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Research article

Association between vitamin D receptor genes polymorphisms with systemic lupus erythematosus in children

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Abstract

Background: Several vitamin D receptor polymorphisms have been associated with SLE development and clinical manifestations. So, the aim of the study was to evaluate the possible association between the common VDR gene polymorphisms and SLE susceptibility and severity in children. **Patients and methods**: Thirty six lupus children, 29 females (80.6%) and 7 males (19.6 %) were recruited from the Collagen Clinic, Children Hospital, Cairo University between February and November, 2016. Forty three healthy age and sex matched children were included as a control group. Five ml venous blood sample was withdrawn under aseptic conditions from the studied groups. Two ml were put in a sterile tube containing EDTA for genotyping of BsmI (rs1544410), ApaI (rs7975232), TaqI (rs731236) and FokI (rs2228570) polymorphisms by real time PCR. The rest was centrifuged and serum was used for assessment of: serum calcium, vitamin D level, total and intact Parathormone (IPTH) level, and fibroblast growth factor 23(FGF23). All were done by ELISA. **Results**: The serum calcium, vitamin D level and IPTH were significantly lower in patients than that in controls (p=0.001, p=0.001 & p=0.007) respectively, while FGF23 was significantly higher in the patients (p=0.02). The risk of SLE was significantly higher among patients carrying (Fok1 AA) (OR=2.6, & p =0.04). SLICC values >3 were higher in SLE patients with FokI AA and BsmI CT genotypes (p=0.03 & p=0.1 respectively). GACC haplotype was significantly higher in patients (p=0.02, OR=3.24). **Conclusion:** The VDR gene polymorphisms and haplotypes were associated with SLE. FokI AA and BsmI CT genotypes carry a worse prognosis.

Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder with heterogeneous clinical manifestations. Female are the main victims of this disorder with approximately, female/male ratio is 9:1 [1]. The main feature of this disorder is heterogeneity of its clinical characteristics; therefore four out of the 11 criteria defined by the American College of Rheumatology are simultaneously required for its defenitive diagnosis [1]. SLE phenotype can range from different organ manifestations to autoantibody production [2]. The interplay of environmental, genetic and hormonal factors can affect both the phenotype and the progression of this disorder [1, 3]. One of the environmental factors is vitamin D. Although, vitamin D is a critical regulator of bone mineral homeostasis, yet many data declared its immunosuppressive hormonal effect where it inhibits T helper (Th) cell immunoglobulin production. Interferone-γ (IFN-γ) secretion by Th1 cells have been elicited to be suppressed by 1, 25-dihydroxy vitamin D3 ($1,25(OH)_2D3$) which also negatively regulates interleukin-12 (IL-12) production [4].

Long suffering photosensitivity, with consequent sun avoidance may justify the increased risk of low vitamin D status in SLE patients [5, 6].

Fibroblast growth factor-23 (FGF-23), derived from osteocytes and osteoblasts, has been found to facilitate urine phosphate excretion by inhibition of sodium/ phosphate exchangers in renal epithelial cells [7, 8]. FGF-23 exhibits fundamental roles in bone metabolism. It is clamed to inhibit 1α-hydroxylase & enhance 24-hydroxylase with consequent reduction of the active vitamin D ($1,25(OH)_2D3$) level and induction of its inactive metabolite, ($24,25(OH)_2D3$) [7]. The relationships between vitamin D level, FGF-23, Bone mineral density (BMD) and parathyroid hormone (PTH) in patients with SLE have been illustrated [9, 10].

Vitamin D displays its effect through the vitamin D receptor (VDR) [11]. Therefore, polymorphisms in the VDR sequence may have an influence on the gene function and consequently vitamin D3 action. Nevertheless, numerous VDR gene single nucleotide

polymorphisms (SNPs) have been described, yet four namely TaqI, BsmI, ApaI and FokI, have been intensively studied [12]. Controversial results have been reported upon studying the association between VDR polymorphisms and SLE susceptibility [13-15]. So, the aim of the present study was to evaluate the possible association between the common VDR gene polymorphisms (TaqI, BsmI, ApaI and FokI), vitamin D3 levels, FGF-23 levels and SLE susceptibility and severity in children complaining of SLE.

Patients and Methods

The patients

This a case–control study included thirty six lupus patients, 29 female (80.6 %) and 7 males (19.6 %) were recruited from the Collagen Clinic, Children Hospital, Cairo University between February and November, 2016. The SLE patients were diagnosed according to the 1997 and 2012 revised American College of Rheumatology (ACR) criteria for SLE) [16]. Forty three healthy age and sex matched children were included as a control group. The mean age of the SLE patients was 15.09±1.7 years and the mean disease duration was 9.9±2.24 years. The mean age of the healthy control was 14.6±8.3 years.

Disease activity was assessed using the SLE Disease Activity Index (SLEDAI) [17]. Cumulative damage was evaluated with the Systemic Lupus International Collaborative Clinics (SLICC)/ACR Damage Index [18].

Through history, clinical examination and laboratory investigation of the patients (kidney involvement, central nervous system (CNS) involvement, peripheral nervous system (PNS) involvement, articular involvement, malar rash, photosensitivity, double-stranded DNA (dsDNA), leucopenia, lymphopenia, pericarditis, pleuritis, lung, myositis, ascitis, serositis, haematological and skin involvement) were established using a standard protocol according to revised American College of Rheumatology (ACR) glossaries for SLE classification criteria and European League Against Rheumatism (EULAR) recommendations for neuropsychiatric, nephritis and SLE management [19-21].

All the lupus patients had controlled blood pressure with medication. They were all on corticosteroid medication, 50% on immunosuppressive drugs and 10.6% on chloroquine in addition according to her or his condition severity. All the patients were on regular vitamin D and calcium supplementation.

Blood samples collection

Five ml venous blood samples were withdrawn under aseptic condition from the studied groups. Two ml were put in a sterile tube containing EDTA, and were stored at – 80ºC till DNA extraction for genotyping of BsmI (rs1544410), ApaI (rs7975232), TaqI (rs731236) and FokI (rs2228570) polymorphisms. The rest was put in clean tubes centrifuged, and the obtained serum aliquoted and stored at -20°C for further assessment of the following: serum calcium, vitamin D levels, total and intact Parathormone (PTH) level and FGF-23 level.

Laboratory investigation

Laboratory investigation were done for all cases and controls including serum levels of Calcium, Phosphorus and Alkaline phosphatase using the automated clinical chemistry analyzer (Olympus AU 400 analyzer-Germany). ELISA kit for; FGF-23 [Uscan Life Cat.no. #ED746h (Ray Biotech, inc., USA)], iPTH [intact DRG International, Inc., USA. (EIA- 3645)], PTH (BioVendor, Cat.no.R15003R) and Vit. D [The DRG 25-OH Vitamin D (total) ELISA Kit EIA-5396; DRG Diagnostics GmbH, German] were performed and all were done in the laboratory of NRC in Egypt.

DNA was extracted using QIAamp DNA Blood Mini Kits -50- Catalog no.51104 supplied by QIAGEN. DNA concentration was determined by Nano Drop 2000c Spectrophotometer (Thermo Fisher).

The BsmI, TaqI, FokI and ApaI polymorphisms were detected by polymerase chain reactions (PCR) using the Quantistudio 12 Flex real- time PCR system (Applied Biosystems, CA 94404, USA). Genotyping for the single nucleotide polymorphisms (SNPs) of BsmI C/T (rs15444410), TaqI A/G (rs731236), FokI A/G (rs2228570) and ApaI G/T (rs7975232) were performed using the TaqMan genotyping protocol (Applied Biosystems, Foster City, CA, USA).

Real time PCR was performed using 10 µl TaqMan Universal Genotyping Master Mix, 1 µl TaqMan SNP Genotyping Assay, 8 µl Dnase free water and 1µl DNA (20-30 ng) to bring the final reaction volume 20 µl. The PCR assay was carried out according to manufacturer's instructions including one step of 10 min at 95°C followed by 40 cycles of DNA denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Final products were analyzed by Taq Man Genotyper software.

Written informed consent was taken from the guardians of all children. The study was approved by National Research Centre and Cairo University ethical committee Centre according to World Medical Association Declaration of Helsinki (2013) [22].

Statistical analysis

Data were coded and entered using the statistical package SPSS version 15. Data was summarized using mean, standard deviation, for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between quantitative variables were done using independent Student t-test. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5[23]. Genotype and allele frequencies were compared

between the disease and the control groups using chi-square tests.

The VDR gene variants were evaluated for deviation from Hardy–Weinberg equilibrium by comparing observed and expected genotype frequencies by means of Chi square (x2) test in case and control groups. The same test was used for determination of the haplotypes. Odds ratio (OR) with 95% confidence intervals was calculated. Correlation between various variables was done using Pearson correlation coefficient. P-values less than 0.05 were considered as statistically significant.

Results and Discussion

Results

The demographic and clinical features of the SLE patients according to revised ACR criteria for classification of SLE, were presented in table 1.

Table 1. The demographic and clinical data of the systemic lupus patients

Patients parameters	Frequency	%
Number	36	
Males	5/36	13.9
Females	31/36	86.1
Mucocutaneous affection	15/36	41.7
Articular affection	24/36	66.7
Nervous system affection	8/36	22.2
Arrhythmias	3/36	8.3
Malar rash	17/36	47.2
Discoid	3/36	8.3
Photosyntivety	19/36	52.8
Oral ulcer	7/36	19.4
sororities	17/36	47.2
Seizure	6/36	16.7
RF	5/36	13.9
Psychosis	7/36	19.4
ANA	36/36	100
Anti DNA	24/36	66.7
APS	4/36	11.1
ITP	5/36	13.9
Renal Biopsy		
Type II	4/36	11.1
Type III	11/36	30.6
Type IV	16/36	44.4
Type V	3/36	8.3
Focal necrotizing GN	2/36	5.6

RF=Rheumatoid Factor, ANA=Anti nuclear antibody, APS= Antiphospholipid syndrome, ITP= Idiopathicthrombocytopenia

The comparison between systemic lupus patients and controls regarding demographic and laboratory data were presented in table 2. We found that, the serum calcium, vitamin D level and intact parathrmon hormone (IPTH) were significantly lower in patients than that in controls (p=0.001, p=0.001 & p=0.007) respectively. While FGF-23 was significantly higher in the patients (p=0.02).

Normal range for 25-hydroxy vitamin D is 30-60 ng/ml. Insufficiency was diagnosed at level <30 ng/ml, deficiency at level <20 ng/ml and severe deficiency at level <10 ng/ml [24]. We found that, 28.6% of patients had critically low vitamin D <10ng/ml, 46.6% had level 10-20, 25 % had levels 20-30 ng/ml and no one had level >30 ng/ml. The comparison between patients and controls according to vitamin D level were shown in figure 1.

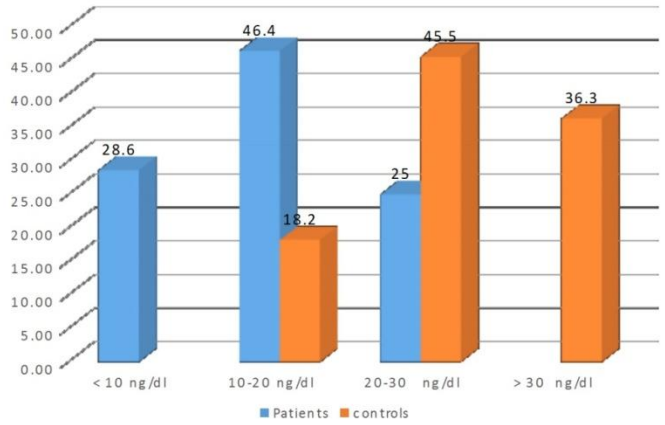

Figure 1. Distribution of patients and controls according to vitamin D level.

No correlation was found between vitamin D level, PTH (total and intact) and FGF-23.

Our result showed that the genotype distributions were in Hardy–Weinberg equilibrium in each studied group.

When assessing the frequency of VDR polymorphisms in SLE patient compared to controls, we found that the risk of SLE was significantly higher among patients carrying FokI AA (OR=3.886, & p =0.025). The risk of SLE was significantly higher among patients carrying A alleles of FokI (OR=2.32 &p =0.01) as shown in table 3.

SLICC values >3 were higher in SLE patients with FokI AA and BsmI CT genotypes (p=0.03 & p=0.01 respectively).

The association between VDR polymorphisms and disease expression (mucocutaneous, articular affection, nervous system affection, arrhythmias, malar rash, discoid, photosyntivety, oral ulcer, serositis, seizure, psychosis, RF, ANA, Anti DNA) were analyzed in the patients. No associations were found between VDR SNPs and all of the clinical features previously referred to, except with mucocutaneous affection for (BsmI CC) genotype (OR=7.1, P =0.006); (ApaI AA) genotype (OR=8.8, P =0.003); and (FokI AA genotype) (OR =8.8, P =0.003); and oral ulcer for (ApaI CC) genotype (OR=11.56, P =0.01) as shown in table 4.

According to genotypes distribution, no significant differences were found between the level of vitamin D, Parathrmon hormone or FGF-23 among the different VDR SNPs.

Haplotype frequencies of VDR (Taq I ,Fok I, Bsm I, and Apa I) polymorphisms in SLE patients versus controls were (16.7% Vs 5.8%, p=0.02) for GACC, (0% Vs 12.8%, p=0.002) for AGCA and (0% Vs 8.1, p=0.016)

for AGCC, respectively. On other hand, AATC haplotype was absent in both patients and controls (Table 5).

We investigated the association of VDR gene haplotypes and all the data of SLE in table 1. We found that, GACC is significantly higher in patients complaining of mucocutaneous affection (P=0.001). Otherwise, no associations were found between different haplotypes and all the clinical features previously referred to.

We did not found an influence of VDR haplotypes on $25(OH)_2$ D3 levels (p=0.227).

Table 2. The demographic and laboratory data of the studied groups

	Patients	Controls	P value
Age(years)	15.09±9.9	14.6±8.3	0.8
Durations of disease(years)	9.9±2.24	-	
BMI	20.7±4.16	19.5±3.5	0.2
SBP	106.6±15.9	110.4±8.7	0.3
DBP	74.1±13.6	79±7.2	0.07
Males /Females	5/31	8/35	0.6
Serum creatinine (mg/dl)	0.8±0.9	0.7±0.1	0.7
Serum calcium (mg/dl)	6.7±3.4	10.1±0.37	0.001
Serum Phosphorus(mg/dl)	3.2±1.6	4.2±0.16	0.02
Serum Alkaline phosphatase(U/L)	161.6±139	138±121	0.6
Vitamin D(ng/ml)	15.77±6.15	27.85±6.67	0.001
Human PTH(pg/ml)	49.3±28.2	52.1±32.1	0.8
Intact PTH(pg/ml)	56.7±28.4	88.69±39.03	0.007
FGF(pg/ml)	66.05±108	19.33±14.28	0.02

BMI=Body mass index, SBP=systolic blood pressure, DBP= diastolic blood pressure, PTH= Parathrmon hormone, FGF23= Fibroblast Growth Factor 23.

Table 3. Genotype and phenotype frequencies of VDR polymorphisms in patients and a control population

		Patients		Controls		P value	OR	95%Confidence Interval	
		N	%	N	%				
TaqI									
Genotypes	AA	4	11.1%	14	32.6%	.124	.352	.093	1.330
	AG	19	52.8%	13	30.2%	.258	1.799	.651	4.971
	GG	13	36.1%	16	37.2%		Reference		
Alleles	A	27	37.5%	41	47.7%	.199	.659	.348	1.246
	G	45	62.5%	45	52.3%		Reference		
FokI									
Genotypes	AA	16	44.4%	10	23.3%	.025	3.886	1.191	12.681
	AG	13	36.1%	16	37.2%	.245	1.973	.628	6.199
	GG	7	19.4%	17	39.5%		Reference		
Alleles	A	45	62.5%	36	41.9%	.010	2.315	1.219	4.395
	G	27	37.5%	50	58.1%		Reference		
Bsm1									
Genotypes	TT	7	19.4%	3	7.0%	.154	3.051	.659	14.137
	CT	16	44.4%	23	53.5%	.847	.910	.347	2.384
	CC	13	36.1%	17	39.5%		Reference		
Alleles	T	30	41.7%	29	33.7%	.304	1.404	.735	2.683
	C	42	58.3%	57	66.3%		Reference		
ApaI									
Genotypes	CC	7	19.4%	6	14.0%	.571	1.441	.407	5.102
	AC	13	36.1%	16	37.2%	.879	.926	.346	2.480
	AA	16	44.4 %	21	48.8%		Reference		
Alleles	C	26	36.1%	28	32.6%	.639	1.171	.606	2.264
	A	46	63.9%	58	67.4%		Reference		

Table 4. Systemic lupus erythematosus patients' clinical manifestation (%) accordingly to SNPs VDR gene associated genotype

	Bsm I rs1544410			Apa I rs7975232			Taq I rs 731236			FokI rs 731236		
	CC (13)	CT (16)	TT (7)	AA (16)	AC (13)	CC (7)	AA (4)	AG (19)	GG (13)	AA (16)	AG (13)	GG (7)
Patients feature												
Mucocutaneous	9[a]	4	2	11[b]	3	1	2	7	6	11[d]	2	2
Articular affection	10	9	5	11	7	6	2	11	11	12	7	5
Nervous system affection	2	4	2	5	1	2	0	5	3	2	3	3
Arrhythmias	0	2	1	3	0	0	0	2	3	0	2	1
Malar rash	7	8	2	5	7	5	3	9	5	10	5	2
Discoid	1	2	0	2	0	1	1	1	1	2	0	1
Photosyntivety	7	8	4	9	5	5	2	11	6	11	5	3
Oral ulcer	4	3	0	2	1	4[c]	0	3	4	4	2	1
serositis	8	6	3	6	6	5	3	7	7	8	6	3
Seizure	0	4	2	5	1	0	0	3	3	0	4	2
Rheumatoid Factor	2	2	1	2	2	1	0	2	3	2	1	2
Psychosis	4	3	0	2	3	2	0	4	3	4	2	1
Anti nuclear antibody	13	16	7	16	13	7	4	19	13	16	13	7
Anti DNA	9	11	4	12	7	5	1	13	10	10	9	5
Antiphospholipid syndrome (APS)	2	1	1	2	2	0	2	2	1	1	3	0

SNP: single nucleotide polymorphism; VDR: vitamin D receptor; OR: odds ratio; CI: confidence interval.
[a]OR=7.1, CI=1.6-31.7, P value=0.006, [b]OR=8.8, CI=1.9-40.33, P value=0.003, [c]OR=11.56, CI=1.7-78.46, P value=0.01, [d]OR =8.8, CI=1.9-40.3, P value=0.003.

Table 5. Haplotype frequencies of VDR polymorphisms in SLE patients and controls

Taq1	Fok1	Bsm1	Apa1	Patients		Controls		P value	OR	95% CI	
				Count	%	Count	%			Lower	Upper
A	A	C	A	16	22.2%	14	16.3%	0.343	1.469	0.662	3.263
A	A	C	C	5	6.9%	5	5.8%	1	1.209	0.336	4.353
A	A	T	A	3	4.2%	1	1.2%	0.331	3.696	0.376	36.325
A	A	T	C	0	0%	0	0%	---	---	---	---
A	G	C	A	0	0%	11	12.8%	0.002	---	---	---
A	G	C	C	0	0%	7	8.1%	0.016	---	---	---
A	G	T	A	2	2.8%	0	0%	0.206	---	---	---
A	G	T	C	1	1.4%	3	3.5%	0.626	0.390	0.040	3.830
G	A	C	A	6	8.3%	9	10.5%	0.649	0.778	0.263	2.3
G	A	C	C	12	16.7%	5	5.8%	0.028	3.24	1.083	9.689
G	A	T	A	1	1.4%	1	1.2%	1	1.197	0.074	19.485
G	A	T	C	2	2.8%	1	1.2%	0.592	2.429	0.216	27.344
G	G	C	A	2	2.8%	5	5.8%	0.456	0.463	0.087	2.461
G	G	C	C	1	1.4%	1	1.2%	1	1.197	0.074	19.485
G	G	T	A	16	22.2%	17	19.8%	0.705	1.160	0.538	2.5
G	G	T	C	5	6.9%	6	7.0%	0.994	0.995	0.291	3.406

Discussion

Broad scope on the role of vitamin D function have been elucidated upon identification of VDR over three decades ago with description of more than 50 targets [11].

The general immunologic effect of D3 includes: down regulation of Th1 immune responses, modulation of dendritic cells differentiation, depressing activated B cell proliferation, up regulation of regulatory T cells and, particularly, preserving immune response [25].

Although, our patients were on regular vitamin D and calcium supplementation. All of them had calcium and vitamin D deficiency. 28.6% of the patients had critical low level of vitamin D (< 10) in agreement with many other studies [9, 26, 27].

Long lasting Photosensitivity with consequent protection against sunlight, impairment of renal function and the current production of auto antibodies against vitamin D that probably contribute to its clearance were risk factors for vitamin D insufficiency and deficiency. It is clearly that treatment with calcium and vitamin D did not completely protect against vitamin D deficiency [28, 29]. Also, the chronic use of glucocorticoids and chloroquine were risk factors for vitamin D deficiency in SLE patients [4].

FGF-23 is a 32-kDa protein with 251 amino acids that is synthesized and secreted by bone cells, mainly osteoblasts. FGF-23 induces urinary phosphate excretion by suppressing the abundance of the Na/Pi II a and II c cotransporters in the brush border of renal proximal tubules [30]. FGF-23 exhibits critical roles in bone metabolism. Where FGF-23 not only decreases the level of active vitamin D ($1,25(OH)_2D_3$) but also increase its inactive metabolites ($24,25(OH)_2D_3$) through 1α-hydroxylase inhibition and enhancement of 24-hydroxylase, respectively [30, 31]. In addition, parathrmon hormone is another target for FGF-23 by inhibiting its the production in vitro [32]. Celik *et al.,* and Mirza *et al.,* [33, 34] found that, elevated FGF-23 level in SLE patients who treated with cyclosporine A and/or Glucocorticoids and lead to decreased bone turnover and osteoporosis in the long run. Our results showed significant elevation of GF23 than controls in agreement with Lai *et al.,* [35]. Increased production of FGF-23 have been demonstrated in other circumstances such as -phosphate diet, chronic renal failure and cumulative dose of cyclosporine A [36]. These factors may present alone or together in our patients, that can explain the high levels of FGF in them.

The immune modulatory feature of vitamin D have been high lightened with its expression in multiple types of immune cell, such as activated T cells, monocytes and dendritic cells, accentuating its prospective relevance in the susceptibility and development of many autoimmune diseases [37] .

VDR protein structure and transcriptional activity pretended to be affected by the FokI polymorphism [38]. Therefore, immunomodulatory action of vitamin D can be modified by VDR gene polymorphisms with consequent impact on SLE clinical manifestations and autoantibody production [39].

Positive association between SLE susceptibility and BsmI and FokI VDR polymorphisms have been proclaimed in Asian population studies [40, 41]. In Egypt, Emerah *et al.,* found a significant association between FokI FF, BsmI Bb, Bb and ApaI AA polymorphism and SLE disease [42]. We found that Fok1 AA genotype were significantly associated with Egyptian SLE children, while no association were found with BsmI, ApaI and TaqI genotypes.

Conversely, other studies showed no significant differences in VDR Fok I allele and genotype frequencies in patients with SLE and healthy controls [43-45].

Carvalho *et al.,* [46] found that, FokI CT and TaqI TT genotypes seems to be a risk of long-term cumulative damage in SLE. Our result showed that, the presence of FokI AA and BsmI CT genotypes were risk factors for higher long term cumulative damage and worse prognosis. These finding can explain that, not only the disease activity itself is accused, but the damage is multifactorial.

When considering VDR SNPs and SLE clinical manifestations, the following associations have been found between mucocutaneous affection and each of BsmI CC; ApaI AA; and FokI AA genotypes, also we found an association between oral ulcer and ApaI CC genotype. Our result were in agreement with Carvalho *et al.,* [46] who found that, there was an association of between skin affection and ApaI TC genotype frequency. Emerah *et al.,* [42] elucidated significant linkage between VDR gene polymorphism; ApaI AA, BsmI BB, and FokI FF genotypes and lupus nephritis as well as higher activity score of Egyptian SLE. In contrary to our results, Monticielo *et al.,* [45], and Mostowska *et al.,* [43] failed to demonstrate any impact of BsmI, FokI or ApaI polymorphisms on either the clinical or the laboratory expressions of SLE.

The studies on the distribution of VDR polymorphism had conflicting results, and this could be attributed to the inter play of many factors; ethnicity of populations, sample size, environmental participators and geographical location [47].

According to genotypes distribution, no significant differences were found between the levels of vitamin D among the different VDR SNPs. Our results were parallel with Emerah *et al.,* [42] who found that, 25(OH) D level were not significantly different in ApaI or BsmI genotypes among SLE patients. However, they found that vitamin D was significantly higher in patients with ff genotype when compared with those carrying FF genotype of Fok1 which unlike our results.

The significant higher of GACC haplotype and the absence of haplotype AGCA and AGCC in SLE, also the association of GACC with mucocutaneous affection may suggest that, their were an association between haplotypes and the disease-risk. Our result is in agreement with Emerah *et al.,* and Luo *et al.,* [42, 47]. On other hand, J de Azeveˆ *et al.,* [47] reported that, the haplotype combination did not display any impact on either the SLE susceptibility or its clinical manifestations.

We found a significant association between the haplotype GACC of TaqI, BsmI, FokI and ApaI genes polymorphism combination and the incidence of SLE. however, in the same time, we failed to detect the any associations of VDR TaqI, BsmI and ApaI with SLE when analyzing each polymorphism individually. Our

results go with Luo *et al.,* and Acikbas *et al.,* [47, 49]. Acikbas *et al.,* [49] suggesting that the haplotypes are tags which are sum of the marker polymorphisms in a gene. Individually, SNPs and genotypes may not have significance and association, but together as haplotype they can. They added that, the haplotype is a special final VDR protein changing RNA splicing, processing and editing with consequent impact on disease susceptibility. Not only can the receptor protein folding be changed by the haplotype but also the receptor affinity and binding specifically to DNA response elements or other nuclear receptors. Therefore, the regulatory function of VDR protein, and its final haplotype may be disrupted complementing the other disturbing factors. However, this finding may be confirmed by studying the interaction as well as the activity characteristics of VDR protein in these subjects.

Our study spotlights the important of haplotypes that must be corroborative by many studying interaction and activity characteristics of VDR protein in these subjects. VDR SNPs and haplotype associated with SLE clinical manifestations in this study may reinforce the suggestion that VDR should be implicated in SLE etiopathogenesis.

The limited size of our sample may have lacked the ability to find more possible association between VDR polymorphisms and SLE. Thus, further studies in larger numbers of subjects are necessary to reinforce our results and to clarify the role of VDR polymorphisms and haplotype in SLE.

Conclusion

There is an association between VDR genes polymorphisms and haplotypes and SLE. The presence of AA genotype of FokI and CT genotype of BsmI seems to confer a worse prognosis

Recommendation

Further study on a larger number of patients and controls to prove the results

Conflict of interest

None

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