International Journal of Research in Medical Sciences
El Omri N et al. Int J Res Med Sci. 2017 Mar;5(3):811-815
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20170467

Original Research Article

Serum vitamin D and vitamin D receptor gene polymorphism in Moroccan patients with systemic lupus erythematosus

Naoual El Omri[1]*, Fadwa Mekouar[1], Youssef Sekkach[1], Mohamed Jira[1], Mohamed El Qatni[1], Naoufal Assoufi[1], Salaheddine El Khader[1], Taoufik Amezyane[1], Driss Ghafir[1], Rachid Eljaoudi[2]

[1]Internal Medecine B Departement, Military Teaching Hospital Mohamed V, University Mohammed V, Rabat, Morocco, Africa
[2]Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V, Rabat, Morocco, Africa

Received: 21 January 2017
Accepted: 31 January 2017

***Correspondence:**
Dr. Naoual El Omri,
E-mail: elomrinaoual@gmail.com

ABSTRACT

Background: Vitamin D plays an important role in the immunomodulation and could be involved in the development of autoimmune diseases such as systemic lupus erythematous (SLE). The study of the polymorphism of the Vitamin D Receptor (VDR) gene may be of interest in explaining the pathophysiology of SLE.
Methods: In this study, we aimed to examine the characteristics of VDR gene BsmI polymorphism for the first time in Moroccan patients with SLE and their relationship with clinical manifestations of the disease. We also measured the serum level of 25-hyroxyvitamin D_3 to assess its relation to such polymorphism.
Results: The study included 66 SLE patients and 91 healthy controls. Our results showed that there were no differences observed in VDR genotypes and allelic distribution within the two groups. Both groups were in Hardy-Weinberg equilibrium, with no significant P values for the observed and expected genotype frequencies. 25-hyroxyvitamin D3 serum levels were the same in the two groups.
Conclusions: Based on the results of the present study. We cannot verify any association between VDR gene BsmI polymorphism and SLE. This polymorphism could not be regarded as a genetic marker of the SLE. A larger study examining BsmI and other VDR gene polymorphisms is needed.

Keywords: Gene polymorphism, Lupus, Vitamin D, Vitamin D receptor

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic antibody-mediated autoimmune disease characterized by cutaneous, articular, haematological and visceral manifestations. The prevalence of SLE is 30 to 40 cases per 100,000 people in northern Europe and more than 200 cases per 100,000 people in black populations. Women are more affected by SLE than men, especially between the ages of 20-40 years, with a preponderance of 9 to 1.[1]

SLE is a complex multifactorial disease and fully understanding of its etiopathogeny has not been reached yet.

There is enough data nowadays showing a different behaviour of SLE in different populations. Environmental, genetic and hormonal factors have been linked to the onset and evolution of this disease. Many studies demonstrate association between SLE and genes which crucial to immunological response.[2,3] Vitamin D,

in addition to the maintenance of phosphocalcic homeostasis, has other biological activities, in particular in the immune system. It plays an immunomodulatory role on lymphocyte cells by decreasing the production of immunoglobulins by B lymphocytes, T lymphocyte proliferation and cytotoxic function of killer cells.[4,5] It has been demonstrated that patients with SLE have a lower level of 25-hyroxyvitamin D_3 [25(OH)D_3] compared to the healthy controls.[6-8] Active form of vitamin D, 1,25-dihyroxyvitamin D_3, exerts action by binding to the vitamin D receptor (VDR) which acts as a ligand-dependent transcriptional factor. This protein is produced by VDR gene located on the chromosome 12 and many mutations and deletions of this gene have been found in patients with various diseases; these alterations often leading to a disability of VDR to bind 1,25-dihyroxyvitamin D_3. More than 63 polymorphisms have been described; among the most studied polymorphisms, BsmI, FokI, TaqI and ApaI may be responsible in part for variations in the immune balance.[9, 10]

The purpose of this study is to test, for the first time in Morocco, for the presence of an association between VDR gene BsmI (rs1544410) polymorphism and susceptibility to SLE patients. We also measured the serum level of 25 (OH)D_3 to assess its relation to such polymorphism.

METHODS

The study involved 66 Moroccan patients with SLE and 91 healthy controls. All SLE patients were treated in the department of Internal Medicine B, Mohammed V Military Teaching Hospital of Rabat, Morocco, Africa and fulfilled at least four of eleven criteria for SLE classification.[11] The healthy controls did not meet criteria for SLE and other autoimmune diseases. This study was conducted according to the principles of the declaration of Helsinki and was approved by the local Ethical Committee from the Faculty of Medicine and Pharmacy in Rabat, Morocco, Africa.

For vitamin D assays, blood was drawn into a 5ml gel and clot activator tube. After centrifugation at 3000 rpm, serum was collected and stored at -25°C. Serum 25(OH)D_3 levels were measured by Acquity ultraperformance liquid chromatography coupled to a Tandem mass spectrometer (TQD, Waters) in multiple reaction monitoring mode and using deuterated internal standards. Concentrations are reported in ng/mL (to convert in nmol/l, multiply by 2.496).

Genomic DNA for genotyping was extracted from peripheral blood, which was collected in EDTA vacutainers, using a QiaAmp DNA Blood Mini Kit (Qiagen GmbH, Germany). DNA was quantitated by UV absorption at 260 nm and 280 nm and stored at -20°C until analyzed. Genotyping of the BsmI Polymorphism was carried out in Real Time PCR-amplified DNA by allelic discrimination using TaqMan from Applied Biosystems (Foster City, CA, USA). Real Time PCR was performed using 12.5µl TaqMan® Universal PCR Master Mix, 1.25µl SNP Genotyping Assay mix (TaqMan probes) (20x), 10.25µl Dnase Free Water and 1µl DNA (20 ng), to bring the final reaction volume to 25µl. The cycling condition was set as follows: initial denaturation at 95°C for 10 minutes, 40 cycles of 92°C for 15 seconds (denaturation) and 60°C for 1 minutes (annealing/extension). PCR was performed using 7500 fast real time PCR system (applied biosystems). The genotypes were classified as homozygote major allele (GG), heterozygote (GA), and homozygote minor allele (AA),

Statistical analysis was performed with SPSS 13.0 for windows (SPSS, Inc., Chicago, IL, USA) from SLE patients and healthy controls. Quantitative data were expressed as mean ± SD and qualitative data as absolute numbers and percentage. Comparison between variables was performed using the t-test, or the chi square test. Hardy-Weinberg equilibrium (HWE) was determined by Pearson's goodness-of-fit test. In all cases, p values ≤0.05 (two-sided) were considered statistically significant.

RESULTS

Present study included 66 patients with SLE, 64 (97%) were females and 2 (3%) were males and their mean age was 35.5±11,7 years. The control group were age- and sex-matched to patients with SLE (P>0.05) (Table 1). There were no statistical differences between the two groups in serum 25 (OH)D_3 levels.

Table 1: Characteristic and serum 25-hydroxyvitamin D levels of SLE patients and control subjects.

	SLE	Control	P
Number of subjects	66	91	
Female/male	64/2	84/7	0.53
Age (years)	35.5±11.7	33.5±7.1	0.66
25-hydroxyvitamin D (ng/mL)	12.27±8.1	14.1±7.2	0.35

Table 2: Distribution of the VDR BsmI genotypes, alleles in patients with systemic lupus erythematosus and controls and the results of the Hardy-Weinberg Equilibrium (HWE) tests.

	Genotype n (%)			Alleles n (%)	
	GG	GA	AA	G	A
SLE[1] (n=66)	29 (44)	26 (39)	11 (17)	84 (64)	48 (36)
Control[2] (n=91)	38 (42)	34 (37)	19 (21)	110 (60)	72 (40)
Statistics	χ^2=2.11; P=0.38			χ^2=2.37; P=0.20	

[1]HWE: χ^2=2.83; P=0.18, [2]HWE: χ^2=2.07; P=0.23.

Table 2 presents VDR BsmI genotypes, alleles and the results of the HWE in patients with SLE and in control

group. The distribution of genotypes was 44% for GG, 39% for GA, and 17% for AA in patients with SLE and, respectively, 42%, 37%, and 21% in control group. There was no statistically significant difference between these groups (P=0.38). The allelic distribution of G and A was similar within the two groups (P=0.20). Both groups were in Hardy-Weinberg equilibrium, with no significant P values for the observed and expected genotype frequencies.

Table 3: Relationship between BsmI genotypes with clinical, laboratory features, and serum 25-hydroxyvitamin D levels in patients with systemic lupus erythematosus.

	GG n=29	GA n =33	AA n=11	Total N=66	P*
Cutaneous disorder	21/29	27/33	8/11	56/66	0.63
Mucosal ulcers	2/29	4/33	1/11	7/66	0.55
Arthritis	25/29	30/33	8/11	63/66	0.84
Serositis	6/29	7/33	3/11	16/66	0.72
Nephritis	15/29	14/33	5/11	34/66	0.91
Neuropsychiatric disorder	8/29	7/33	2/11	17/66	0.66
Hematologic disorder	19/29	21/33	3/11	43/66	0.61
Anti-dsDNA	23/29	25/33	2/11	50/66	0.58
Anti-Sm	5/29	5/33	1/11	11/66	0.68
ANA presence	25/29	28/33	10/11	63/66	0.73
Serum 25(OH)D, ng/mL	11.53±7.7	12.63±9.1	12.33±7.3	-	0.43

ANA: Antinuclear antibodies; dsDNA: double stranded deoxyribose nucleic acid; Sm: Smith.

Table 3 shows the relationship between VDR BsmI genotypes and clinical manifestations, laboratory profiles of SLE and 25 (OH)D3 serum levels. There is no relationship between BsmI genotypes and studied parameters.

DISCUSSION

VDR polymorphisms and the status of vitamin D may have a role in the development of autoimmune diseases such as SLE due to their immunomodulatory effects. In this study, we investigated the association between the BsmI polymorphisms, 25 (OH)D_3 status and SLE in the Moroccan patients.

Numerous studies have shown that VDR polymorphism can modify the immunomodulatory action of vitamin D, which could have an impact on the clinical picture of SLE.[12,13] VDR gene BsmI polymorphisms have been used as genetic markers to determine their association with SLE in Asian patients.[2,3,14-16] A Japanese study found that the AA genotype might trigger the development of SLE and the GG genotype was associated with lupus nephritis.[17]

In a recent Egyptian study, authors found that the AA genotype was overrepresented in patients with SLE compared to the control group and the homozygous individuals with the AA genotype had a 5.5-fold higher risk for developing SLE.[18] A Taiwanese study also found an increased distribution of the VDR AA genotype in SLE but indicated no association between the frequency of VDR allelic variations and clinical manifestations or laboratory profiles.[19] In present study we did not find statistically significant differences in the frequency of BsmI genotypes and alleles in SLE patients and healthy controls. Present results are consistent with the findings of previous published studies, who reported no association of the BsmI polymorphism with the development of SLE in the Brazilian, Iranian, Thai, and European populations.[9,20,21] It is difficult to explain the divergence of these results, but it is may be due to group size, gene-gene interactions, and environmental factors.

In the other hand, we could not find any significant association between VDR BsmI genotypes or alleles and serum 25(OH)D_3 levels among SLE patients. These results were concordant with previous studies.[18] The mean 25(OH)D_3 serum level was 12.27±8.1 ng/mL in SLE group; all the concentrations were above of the adequate levels (≥30 ng/ml).[22] In SLE patients, the prevalence of vitamin D deficiency ranges from 16% to 96%.[23] Most reports have been designed as cross-sectional studies, the prevalence of vitamin D deficiency among SLE patients is higher than in healthy groups.[24] This may be because people with lupus are sensitive to the sun and have to avoid sun exposure. The kidney failure, and treatment with corticoids may contribute to the vitamin D deficiency in SLE. However, in present study, there were no statistical differences between the two groups in serum 25(OH)D_3 levels; the healthy controls were also vitamin D deficient. Vitamin D deficiency was reported by other studies concerning Moroccan population.[25-27] It is perhaps due to low quality of exposure and covering dress habits is recognized predisposing factor for hypovitaminosis D.[26]

This study is, to the best of our knowledge, the first of its kind in Morocco; however, the relative small sample size was one of our limitations in this study; we suggest that multicentre approaches may be necessary to attain larger sample size. The study of other polymorphisms of VDR gene such as TaqI, ApaI and FokI might be useful to complete our findings.

CONCLUSION

We examined for the first time in Morocco the relationship between the VDR gene BsmI polymorphism, vitamin D status and SLE patients. Our data did not reveal that VDR gene BsmI polymorphism could be regarded as a genetic marker of the SLE. The BsmI genotype frequencies in Moroccan patients with SLE are not different from healthy controls. The serum levels of 25 $(OH)D_3$ were similar between the two groups.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Pons-Estel GJ, Alarcón GS, Scofield L, Reinlib L, Cooper GS. Understanding the epidemiology and progression of systemic lupus erythematosus. Semin Arthritis Rheum. 2010;39:57-68.
2. Lee HS, Bae SC. What can we learn from genetic studies of systemic lupus erythematosus? Implications of genetic heterogeneity among populations in SLE. Lupus. 2010;19:1452-9.
3. Moser KL, Kelly JA, Lessard CJ, Harley JB. Recent insights into the genetic basis of systemic lupus erythematosus. Gene Immun. 2009;10:373-9.
4. Cantorna MT, Snyder L, Lin YD, Yang L. Vitamin D and $1,25(OH)_2D$ regulation of T cells. Nutrients. 2015;7:3011-21.
5. Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M et al. 1,25-dihydroxyvitamin D_3 and interleukin-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol. 2009;183:5458-67.
6. Ritterhouse LL, Crowe SR, Niewold TB, Kamen DL, Macwana SR, Roberts VC et al. Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. Ann Rheum Dis. 2011;70:1569-74.
7. Cutillas-Marco E, Morales-Suárez-Varela MM, Marquina-Vila A, Grant WB. Serum 25-hydroxyvitamin D levels in patients with cutaneous lupus erythematosus in a Mediterranean region. Lupus. 2010;19:810-4.
8. Mandal M, Tripathy R, Panda AK, Pattanaik SS, Dakua S, Pradhan AK et al. Vitamin D levels in Indian systemic lupus erythematosus patients: association with disease activity index and interferon alpha. Arthritis Res Ther. 2014;16:R49.
9. Mostowska A, Lianeri M, Wudarski M, Olesińska M, Jagodziński PP. Vitamin D receptor gene BsmI, FokI, ApaI and TaqI polymorphisms and the risk of systemic lupus erythematosus. Mol Biol Rep. 2013;40:803-10.
10. Erten G, Kalkan M, Gazioğlu SB, Akdeniz N, Ozkok E, Vural B. TaqI, FokI, and ApaI Polymorphisms in the Vitamin D Receptor in Behçet's disease in Turkish population. Dis Markers. 2016;2016:7475080.
11. Tan EM, Cohen AS, Fries JF. The 1982 revised criteria for the classification of systemic lupus erythrematosus. Arthritis Rheum. 1982;25:1271-7.
12. Ceccarelli F, Perricone C, Borgiani P, Ciccacci C, Rufini S, Cipriano E, et al. Genetic factors in systemic lupus erythematosus: contribution to disease phenotype. J Immunol Res. 2015;2015:745647.
13. Vasile M, Corinaldesi C, Antinozzi C, Crescioli C. Vitamin D in autoimmune rheumatic diseases: A view inside gender differences. Pharmacol Res. 2016;31:228-41.
14. Vollmert C, Illig T, Altmüller J, Klugbauer S, Loesgen S, Dumitrescu L et al. Single nucleotide polymorphism screening and association analysis-exclusion of integrin beta 7 and vitamin D receptor (chromosome 12q) as candidate genes for asthma. Clin Exp Allergy. 2004;34:1841-50.
15. Ozaki Y, Nomura S, Nagahama M, Yoshimura C, Kagawa H, Fukuhara S. Vitamin-D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. Nephron. 2000;85(1):86-91.
16. Huang CM, Wu MC, Wu JY, Tsai FJ. Non-association of vitamin D receptor gene start codon FokI polymorphism in Chinese patient with systemic lupus erythematous. J Rheumatol. 2002;29:1211-3.
17. Ozaki Y, Nomura S, Nagahama M, Yoshimura C, Kagawa H, Fukuhara S. Vitamin-D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. Nephron. 2000;85:86-91.
18. Azab SF, Ali YF, Farghaly MA, Hamed ME, Allah MA, Emam AA et al. Vitamin D receptor gene BsmI polymorphisms in Egyptian children and adolescents with systemic lupus erythematosus. Medicine (Baltimore). 2016;95:e5233.
19. Huang CM, Wu MC, Wu JY, Tsai FJ: Association of vitamin D receptor gene BsmI polymorphisms in Chinese patients with systemic lupus erythematosus. Lupus. 2002;11:31-4.
20. Monticielo OA, Brenol JC, Chies JA, Longo MG, Rucatti GG, Scalco R et al. The role of BsmI and FokI vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D in Brazilian patients with systemic lupus erythematosus. Lupus. 2012;21:43-52.

21. Sakulpipatsin W, Verasertniyom O, Nantiruj K, Totemchokchyakarn K, Lertsrisatit P, Janwityanujit S. Vitamin D receptor gene BsmI polymorphisms in Thai patients with systemic lupus erythematosus. Arthritis Res Ther. 2006;8:R48.
22. Holick MF. Optimal vitamin D status for the prevention and treatment of osteoporosis. Drugs Aging. 2007;24:1017-29.
23. Mok CC. Vitamin D and systemic lupus erythematosus: an update. Expert Rev Clin Immunol. 2013;9:453e63.
24. Borba VZ, Vieira JG, Kasamatsu T, Radominski SC, Sato EI, Lazaretti-Castro M. Vitamin D deficiency in patients with active systemic lupus erythematosus. Osteoporos Int. 2009;20:427e33.
25. Handor N, Elalami S, Bouabdellah M, Srifi A, Esselmani H, Benchekroun L et al. Dosage of 25-OH vitamin D: experience of central clinical biochemistry laboratory of Ibn Sina Hospital. Pan Afr Med J. 2014;17:152.
26. Bouaddi I, Rostom S, El Badri D, Hassani A, Chkirate B, Abouqal R, et al. Vitamin D concentrations and disease activity in Moroccan children with juvenile idiopathic arthritis. BMC Musculoskelet Disord. 2014;15:115.
27. El Maataoui A, Biaz A, El Machtani S, Bouhsain S, Dami A, El Maghraoui A et al. Vitamin D status in healthy Moroccan men and women aged 50 years and older: a cross-sectional study. Arch Osteoporos. 2016;11:24.

Cite this article as: El Omri N, Mekouar F, Sekkach Y, Jira M, Qatni ME, Assoufi N, et al. Serum vitamin D and vitamin D receptor gene polymorphism in Moroccan patients with systemic lupus erythematosus. Int J Res Med Sci 2017;5:811-5.