Vitamin D Receptor rs2228570 and rs731236 Polymorphisms are Susceptible Factors for Systemic Lupus Erythematosus

Abstract

**Background:** The Vitamin D receptor (VDR) polymorphisms are the candidate genetic variants for susceptibility to different disease including autoimmune disorders. In the present study, we aimed to assess the association between VDR polymorphisms and systemic lupus erythematosus (SLE) susceptibility in Southeast Iranian population. **Materials and Methods:** One hundred and twenty-seven patients with SLE and 139 controls were genotyped for VDR rs2228570, rs731236, and rs7975232 polymorphisms using polymerase chain reaction-restriction fragment length polymorphism method. **Results:** The VDR rs2228570 polymorphism was associated with higher risk of SLE in codominant, dominant, and overdominant models. Moreover, higher risk of SLE was observed in individuals with VDR rs731236 polymorphism in codominant, dominant, overdominant, and allelic models. The tAf haplotype of rs731236/rs7975232/rs2228570 polymorphisms was associated with higher risk of SLE. **Conclusion:** In conclusion, VDR rs2228570 and rs731236 polymorphisms and tAf haplotype were associated with SLE risk.

**Keywords:** Genetic, lupus erythematosus, polymorphism, systemic, Vitamin D receptor

Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder, characterized by autoantibody production, complement activation, and damage of immune tolerance to self-antigens, and may lead to diverse clinical symptoms encompassing most organs and tissues. Although the etiology and pathogenesis of this autoimmune disease remain unclear, various environmental, hormonal, and genetic factors are found to be associated with SLE pathogenesis. Proteomic analysis reveals altered pattern of different proteins in SLE patients. Moreover, numerous studies have reported the effects of various genetic polymorphisms on SLE susceptibility.

Vitamin D as an environmental factor has multiple immunosuppressant properties. Indeed, the available evidences have introduced Vitamin D as an immune modulator for the immune system. Previous reports showed that Vitamin D deficiency/insufficiency was high in SLE patients. The promising results reported for Vitamin D administration in several animal models suffered from inflammatory bowel disease, autoimmune thyroiditis, Type I diabetes mellitus, and SLE. Vitamin D interacts with the immune system and either directly or indirectly regulates proliferation and differentiation of immune cells. Indirectly, both expression of the immune suppressive cytokine and Interleukin-10 and Interleukin-12 production are regulated through effects of Vitamin D on dendritic cells. In direct mode, Vitamin D inhibits T lymphocytes proliferation and cell cycle progression from G<sub>0</sub> to G<sub>1</sub>. Furthermore, it also suppresses B cell proliferation and differentiation and decreases interleukin-17 levels. Vitamin D receptor (VDR) is the mediator of the Vitamin D activity located in the nucleus of target cells, including immune cells (antigen-presenting cells, natural killer cells, and B and T lymphocytes). The VDR gene is located on chromosome 12q13, and the expression of genes in various Vitamin D-responsive tissues is regulated by it.

Results of the study on VDR knockout mice have shown that the VDR expression plays an important role in T helper-1 type
immune response in spleen cells.\[^{[17]}\] In addition, VDR genetic variants have been associated with endocrine autoimmune disease. VDR polymorphism is one of the multiple polymorphisms that predispose individuals to autoimmunity, but their influences on VDR function are still unknown.\[^{[18]}\] Evidence showed that four important polymorphisms of VDR (rs2228570, rs1544410, rs731236, and rs7975232) may be involved in autoimmune disorders.\[^{[18,19]}\] Several population-based studies have shown that VDR polymorphisms are implicated in SLE and SLE severity (chronic damage) and may be accounted for worse prognosis and enhanced risk for organ damage in SLE patients.\[^{[20]}\] In the present study, we investigated the possible effects of VDR polymorphisms on SLE susceptibility in Southeast Iranian population.

Materials and Methods

One hundred and twenty-seven SLE patients (13 males and 116 females, mean age 31.6 ± 8.3 years), who referred to Rheumatology Clinic of Ali-ibn Abi-Talib University Hospital in Zahedan and fulfilled the 1998 American College of Rheumatology criteria, were recruited in this study. The protocol of the study was approved by the Local Ethics Committee of the Zahedan University of Medical Sciences, and written informed consent was obtained from all patients and controls. One hundred and thirty-nine age-, sex-, and ethnically matched controls (14 males and 125 females, mean age 31.2 ± 10.2 years) were selected. The patients had no history of systemic diseases, other rheumatic diseases, infections, and malignancies. The control group with negative ANA test had no history of autoimmune diseases and family relation with SLE patients.

Genotyping

Genomic DNA was extracted from 500 µl ethylenediaminetetraacetic acid-treated whole blood using the salting out method and frizzed at −20°C until genotyping. Genotyping of VDR rs2228570 (FokI), rs731236 (TaqI), and rs7975232 (ApaI) polymorphisms were performed using polymerase chain reaction (PCR) restriction fragment length polymorphism method. The primer sequences, annealing temperatures, and fragment sizes are presented in Table 1.\[^{[18,21]}\]

PCR reactions were done in a 15-µl volume with 7 µl master mix (SinaClon, Tehran, IR Iran), 100 ng DNA, and 40 pmol of each primer (SinaClon, Tehran, IR Iran). Amplification was carried out by a MyCycler™ Thermal Cycler (Bio-Rad, USA). PCR was improved with the program as follows: 95°C for 5 min, 35 cycles of 95°C for 30 s, 30 s at annealing temperatures, and final extension at 72°C for 5 min. All products were digested with corresponding restriction enzyme (Fermentas, Lithuania), electrophoreosed on 2% agarose gel, and visualized by safe stain (SinaClon, Tehran, IR Iran) [Figure 1].

Statistical analysis

All statistical analyses were performed using SPSS software (Version 20; SPSS Inc., Chicago, IL, USA). The clinical data were compared using the independent samples t-test or Fisher’s exact test. The alleles’ frequency was compared using the Fisher’s exact test. The independent effect of VDR gene polymorphisms and SLE was estimated by calculating the odds ratio (OR) and 95% confidence intervals (CI) from a logistic regression analysis model after adjustment for age, sex, and ethnicity. Haplotype frequency and linkage disequilibrium (LD) were analyzed using Haploview software (version 4.2). P < 0.05 was considered statistically significant.

| Polymorphism | Primer sequences | Restriction enzyme | Annealing temperature (°C) | PCR products | RFLP fragments |
|--------------|------------------|--------------------|-----------------------------|--------------|----------------|
| rs2228570    | Forward: 5’-AGCTGGCCCTGGGACTGACTCTGGCCT-3’  
                Reverse: 5’-ATGGAAACACCTTGTCTTCCCTCCCTC-3’ | FokI | 69 | 267 | F(C): 267 |
|              |                   |                   |                             |              | f(T): 197, 70 |
|              |                   |                   |                             |              | T(T): 512, 204 |
| rs731236     | Forward: 5’-GGGGAGTAGGAGGTGAGGACAGAAGC-3’  
                Reverse: 5’-GAAAGAGGTGAGGTGAGGACAGAAGC-3’ | TaqI | 68 | 716 | t(C): 311, 204 |
|              |                   |                   |                             |              | a(C): 528, 217 |
| rs7975232    | Forward: 5’-CAGAGCATGGGAGAGAGGACAGAAGC-3’  
                Reverse: 5’-GCCACTCTCTGGGACTGACTCTGGCCT-3’ | ApaI | 68 | 745 | A(A): 745 |
|              |                   |                   |                             |              | a(C): 528, 217 |

PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

![Figure 1: Agarose gel electrophoresis images for Vitamin D receptor polymorphisms](image-url)
Results

The demographic and clinical characteristics of SLE and control groups are presented in Table 2. Various clinical manifestations in SLE patients were as follows: joint symptoms (84%), dermomois disorders (81%), hematological disorders (60%), oral ulcers (28%), renal involvement (26%), and neurological disorders (19%). The positive results of antinuclear antibodies (ANAs) and anti-dsDNA antibodies tests were 91% and 78% in the SLE patients, respectively.

VDR rs2228570 and rs7975232 polymorphisms were conformed to the Hardy–Weinberg equilibrium in control and SLE groups ($P > 0.05$), but rs731236 polymorphism was deviated from Hardy–Weinberg equilibrium only in SLE group ($P < 0.001$).

The frequency of $VDR$ rs2228570Ff genotype was significantly higher in SLE patients than that in controls (46.5 vs. 32.4), and this genotype was associated with a 1.8-fold increased risk of SLE (OR, 1.8 [95% CI, 1.1–3.1], $P = 0.02$). Moreover, the rs2228570 polymorphism was associated with SLE in dominant model but not recessive and allelic models. A significant increase in frequency of rs731236Tt genotype was observed in SLE patients compared to controls (68.5 vs. 47.5) and might increase SLE susceptibility almost 2.8 fold (OR, 2.8 [95% CI, 1.6–5], $P = 0.0002$). The rs731236 polymorphism could increase SLE risk in dominant and allelic models but not recessive model. There was no significant association between rs7975232 polymorphism and SLE neither in dominant nor in recessive models and allelic models [Table 3].

Haplotype analysis indicated that among eight haplotypes observed in rs731236/rs7975232/rs2228570 polymorphisms, only tAf haplotype was associated with increased SLE risk. In addition, we found no relation between $VDR$ polymorphisms and SLE manifestations.

Discussion

In the present study, we observed higher frequency of $VDR$ rs2228570Ff genotype in SLE patients. In addition, $VDR$ rs2228570 was associated with a higher risk of SLE in dominant model but not recessive and allelic models. The $VDR$ rs731236Tt genotype frequency was higher in SLE patients, and rs731236 polymorphism was associated with the higher risk of SLE in dominant and allelic models but not recessive model. There was no significant association between rs7975232 polymorphism and SLE in each model. The results of haplotype analysis showed that the tAf haplotype was associated with increased SLE risk. In addition, we found no relation between $VDR$ polymorphisms and SLE manifestations.

Vitamin D is a key factor in inflammatory and autoimmune rheumatic diseases. Since Vitamin D has endocrine effects and regulates transcription of genes involved in rheumatic diseases, its effect on inflammatory autoimmune diseases is expectable.[7] It is well known that the active form of Vitamin D plays a key role in inflammatory and autoimmune rheumatic diseases. The effects of Vitamin D are mediated by high-affinity binding of VDR which binds to the active form of Vitamin D ($1,25(OH)_2D$) and distributes in various tissues. $VDR$ gene is highly polymorphic; however, rs7975232 and rs7975232 polymorphisms in intron 8, rs731236 in exon 9, and rs2228570 in the start codon are the most studied variants.[22]

Although different reports on the effects of $VDR$ polymorphisms on SLE susceptibility have been published, their results are inconsistent. Moreover,

Table 2: Demographic and clinical characteristics of systemic lupus erythematosus patients and controls

| Parameter                  | SLE patients (n=127) | Controls (n=139) | $P$  |
|----------------------------|----------------------|------------------|------|
| Age (year)                 | 31.6±8.3             | 31.2±10.2        | NS   |
| Sex, n (male/female)       | 13/116               | 14/125           | NS   |
| Joint symptoms, n (%)      | 105 (84)             | -                |      |
| Renal diseases, n (%)      | 33 (26)              | -                |      |
| Dermomucus disorders, n (%)| 103 (81)             | -                |      |
| Neurological disorders, n (%)| 24 (19)            | -                |      |
| Hematological disorder, n (%)| 76 (60)            | -                |      |
| Oral ulcer, n (%)          | 36 (28)              | -                |      |
| ANA                        | 116 (91)             | -                |      |
| Anti-dsDNA antibodies      | 99 (78)              | -                |      |

ANA: Antinuclear antibodies, NS: Not significant, SLE: Systemic lupus erythematosus

Although the frequency of $VDR$ rs2228570Ff genotype was higher in SLE patients suffered from joint symptoms, the difference was not statistically significant. There was no significant association between the $VDR$ rs2228570, rs731236, and rs7975232 polymorphisms and SLE manifestations and laboratory findings.
The results obtained from the meta-analysis of Xiong et al. performed on 11 studies (1683 cases and 1883 controls) showed that the rs1544410BB + Bb and rs2228570FF + Ff genotypes were significantly higher in SLE patients in the overall population and Asians. No significant association was observed between rs7975232 and rs731236 polymorphisms and SLE neither in the overall populations nor in Asians and Caucasians. In another meta-analysis (13 studies), Zhou et al. observed the association between rs1544410B and rs2228570f alleles and SLE susceptibility in the overall population, Asians, and Africans but not Caucasians. In addition, the rs1544410 bb, rs2228570ff, and rs7975232aa genotypes were associated with SLE risk in the overall population. However, they found the higher frequencies of rs1544410bb and BB and rs2228570ff genotypes but not rs7975232aa genotype in Asian SLE patients. In Africans, the rs1544410BB/bb, rs2228570ff, and rs7975232AA/aa genotypes were significantly higher in SLE patients than in control groups.

**Table 3: Comparison of genotypic and allelic frequency of Vitamin D receptor polymorphisms in systemic lupus erythematosus patients and control group**

| VDR                  | SLE (n=127) | Control (n=139) | P   | OR (95% CI) |
|----------------------|-------------|-----------------|-----|-------------|
| rs2228570(Fok1)      |             |                 |     |             |
| Codominant           |             |                 |     |             |
| FF, n (%)            | 58 (45.7)   | 81 (58.2)       | 1.0 |             |
| Ff, n (%)            | 59 (46.5)   | 45 (32.4)       | 0.02| 1.8 (1.1-3.1)|
| ff, n (%)            | 10 (7.8)    | 13 (9.4)        | 0.88| 1.1 (0.4-2.6)|
| Dominant (Ff + ff vs. FF) |     |                 | 0.04| 1.7 (1-2.7) |
| Recessive (Ff vs. FF + FF) |   |                 | 0.67| 0.8 (0.4-2)  |
| Over-dominant (Ff vs. FF + ff) | |                 | 0.02| 2.8 (1.1-3)  |
| Allele               |             |                 |     |             |
| F, n (%)             | 175 (69)    | 207 (74)        | 1.0 |             |
| f, n (%)             | 79 (31)     | 71 (26)         | 0.18| 1.3 (0.9-1.9)|
| rs731236(Taq1)       |             |                 |     |             |
| Codominant           |             |                 |     |             |
| TT, n (%)            | 26 (20.5)   | 56 (40.3)       | 1.0 |             |
| Tt, n (%)            | 87 (68.5)   | 66 (47.5)       | 0.002| 2.8 (1.6-5) |
| tt, n (%)            | 14 (11)     | 17 (12.2)       | 0.19| 1.8 (0.8-4.1)|
| Dominant (Tt + tt vs. TT) |         |                 | 0.001| 2.6 (1.5-4.5)|
| Recessive (tt vs. Tt + TT) |   |                 | 0.8 | 0.9 (0.4-1.9) |
| Overdominant (Tt vs. TT + tt) | |                 | 0.001| 2.4 (1.5-4)  |
| Allele               |             |                 |     |             |
| T, n (%)             | 139 (55)    | 178 (64)        | 1.0 |             |
| t, n (%)             | 115 (45)    | 100 (36)        | 0.03| 1.5 (1-2.1)  |
| rs7975232 (Apa1)     |             |                 |     |             |
| Codominant           |             |                 |     |             |
| AA, n (%)            | 43 (33.9)   | 45 (32.4)       | -   | -           |
| Aa, n (%)            | 70 (55.1)   | 77 (55.4)       | 0.85| 1 (0.6-1.6)  |
| aa, n (%)            | 14 (11)     | 17 (12.2)       | 0.72| 0.9 (0.4-2)  |
| Dominant (Aa + aa vs. AA) | |                 | 0.80| 0.9 (0.6-1.6)|
| Recessive (aa vs. Aa + AA) |     |                 | 0.76| 0.9 (0.4-1.9)|
| Overdominant (Aa vs. AA + aa) | |                 | 1   | 1 (0.6-1.6)  |
| Allele               |             |                 |     |             |
| A, n (%)             | 156 (61)    | 167 (60)        | 0.80| 1 (0.7-1.3)  |
| a, n (%)             | 98 (39)     | 111 (40)        | 0.80| 1 (0.7-1.3)  |

**Table 4: Haplotype analysis of Vitamin D receptor rs731236, rs7975232, and rs2228570 polymorphisms in systemic lupus erythematosus patients and control group**

| Haplotype | SLE (n=127) | Control (n=139) | P   | OR (95% CI) |
|-----------|-------------|-----------------|-----|-------------|
| TAF       | 90 (0.354)  | 107 (0.385)     | 0.465| 0.7 (0.4-1.3)|
| taF       | 44 (0.173)  | 47 (0.169)      | 0.908| 1 (0.6-1.7)  |
| tAF       | 28 (0.110)  | 28 (0.100)      | 0.779| 1.1 (0.6-2)  |
| TaF       | 22 (0.087)  | 25 (0.089)      | 0.914| 1 (0.6-1.5)  |
| taf       | 27 (0.106)  | 18 (0.065)      | 0.907| 1.8 (1-3.5)  |
| TaF       | 13 (0.052)  | 25 (0.090)      | 0.105| 0.5 (0.3-1.1)|
| Taf       | 13 (0.052)  | 21 (0.076)      | 0.269| 0.6 (0.3-1.3)|
| tAf       | 16 (0.063)  | 7 (0.025)       | 0.025| 2.7 (1.1-6.8)|

SLE: Systemic lupus erythematosus, OR: Odds ratio, CI: Confidence interval

Various meta-analyses have been performed regarding the association of VDR polymorphisms with SLE; however, the results were different in various races and ethnicities.\[^{19,23,24}\]
associated with SLE. However, there was no significant association between VDR rs1544410, rs2228570, rs7975232, and rs731236 polymorphisms and SLE susceptibility in Caucasians. In the recent meta-analysis of Bae et al., performed on 12 studies (1974 patients and 2506 controls), there was no association between VDR rs2228570, rs731236, and rs7975232 polymorphisms and SLE susceptibility in the overall population and European and Asian populations in allelic, recessive, and dominant models; however, only VDR rs2228570 polymorphism was associated with SLE risk in the above-mentioned models in the Arab population. With regard to different meta-analysis results in Asians, Caucasians, Africans, and Arab population and inconsistent results of the performed meta-analyses (because of low number of studies), further studies in different ethnicities are needed to validate the results of a meta-analysis.

Moreover, we did not find significant association between VDR polymorphisms and SLE manifestations. Kaleta et al. found the relation between rs7975232 AA genotype and the higher levels of ANAs in SLE patients. In a study conducted by Luo et al., VDR rs1544410 polymorphism was associated with SLE and SLE nephritis in Han Chinese patients. Furthermore, they found that the rs1544410B allele might lead to the downregulation of VDR mRNA levels in SLE group. However, Huang et al. showed the association of VDR rs1544410B allele with SLE but not SLE manifestations and laboratory findings or lupus nephritis in Taiwan. VDR rs1544410 and rs2228570 polymorphisms were not associated with SLE in the European population in a study conducted by Monticciolo et al., but they showed the higher concentration of 25(OH)D in patients carrying rs2228570ff genotype. In their study, Mostowska et al. observed no relation between the VDR rs2228570, rs1544410, rs7975232, and rs731236 polymorphisms and SLE; however, despite the results of the current study, they found higher frequencies of rs2228570FF and FF genotypes of VDR gene in SLE patients with renal disease.

In their study, Emerah et al. observed higher frequencies of rs2228570FF, rs7975232AA, and rs1544410BB/BB genotypes as well as aBF and ABF haplotypes in the SLE group. In addition, rs7975232AA, rs1544410BB, and rs2228570FF genotypes were associated with SLE activity and lupus nephritis. The serum 25(OH)D levels were significantly higher in SLE patients with rs2228570FF genotype. There was an association between VDR rs2228570FF genotype and childhood-onset SLE and lupus nephritis and lower serum (25(OH)D) levels in Egyptian patients in a study conducted by Azab et al.

There are some limitations in our study that may affect the results. One of the limitations is relatively small sample size. Moreover, if serum Vitamin D levels in SLE patients and controls had been assayed, the findings would have become more valuable. Since the results of the association between VDR polymorphisms and SLE were different in various races and ethnicities, more investigations are necessary.

**Conclusion**

The present study showed the association between VDR rs2228570 polymorphism and higher risk of SLE in dominant model. The VDR rs731236 polymorphism was associated with higher risk of SLE in dominant and allelic models. There was no association between rs7975232 polymorphism and SLE. The frequency of tAf haplotype of rs731236 polymorphisms was higher in SLE group. In addition, we found that there was no association between VDR polymorphisms and SLE manifestations. Further studies are necessary to evaluate the effects of these polymorphisms on SLE, especially its manifestations in other ethnic groups with the larger sample sizes to confirm or refute our findings.

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**Conflicts of interest**

There are no conflicts of interest.

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