ± 6.4 ng/ml) compared to controls (40.16 ± 17.01 ng/ml) (P -value < 0.05) (Figure 1b). In addition, there was not any significant association between serum Vit D and the age of studied subjects (P -value = 0.215). We found an indirect significant correlation between Vit D with ASST (Spearman's $\rho = -0.615$, P -value < 0.001) and UAS (Spearman's $\rho = -0.488$, P -value = 0.001) and also a direct strong correlation with serum Ca concentration (Spearman's $\rho = 0.92$, P -value < 0.0001) and a direct but NOT significant correlation with serum total IgE (Spearman's $\rho = 0.270$, P -value = 0.083) (Figure 2a–d).

We also measured serum VDBP in both case and control groups. CU patients had higher (1317.3 ± 183.71 μ g/ml) VDBP when compared to healthy subjects (395.77 ± 12.96 μ g/ml) (Table 2). As shown in Table 2, a noticeable increase in serum VDBP was observed in CU patients compared to controls (P -value < 0.0001). Increased levels of serum VDBP had a significant correlation with progression of CU (Spearman's $\rho = 0.426$, P -value = 0.01). In addition, the difference between males and females with regard to VDBP was not significant (1129.7 ± 236.75 vs. 933.33 ± 148.95 ng/ml) (P -value = 0.462). Furthermore, we could find a significant association between serum VDBP and the age of studied subjects (Spearman's $\rho = -0.215$, P -value = 0.02). Besides, our data showed that Vit D concentration had an indirect, but NOT STRONG, significant correlation with VDBP (Spearman's $\rho = -0.371$, P -value = 0.049). Serum VDBP had no significant correlation with clinical and laboratory findings including ASST, UAS, serum total IgE and total Ca concentration (data not shown).

Genotype analysis

We determined the frequencies of VDR rs1544410 and rs2228570 and VDBP rs7041 SNPs in all 110 CU patients as well as in healthy control subjects (Table 3).

The VDR rs1544410 SNP in patient and control groups had no significant deviation from a Hardy–Weinberg distribution

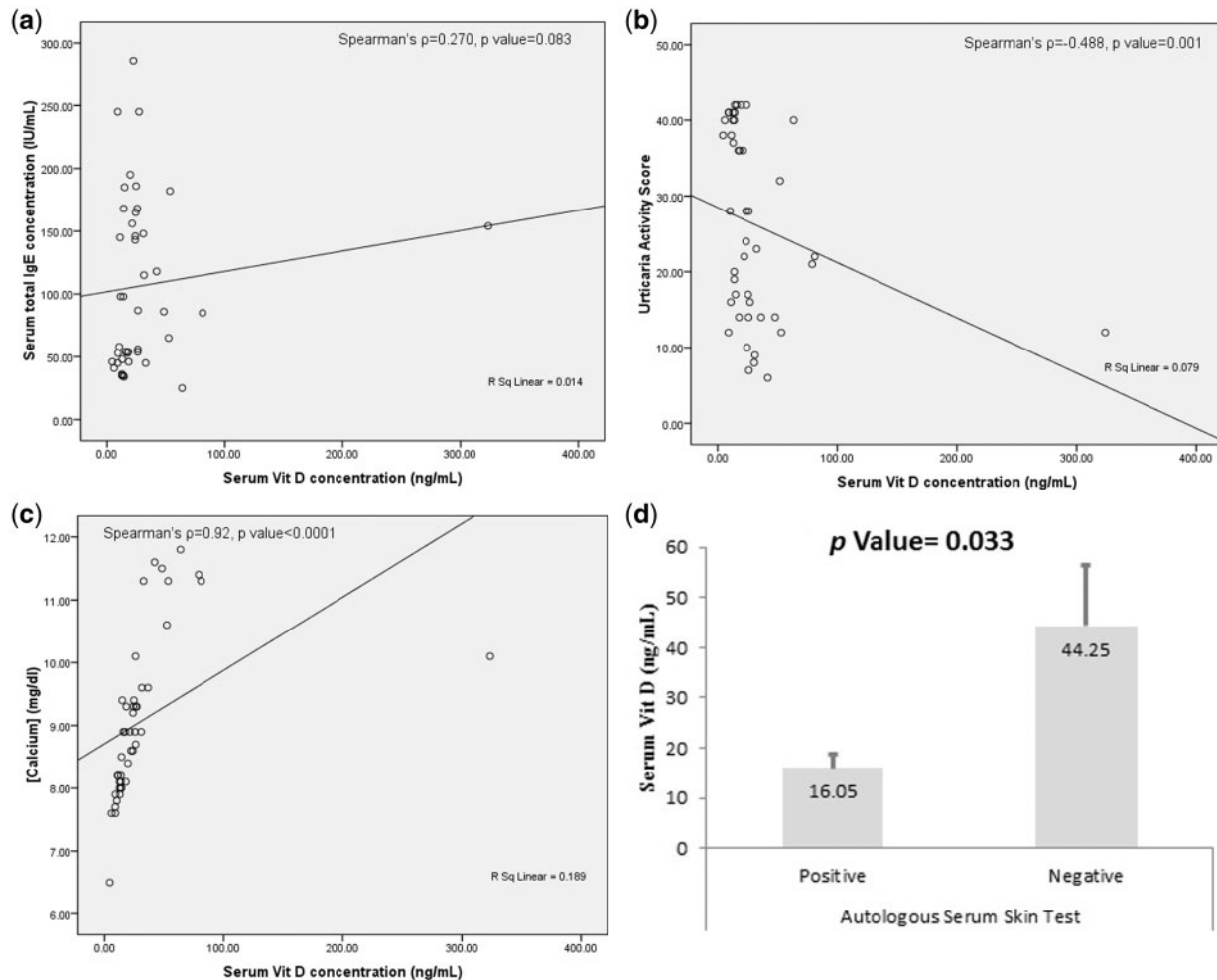


Figure 2. Association of serum Vit D with clinical and laboratory findings in studied subjects. (a) Correlation of serum Vit D with serum total IgE was not statistically significant in studied subjects. (b) Vit D had a statistically significant correlation with UAS in studied subject and linear regression analysis showed that this correlation is indirect. (c) Vit D had a statistically significant correlation with serum total Ca concentration in studied subject and linear regression analysis showed that this correlation is direct. (d) Serum Vit D concentration according to ASST had a statistically significant difference (16.05 ± 2.71 vs. 44.25 ± 12.21 ng/ml).

(P -value >0.05). As shown in Table 3, the frequency of the AA genotype of rs1544410 was higher in CU patients than in controls (39.09% vs. 9.99%, P -value <0.0001). Besides, the homozygous AA genotype of VDR rs1544410 was statistically associated with the progression of CU when compared to GA genotype [OR 4.3434, 95% CI (1.7331–10.8852), Z -statistic = 3.133, P -value = 0.0017]. In addition, subjects carrying A allele (61 patients compared to 46 healthy subjects) were associated with a higher risk to develop CU when compared to the G allele carriers (39 patients compared to 54 healthy controls) [OR 1.8361, 95% CI (1.0464–3.2217), Z -statistic = 2.118, P -value = 0.0342]. However, we could not find any other association between this SNP with clinical and laboratory findings of studied subjects. ANOVA analysis showed that there were not any significant differences between VDR rs1544410 genotypes for serum Vit D, VDBP and total IgE concentration and also ASST and UAS scores (data not shown). However, we found that subjects with AA genotype had lower serum total Ca concentration compared to GG genotype (but NOT FOR GA genotype) (8.76 ± 0.17 vs. 9.62 ± 0.31 mg/dl, P -value = 0.013) (Figure 3).

Genotype distribution for controls, but not for patients, were in agreement with Hardy–Weinberg equilibrium for VDR

rs2228570 polymorphism (P -value >0.05). The CC genotype of VDR rs2228570 SNP was significantly higher in both patients and controls (58.18 and 49.1%, respectively) compared to TT genotype (4.55 and 5.45%) (P -value <0.0001). Attractively, we found that the frequency of carriers of Thr allele (CC and CT genotypes) at position g.30920 of the VDR gene was significantly increased in healthy (72% vs. 28%, P -value <0.0001) and case (77% vs. 23%, P -value <0.0001) subjects as compared to Met allele (TT and CT genotypes). Furthermore, our data revealed that, the VDR rs2228570 had no significant association with risk of CU progression (P -value >0.05). No associations were found between VDR p.Met1Thr SNP with clinical and laboratory features including serum Vit D, VDBP, IgE, Ca and also ASST and UAS (data not shown).

Genotype distribution for patients and controls (P -value >0.05) were also in agreement with the Hardy–Weinberg equilibrium for VDBP rs7041 SNP. There were not any significant differences for genotypes and allele frequency between patients and controls (Table 3). In addition, we could not find any correlation between VDBP rs7041 and progression of CU. Moreover, there was not a significant correlation between rs7041 and Vit D deficiency and insufficiency and there was not any correlation

Table 3. Genotypes and alleles frequencies in patient and control groups

Genotype frequencies	Chronic urticaria patients (n = 110)	Controls (n = 110)	P-value	Allele frequencies (%)	Chronic urticaria patients (n = 110)	Controls (n = 110)	P-value	OR (95% CI)
VDR rs1544410	GG 18 (16.36%) GA 49 (44.55%) AA 43 (39.09%)	20 (18.18%) 79 (71.83%) 11 (9.99%)	0.05 <0.0001 <0.0001	VDR rs1544410	G 39 A 61	54 46	0.0372 0.0372	1.8361 (1.0464–3.2217)
VDR rs2228570	TT 5 (4.55%) TC 41 (37.27%) CC 64 (58.18%)	6 (5.45%) 50 (45.45%) 54 (49.1%)	0.1819 0.218 0.1769	VDR rs2228570	T 23 C 77	28 72	0.9 0.9	1.3019 (0.6876–2.4650)
VDBP rs7041	TT 23 (20.91%) TG 56 (50.91%) GG 31 (28.18%)	29 (26.36%) 59 (53.64%) 22 (20%)	0.34 0.4 0.156	VDBP rs7041	G 46 A 54	53 47	0.2991 0.2991	1.3238 (0.7594–2.3077)

Data are presented as number (%). According to the table, the frequency of the AA genotype of VDR rs1544410 was higher in CU patients than in controls. In addition, subjects carrying A allele were associated with a higher risk to develop CU when compared to the G allele carriers (P -value = 0.0342).

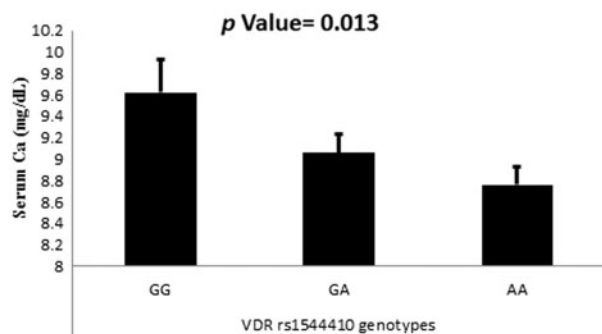


Figure 3. Serum total Ca concentration in different VDR rs1544410 genotypes: Ca level (in mg/dl unit) starting with the AA genotype (8.76 ± 0.17 mg/dl) and increasing over about 9.06 ± 0.17 mg/dl (nearly similar value) for the GA genotype up to about 9.62 ± 0.31 mg/dl for the GG group with acceptable significance (P -value = 0.013). According to the error plot, the mean differences between the illustrated genotypes with GG and AA groups were statistically significant for Ca concentration.

between this SNP and serum VDBP concentration (P -value >0.05). Like other studied SNPs in VDR gene, there were not any significant association between VDBP rs7041 SNP and clinical and laboratory findings.

Discussion

In the present study, we showed that serum Vit D decreased in CU patients compared to healthy subjects. Our results also indicated that VDBP is significantly higher in CU patients than controls. In addition, we found that VDR rs1544410 polymorphism is significant determinant of CU risk in Kurdish population. Our data also revealed that Vit D is not considered as a potential risk factor for development of CU; however, we found that VDBP has a significant positive effect on progression of CU. On the other hands, we found that VDR rs2228570 and VDBP rs7041 SNPs had no statistically significant correlation with development of CU. To the best of our knowledge, this is the first study to evaluate the VDBP and VDR in CU patients.

Vit D directly effect on the regulation of immune system health and low Vit D is an important factor in development of allergic disorders. The role of Vit D on inflammatory pathways applying by VDR expression in various immune cells such as

B cells, T cells, dendritic cells and macrophages.^{39,40} Most of these cells can locally produce the active form of Vit D via expression of CYP27B, and this mechanism enabled them to rapidly increased the level of Vit D potentially needed for regulation of immune response.¹⁰ Vit D can impact the pattern of immune activity by inhibition of Th1 and Th2 immune responses via suppressing the production of IL-12 generated IFN- γ and also IL-4 and IL-4-induced expression of IL-13.⁴¹ However, there are some contradictory studies that do not support this hypothesis; for example, Ozkara et al.⁴² showed that there is a negative correlation between serum Vit D with IgE and IL-4. With regard to CU, decline in serum level of Vit D in patients compared to controls has been revealed in previous studies.^{4,14–17,19,43,44} In line with previous studies, our data showed that serum Vit D is decreased in CU patients compared with healthy controls. On the other hand, there are some contradictory results which showed no significant association between serum Vit D and risk of allergic disorders. Although lower levels of serum Vit D were reported in Yao et al.²¹ and Weisse et al.²² studies, but they did not find any association between decline in Vit D and urticaria. Wu et al.²⁰ study is a unique report in this issue, because in contrast to previous studies, they found higher levels of serum Vit D in patients with CU compared to controls. Although decreased Vit D concentration in CU patients has been well established in many studies, but the association of Vit D level with the severity of disease is not proven yet.⁴⁴ The most likely explanation for these results is derived from variation in the baseline Vit D status, the duration of decline in Vit D concentration¹⁰ and changes in Vit D axis including Vit D, VDBP and VDR. VDBP is not only a Vit D carrier but is also an immunomodulatory protein that may be related to inflammation process.⁴⁵ It seems that low level of Vit D can develop the allergic disease even in the population with low VDBP levels. It can be explained by genetic variations in VDBP gene.²⁶ Binding form of Vit D to VDBP has lower bioavailability in target tissues.²⁵ Moreover, increase VDBP-bound vit D is highly associated with decrease in Vit D function indicators.²⁴ On the other hand, decreased levels of VDBP, even in the low Vit D concentration, may reverse the adverse effects of apparently low serum Vit D.²⁵ To the best of our knowledge, the present study is the first study that evaluates VDBP in patients with CU. Our study clearly showed that VDBP is increased in CU patients compared to controls. However, genotype analysis for VDBP rs7041 SNP revealed that this SNP was not a potential risk factor

for progression of CU in our studied population. In a Canadian cohort study, VDBP rs7041 SNP was associated with higher concentration of serum VDBP and increased binding affinity to Vit D.⁴⁶ In contrast, Mostowska et al.⁴⁷ did not find any correlation between rs7041 and progression of disease. In the current study, no associations were observed between the rs7041 SNP with progression of CU and concentration of VDBP. We also evaluate the VDR rs1544410 and rs2228570 SNPs in studied population. VDR rs2228570 (g.30920T>C) is located on the long arm of chromosome 12 (12q13.1) and change the start codon Met (ATG) to Thr (ACG). Alteration in start codon may lead to different activity in shorter protein (424 aa) compared to longer VDR protein (427 aa).³² We did not observe any significant association between VDR rs2228570 gene polymorphism and CU progression risk. To the best of our knowledge, there is not any other study regarding the relationship between VDR rs1544410 polymorphism and CU so far. It is well known that SNPs in the VDR gene highly change VDR expression and activity.³² The results of our study showed the impacts of AA genotype with occurrence of CU. Individuals with AA genotype were found to be at increased risk to develop CU. Previous studies showed the association of G allele of VDR rs1544410 SNP with increased risk of asthma and atopy.⁴⁸ There are also too many studies that revealed the potential role of G allele of this polymorphism with risk of different diseases including high bone-mass density,⁴⁹ sporadic hyperparathyroidism.⁵⁰ On the other hand, some studies revealed that A allele of VDR G63980A SNP is a risk factor for fast acquired immunodeficiency syndrome progression⁵¹ and type 1 diabetes risk and acute-onset type 1 diabetes.⁵² Poon et al.⁴⁸ revealed that VDR rs1544410 SNP is a regulatory polymorphism involved in immune-mediated diseases.

Although it was well known that VDBP is stable in adulthood over the period of 1–3 years and its seasonal changes are negligible,^{53,54} but one potential limitation in our study is that serum Vit D and VDBP measured in a single blood sample collected and this sampling may not reflect typical concentrations over time. Strengths of our study included measurement of VDBP concentration along with Vit D and polymorphism analysis. Previous studies did not evaluate VDBP and VDR in CU patients; however, there are too many investigations which assessed the association of Vit D with urticaria. So to the best of our knowledge, our study is the first study evaluated the VDBP and VDR at the level of protein and gene in CU patients. Limitations include the lack of study of the others most important polymorphism in VDR and VDBP genes, which is an important area for future researches.

In summary, we identified a strong association between decrease of Vit D, increased VDBP and also genetic variations at the VDR 63980 locus and CU in a Kurdish population. Along with other known CU risk factors identified such as foods, drugs, aeroallergens, infections, contact allergens and autoantibodies to the IgE, the addition of Vit D pathway involvement in the understanding of CU pathogenesis will shed light for better control and treatment.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethics committee of Kurdistan University of Medical Sciences and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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References

1. Shear S. Andrews' diseases of the skin: clinical dermatology. *JAMA* 2012; **307**:92.
2. Beck LA, Bernstein JA, Maurer M. A review of international recommendations for the diagnosis and management of chronic urticaria. *Acta Derm Venereol* 2017; **97**:149–58.
3. Gaig P, Olona M, Munoz Lejarazu D, Caballero MT, Dominguez FJ, Echechipia S. Epidemiology of urticaria in Spain. *J Invest Allergol Clin Immunol* 2004; **14**:214–20.
4. Woo YR, Jung KE, Koo DW, Lee JS. Vitamin D as a marker for disease severity in chronic urticaria and its possible role in pathogenesis. *Ann Dermatol* 2015; **27**:423–30.
5. Piemonti L, Monti P, Sironi M, Fraticelli P, Leone BE, Dal Cin E. Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J Immunol* 2000; **164**:4443–51.
6. Rasool R, Masoodi KZ, Shera IA, Yosuf Q, Bhat IA, Qasim I. Chronic urticaria merits serum vitamin D evaluation and supplementation; a randomized case control study. *World Allergy Organ J* 2015; **8**:15.
7. Watkins RR, Lemonovich TL, Salata RA. An update on the association of vitamin D deficiency with common infectious diseases. *Can J Physiol Pharmacol* 2015; **93**:363–8.
8. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nat Rev* 2014; **14**:342–57.
9. Quirk SK, Rainwater E, Shure AK, Agrawal DK. Vitamin D in atopic dermatitis, chronic urticaria and allergic contact dermatitis. *Exp Rev Clin Immunol* 2016; **12**:839–47.
10. Mirzakhani H, Al-Garawi A, Weiss ST, Litonjua AA. Vitamin D and the development of allergic disease: how important is it? *Clin Exp Allergy* 2015; **45**:114–25.
11. Dębińska A, Sikorska-Szaflik H, Urbanik M, Boznański A. The role of vitamin D in atopic dermatitis. *Dermatitis* 2015; **26**:155–61.
12. Ali NS, Nanji K. A review on the role of vitamin D in asthma. *Cureus* 2017; **9**:e1288.

13. Chirumbolo S, Bjorklund G, Sboarina A, Vella A. The role of vitamin D in the immune system as a pro-survival molecule. *Clin Ther* 2017; **39**:894–916.
14. Grzanka A, Machura E, Mazur B, Misiolek M, Jochem J, Kasperski J, et al. Relationship between vitamin D status and the inflammatory state in patients with chronic spontaneous urticaria. *J Inflamm (Lond)* 2014; **11**:2.
15. Thorp WA, Goldner W, Meza J, Poole JA. Reduced vitamin D levels in adult subjects with chronic urticaria. *J Allergy Clin Immunol* 2010; **126**:413.
16. Ozdemir B, Köksal BT, Karakaş NM, Ozbek OY. Serum vitamin D levels decrease in children with acute urticaria. *Allergol Immunopathol* 2016; **44**:512–6.
17. Wang X, Li X, Shen Y, Wang X. The association between serum vitamin D levels and urticaria: a meta-analysis of observational studies. *G Ital Dermatol Venereol* 2017 [Epub Ahead of Print].
18. Oguz Topal I, Kocaturk E, Gungor S, Durmuscan M, Sucu V, Yıldırım S. Does replacement of vitamin D reduce the symptom scores and improve quality of life in patients with chronic urticaria? *J Dermatolog Treat* 2016; **27**:163–6.
19. Rorie A, Goldner WS, Lyden E, Poole JA. Beneficial role for supplemental vitamin D3 treatment in chronic urticaria: a randomized study. *Ann Allergy Asthma Immunol* 2014; **112**:376–82.
20. Wu CH, Eren E, Arden-Jones MR, Venter C. Association between micronutrient levels and chronic spontaneous urticaria. *Biomed Res Int* 2015; **2015**:926167.
21. Yao T-C, Tu Y-L, Chang S-W, Tsai H-J, Gu P-W, Ning H-C, et al. Suboptimal vitamin D status in a population-based study of Asian children: prevalence and relation to allergic diseases and atopy. *PLoS One* 2014; **9**:e99105.
22. Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M, et al. Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. *Allergy* 2013; **68**:220–8.
23. Jassil NK, Sharma A, Bikle D, Wang X. Vitamin D binding protein and 25-hydroxyvitamin D levels: emerging clinical applications. *Endocr Pract* 2017; **23**:605–13.
24. Bhan I. Vitamin d binding protein and bone health. *Int J Endocrinol* 2014; **2014**:561214.
25. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med* 2013; **369**:1991–2000.
26. Koplin JJ, Suaini NH, Vuillermin P, Ellis JA, Panjari M, Ponsonby AL, et al. Polymorphisms affecting vitamin D-binding protein modify the relationship between serum vitamin D (25[OH]D3) and food allergy. *J Allergy Clin Immunol* 2016; **137**:500–6 e4.
27. Allen KJ, Koplin JJ, Ponsonby AL, Gurrin LC, Wake M, Vuillermin P, et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol* 2013; **131**:1109–16.
28. Yang L, Ma J, Zhang X, Fan Y, Wang L. Protective role of the vitamin D receptor. *Cell Immunol* 2012; **279**:160–6.
29. Zhang J, Chalmers MJ, Stayrook KR, Burris LL, Wang Y, Busby SA, et al. DNA binding alters coactivator interaction surfaces of the intact VDR-RXR complex. *Nat Struct Mol Biol* 2011; **18**:556–63.
30. Maestro MA, Molnar F, Mourino A, Carlberg C. Vitamin D receptor 2016: novel ligands and structural insights. *Exp Opin Ther Pat* 2016; **26**:1291–306.
31. Bizzaro G, Antico A, Fortunato A, Bizzaro N. Vitamin D and autoimmune diseases: is vitamin D receptor (VDR) polymorphism the culprit? *Isr Med Assoc J* 2017; **19**:438–43.
32. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. *Gene* 2004; **338**:143–56.
33. Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, et al. The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update. *Allergy* 2014; **69**:868–87.
34. Maurer M, Altrichter S, Bieber T, Biedermann T, Brautigam M, Seyfried S, et al. Efficacy and safety of omalizumab in patients with chronic urticaria who exhibit IgE against thyroperoxidase. *J Allergy Clin Immunol* 2011; **128**:202–9.e5.
35. Maroofi F, Amini S, Roshani D, Ghaderi B, Abdi M. Different frequencies and effects of ABCB1 T3435C polymorphism on clinical and laboratory features of B cell chronic lymphocytic leukemia in Kurdish patients. *Tumour Biol* 2015; **36**:2863–8.
36. Ghafouri H, Ghaderi B, Amini S, Nikkhoo B, Abdi M, Hoseini A. Association of ABCB1 and ABCG2 single nucleotide polymorphisms with clinical findings and response to chemotherapy treatments in Kurdish patients with breast cancer. *Tumour Biol* 2016; **37**:7901–6.
37. Amini S, Javanmardi M, Mokarizadeh A, Maroofi F, Jalali C, Azadi NA, et al. Association of HaeIII single nucleotide polymorphisms in the SLC2A1 gene with risk of diabetic nephropathy; evidence from Kurdish patients with type 2 diabetes mellitus. *QJM* 2016; **109**:399–404.
38. Jalali C, Ghaderi B, Amini S, Abdi M, Roshani D. Association of XRCC1 Trp194 allele with risk of breast cancer, and Ki67 protein status in breast tumor tissues. *Saudi Med J* 2016; **37**:624–30.
39. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1, 25-dihydroxyvitamin D3 receptors in human leukocytes. *Science* 1983; **221**:1181–3.
40. Adorini L, Penna G, Giarratana N, Roncari A, Amuchastegui S, Daniel KC, et al. Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. *J Steroid Biochem Mol Biol* 2004; **89–90**:437–41.
41. Pichler J, Gerstmayr M, Szepefalusi Z, Urbanek R, Peterlik M, Willheim M. 1 alpha, 25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. *Pediatr Res* 2002; **52**:12–8.
42. Ozkara S, Keles E, İlhan N, Gungor H, Kaygusuz I, Alpaly HC. The relationship between Th1/Th2 balance and 1alpha, 25-dihydroxyvitamin D(3) in patients with nasal polyposis. *Eur Arch Otorhinolaryngol* 2012; **269**:2519–24.
43. Movahedi M, Tavakol M, Hirbod-Mobarakeh A, Gharagozlou M, Aghamohammadi A, Tavakol Z, et al. Vitamin D deficiency in chronic idiopathic urticaria. *Iran J Allergy Asthma Immunol* 2015; **14**:222–7.
44. Abdel-Rehim AS, Sheha DS, Mohamed NA. Vitamin D level among Egyptian patients with chronic spontaneous urticaria and its relation to severity of the disease. *Egypt J Immunol* 2014; **21**:85–90.
45. Chishimba L, Thickett DR, Stockley RA, Wood AM. The vitamin D axis in the lung: a key role for vitamin D-binding protein. *Thorax* 2010; **65**:456–62.
46. Larcombe L, Mookherjee N, Slater J, Slivinski C, Singer M, Whaley C, et al. Vitamin D in a northern Canadian first nation population: dietary intake, serum concentrations and functional gene polymorphisms. *PLoS One* 2012; **7**:e49872.
47. Mostowska A, Sajdak S, Pawlik P, Lianeri M, Jagodzinski PP. Polymorphic variants in the vitamin D pathway genes and the risk of ovarian cancer among non-carriers of BRCA1/BRCA2 mutations. *Oncol Lett* 2016; **11**:1181–8.

48. Poon AH, Laprise C, Lemire M, Montpetit A, Sinnott D, Schurr E, et al. Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med* 2004; **170**:967–73.
49. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, et al. Prediction of bone density from vitamin D receptor alleles. *Nature* 1994; **4**: 284–7.
50. Carling T, Rastad J, Akerstrom G, Westin G. Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors. *J Clin Endocrinol Metab* 1998; **83**: 2255–9.
51. Barber Y, Rubio C, Fernandez E, Rubio M, Fibla J. Host genetic background at CCR5 chemokine receptor and vitamin D receptor loci and human immunodeficiency virus (HIV) type 1 disease progression among HIV-seropositive injection drug users. *J Infect Dis* 2001; **184**:1279–88.
52. Motohashi Y, Yamada S, Yanagawa T, Maruyama T, Suzuki R, Niino M, et al. Vitamin D receptor gene polymorphism affects onset pattern of type 1 diabetes. *J Clin Endocrinol Metab* 2003; **88**:3137–40.
53. Bouillon R, Van Assche FA, Van Baelen H, Heyns W, De Moor P. Influence of the vitamin D-binding protein on the serum concentration of 1, 25-dihydroxyvitamin D3. Significance of the free 1, 25-dihydroxyvitamin D3 concentration. *J Clin Invest* 1981; **67**:589–96.
54. Sonderman JS, Munro HM, Blot WJ, Signorello LB. Reproducibility of serum 25-hydroxyvitamin d and vitamin D-binding protein levels over time in a prospective cohort study of black and white adults. *Am J Epidemiol* 2012; **176**:615–21.