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Immunological analysis of vitamin D receptor gene expression in Egyptian patients with rheumatoid arthritis: relation to disease activity and functional disability

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Abstract

Background: Vitamin D (vit D) deficiency has recently been associated with risk of development of rheumatoid arthritis (RA). The aim of this research was to assess vitamin D receptor (VDR) gene expression in Egyptian patients with RA and its relation with the inflammatory state, disease activity, and functional disability.

Results: RA patients had significantly lower vit D level and VDR gene expression compared to controls (mean ± 17.0 ± 6.65, 20.73 ± 8.42 ng/ml, $p < 0.05$ and 3.29 ± 5.47, 14.22 ± 12.60, $p < 0.001$ respectively). Receiver operating characteristic (ROC) curve analysis for VDR gene expression in RA patients revealed (area under the curve 0.826, cutoff value for low VDR expression 1.05 ng/ml). Patients with low VDR expression had significantly higher ESR, CRP, double positive RF+ anti-CCP+, DAS28, and MHAQ ($p < 0.001$, $p = 0.001$, $p < 0.05$, $p < 0.001$, $p < 0.001$) respectively.

Conclusion: Vitamin D and VDR expression are significantly lower in RA patients than controls. Patients with low VDR gene expression had significantly higher disease activity and disability. This may suggest that apart from low vit D levels, low VDR expression is associated with inflammatory process and it has a potential role in RA pathogenesis and prognosis. Further multicenter studies are needed to confirm these findings.

Keywords: RA, Egyptian, VDR gene expression, Vitamin D, DAS score, MHAQ

Background

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases (AIDs) worldwide affecting approximately 0.5 to 1% of total population [1]. If not treated early, RA as a chronic inflammatory disease can lead to joint destruction, systemic complications, progressive disability, or early death [2]. Currently, it is well documented that RA patients have an increased risk of cardiovascular events compared to general population, that to date is the most common cause of death in RA [3].

Like many other AIDs, RA pathogenesis remains incompletely understood. It has been suggested that several environmental factors can trigger the development of RA in genetically predisposed individuals. Therefore, a fundamental need to detect risk factors for early RA diagnosis as well as to determine a novel potential candidate of treating refractory RA is required.

Vitamin D, a secosteroid hormone has a key role in calcium-phosphate homeostasis and bone metabolism [4]. In addition vit D contributes to the regulation of immune system which is referred to as pleotropic effects (extra skeletal effects) of vit D.

Vit D deficiency has been associated previously with risk of the development of RA [5, 6], cardiovascular diseases (CVD), osteoarthritis, infections, as well as type 1

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diabetes, inflammatory bowel disease, and connective tissue disorders [7].

Calcitriol (1, 25 $(OH)_2$ D3), the most active form of Vit D exerts many of its actions through interaction with vitamin D receptor (VDR), which is widely distributed in various immune cells such as T and B lymphocytes, macrophages, monocytes, dendritic cells (DC), and natural killer cells [8]. VDR is a member of the steroid hormone receptor superfamily located on chromosome 12 (12q12-q14). Four adjacent restriction fragment length polymorphisms for BsmI, ApaI, FokI, and TaqI at the 3′ end of VDR regulate transcription of target genes [9].

Most of immunomodulatory effects of vitamin D3 are elicited by genomic mechanisms in which vitamin D3 binds to intracellular VDR that heterodimerizes with retinoic X receptor, then this complex is translocated to nucleus to affect a variety of genes [10]. At the molecular level, vitamin D3 inhibits the accumulation of mRNA for interleukin-2, interferon-γ, and granulocyte-macrophage colony-stimulating factor. At the cellular level, it inhibits T helper (Th-) 1 and Th17 responses, while promoting Th2 cytokines and regulatory T cells (Treg) ability to suppress T cell proliferation, as well as reducing its induction of immunoglobulin production by B cells [11].

In addition, calcitriol inhibits the differentiation and maturation of DC and promotes their apoptosis, thus preventing their transformation into antigen-presenting cells leading to suppression of DC-dependent T cell activation [12].

The role of VDR expression in the development of RA and its clinical manifestations has been demonstrated with conflicting results [13–16]. The aim of this research was to assess vit D receptor gene expression in Egyptian patients with RA and its relation with the inflammatory activity, disease activity and functional disability in order to clarify importance of potential therapeutic effects of vit D supplementation in RA patients.

Methods

Study population

A case-control study was conducted in the period from June 2018 to June 2019 on 100 subjects classified into two groups: 50 RA patients classified according to the 2010 ACR/EULAR classification criteria for RA [17] recruited from Internal Medicine and Rheumatology Department and outpatient clinic in Alexandria University Hospital where the research was conducted; and 50 control subjects with no prior personal or family history of RA or any autoimmune diseases and had no clinical findings suggestive of immunological disorders or chronic infections. RA patients suffering from concomitant autoimmune disease or viral hepatitis B or C were excluded. No one of RA patients or controls was on previous intake of vit D.

Ethics approval and consent to participate

The study received approval from the Medical Ethics Committee of the Faculty of Medicine, from the university where the research was done and the practical work has been carried out in accordance with Helsinki Declaration [IBR NO: 00007555-FWA NO: 00015712, Serial number: 0303546]. All subjects enrolled in this study provided a written informed consent after explaining the nature, steps, and aim of the study. Details that might disclose the identity of the subjects under study were omitted.

History taking and clinical examination

Personal history was taken from all participants, who were subjected to thorough clinical examination. Disease activity score (DAS) in 28 joints (DAS 28 score) was used in measuring disease activity in RA patients. DAS 28 predefined cut offs for remission, mild, moderate and severe disease activity were used [18]. The patients' disease-related disability was assessed using MHAQ assessment questionnaire [19].

Laboratory investigations

Peripheral venous blood samples were aseptically withdrawn by venipuncture into sterile vacutainers. Complete blood picture, liver and kidney functions tests, and erythrocyte sedimentation rate (ESR) were assessed for each individual included in the current study.

Afterwards, total serum 25 OH Vit D analyses were done using the ELFA technique (enzyme-linked fluorescent assay) on VIDAS (BIOMERIEUX).

The report from the International Osteoporosis Foundation (IOF) Nutrition Working Group defined vit D threshold to be : < 50 nmol/L (< 20 ng/ml) is deficient, 50–< 75 nmol/L (20–< 30 ng/ml) insufficient, > 75 nmol/L (> 30 ng/ml) normal and potential toxicity if > 100 ng/ml [20]. The latest IOF classification 2018 for definition of sufficient vit D level < 10 ng/ml is deficient, 10–20 ng/ml is insufficient and > 20 ng/ml is sufficient.

Immunological analysis

Sera were separated immediately and stored at − 20 °C and C-reactive protein (CRP) was measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA). Rheumatoid factor (RF) was determined on BN-Prospec using the nephelometry technique which detects IgM-RF and the normal range was from 0 to 20 IU/ml. Anti-cyclic citrullinated peptide (anti-CCP) was detected in serum samples using ELISA test. The assay was performed according to the manufacturer's instructions. A concentration > 20 IU/ml was considered positive.

Whole blood RNA extraction

After collection of blood samples, total RNA was extracted from the whole blood immediate lysing PureLink RNA Mini Kit (USA) (Invitrogen life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and purity of RNA were measured at 230, 260, and 280 nm using the NanoDrop2000 Spectrophotometer (Thermo Scientific, USA) where A260:A230 ratio greater than 1.8 and A260:A280 ratios greater than 2.0 were considered indicators for high-quality RNA isolated.

Using Invitrogen High Capacity cDNA RT kit (Thermofisher), single-stranded cDNA was synthesized from the purified RNA according to the manufacturer's protocol using Thermo Fisher Scientific-US (Applied biosystem).

RT PCR-based detection of expression of VDR

The real-time PCR amplification of VDR was performed using the RotorGene Q (Qiagen, Germany) Real-Time PCR System. VDR expression was quantified in both patients and controls using TaqMan Gene Expression assay probes. We used beta actin gene from the same samples as our reference internal control gene for normalization (VDR: Hs00172113_m1, B-actin: Hs99999903_M1).

Four microliters of cDNA was added to the PCR reaction mixture with 10 uL Taqman Gene Expression Master Mix, 1 uL assay and 5 uL H2O in a 20 uL reaction. Each sample on RT-qPCR was analyzed in duplicate. The PCR thermal cycling profile was prerun for 2 min at 50 °C followed by incubation at 95 °C for 10 min and then 40 PCR cycles of 95 °C for 15 s followed by 60 °C for 1 min. The relative expression levels of the VDR were determined using the 2−ΔΔCT method as 2−ΔCT (ΔCT = Cttarget-Ctb-actin) [21].

Statistical analysis of the data

Statistical analysis was carried out using IBM SPSS software package version 20.0. Data were presented as number and percentage for categorical variables and mean and standard deviation (SD) for continuous variables. The power of the study was calculated using G-Power 3.1.9.2 software using the mean VDR gene expression of 3.29 ± 5.47 in RA patients vs. 14.22 ± 12.60 in the control group, sample size of 100 subjects (50 cases and 50 controls) at 5% level of significance. The effect size is 1.125 and the calculated power of the study is 99.98%.

For comparisons between studied groups, the Student *t* test was used for normally distributed quantitative variables and the Mann-Whitney test for non-normally distributed variables. For qualitative variables, the chi-squared, Fisher's exact, and Monte Carlo tests were used. Analysis of ROC curve for VDR gene expression as a predictor of RA was done. All results were interpreted at the 5% level of significance.

Results

Subjects' demographic and clinical characteristics

Regarding socio-demographic characteristics: both sexes were equally represented, the mean age of participants in both groups were equivalent (39.88 ± 12.38 and 39.82 ± 9.26 years) and the marital status was comparable, most of the studied subjects were married. Nearly one fifth of either group was smoker (18% of patients and 20% of controls). The mean body mass index (BMI) among patients was 30.46 ± 6.83 compared to 30.64 ± 7.51 in controls. The differences in the socio-demographic variables between patients and control group were statistically insignificant (Table 1).

As regards clinical characteristics of RA, the mean age at diagnosis was 33.45 ± 9.09 years. The average duration of disease was 6.23 ± 6.30 years. DAS28 ranged from 2.1 to 6.88 with a mean of 4.27 ± 1.27 and the mean disease-related disability (MHAQ) score was 1.59 ± 0.62. The prescribed medications included corticosteroids (60%) of patients, SSZ (64%), MTX (44%), LNF (38%), HCQ (76%), AZA (8.3%), and NSAIDs (4%).

Table 1 Comparison between RA patients and control group according to socio-demographic characteristics

Studied variables	RA patients (*n* = 50)		Control group (*n* = 50)		*p*
	No.	%	No.	%	
Sex					
Male	13	26.0	13	26.0	1.0
Female	37	74.0	37	74.0	
Age (years)					0.978
Min.–max.	18–67		24–60		
Mean ± SD	39.88 ± 12.38		39.82 ± 9.26		
Marital status					
Married	45	90.0	43	86.0	0.538
Single	5	10.0	7	14.0	
Smoking					0.799
Yes	9	18.0	10	20.0	
No	41	82.0	40	80.0	
BMI (kg/m^2)					0.90
Min.–max.	18–45		17–46		
Mean ± SD	30.46 ± 6.83		30.64 ± 7.51		
< 18.5 (underweight)	1	2.0	2	4.0	0.871
18.5–< 25 (normal weight)	9	18.0	11	22.0	
25–< 30 (overweight)	10	20.0	8	16.0	
30–< 40 (obese)	24	48.0	21	42.0	
≥ 40 (severe obesity)	6	12.0	8	16.0	

About two thirds (60%) of patients received corticosteroids in addition to DMARDS while 40% took one or more DMARDS only. More than half (52%) of the patients received a combination of two DMARDS, 34% three, and in 14% only one medication was prescribed.

Reduced vitamin D level and VDR gene expression RA patients

Table 2 demonstrates the differences in vit D and VDR gene expression between both groups. More than three quarters (78%) of RA patients and half of the controls had Vit D deficiency; this difference was statistically significant ($p = 0.009$). Moreover, the median level of VITD and VDR gene expression was significantly lower in RA patients ($p = 0.008$ and < 0.001, respectively)

Analysis of ROC curve for VDR gene expression as a predictor of RA (Table 3, Fig. 1) revealed that area under the curve was 0.826, the proposed threshold value (cut-off point) was 1.05 ng/ml with a sensitivity of 82% and specificity of 66%, p value was highly significant ($p < 0.001$).

Relation between VDR gene expression and clinical and biochemical data in RA patients

RA patients were then subdivided, based on the proposed cut-off value for VDR gene expression into: low VDR group: VDR ≤ 1.05 ($n = 33$) and high VDR group; VDR > 1.05 ($n = 17$)

The clinical and biochemical data were compared in these two groups and the results are shown in Tables 4 and 5. The mean age at diagnosis and disease duration did not differ significantly between both subgroups. On the other hand, not only the mean DAS 28 was significantly higher in low VDR group (4.72 ± 1.23) compared to high VDR group (3.41 ± 0.78), $p < 0.001$ with higher significant frequency of disease severity in same group (low VDR group) ($^{MC}p = 0.006^{*)}$. Similarly, the mean MHAQ score was significantly higher in low VDR group (1.82 ± 0.58) compared to high VDR group (1.22 ± 0.41), $p < 0.001$, and patients with lower VDR gene expression showed higher functional limitation ($^{MC}p = 0.001^{*)}$.

More than a third (36.4%) of patients with low VDR received corticosteroids in addition to DMARDs in comparison to 52.9% of patients with high VDR. Around 57.6%, 30.3%, and 12.1% of low VDR group received a combination of three DMARDs, combination of two DMARDs and one DMARD respectively in comparison to 41.2%, 41.2%, and 17.6% of high VDR group (Table 4).

Table 5 reveals that the mean RF and anti-CCP levels were significantly higher in low VDR group ($p = 0.04$, $p < 0.001$) respectively. Patients with double positive RF + anti-CCP+ had lower level of VDR expression $^{FE}p = 0.012^{*}$

The mean platelet count was significantly higher in low VDR group compared to high VDR group ($p = 0.024$) which may be explained by the fact that RA activity may be associated with increased platelet level. No statistically significant differences were found between both groups as regard hemoglobin, white blood cell count, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), urea, creatinine, and uric acid levels.

Whereas, ESR and CRP levels were significantly higher in low VDR $p < 0.001$, $p = 0.001$ respectively. Moreover, the mean vit D level was significantly lower in low VDR group ($p = 0.008$).

Table 2 Comparison between RA patients and control group according to vitamin D and VDR gene expression

Vitamin D and VDR gene expression	RA patients ($n = 50$)		Control group ($n = 50$)		p
	No.	%	No.	%	
Vitamin D (ng/ml)					0.009*
< 20 (vitamin D deficiency)	39	78.0	25	50.0	
20–< 30 (vitamin D insufficiency)	9	18.0	16	32.0	
≥ 30 (vitamin D sufficiency)	2	4.0	9	18.0	
Min.–max.	8.1–36.0		8.2–49.0		0.008*
Median (Q1–Q3)	16.0 (12.15–19.33)		19.9 (14.73–28.0)		
VDR gene expression					< 0.001*
Min.–max.	0.003–19.12		0.02–46.21		
Mean ± SD	0.11 (0.04–4.74)		12.93 (1.56–19.97)		

χ^2 calculated value for chi-square test, Z Z-score of Mann-Whitney test

Analysis of ROC curve for VDR gene expression as a predictor of RA is presented in Table 4 and Fig. 1. It revealed that area under the curve was 0.826, the proposed threshold value (cut-off point) was 1.05 ng/ml with a sensitivity of 82% and specificity of 66%, p value was highly significant ($p < 0.001$).

RA patients were then subdivided, based on the proposed cut-off value for VDR gene expression (1.05), into two groups:

- Low VDR group; VDR ≤ 1.05 ($n = 33$)
- High VDR group; VDR > 1.05 ($n = 17$)

*$p < 0.05$ is significance

Table 3 Analysis of ROC curve for VDR gene expression as a predictor of RA

Variable	Cut-off point (ng/ml)	AUC	*p*	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy (%)
VDR	≤ 1.05	0.826*	< 0.001**	82.0	66.0	78.6	70.7	74.0

AUC area under the curve, *PPV* positive predictive value, *NPV* negative predictive value
*AUC ≥ 0.5
**Statistically significant at $p \leq 0.05$

Discussion

As many other Arabic countries, low Vit D level is prevalent in Egypt especially among women. The present study revealed 74% females (RA and control). Though living in sunny areas, there is low exposure of the skin to sunlight (traditional dressing), dark skin pigmentation, ethnicity, and an insufficient dietary intake of vit D [22]. In the current study, compared with healthy controls, serum Vit D level was significantly lower in RA patients, still only 18% of the controls had sufficient Vit D level (> 30 ng/ml) demonstrating that vit D deficiency and insufficiency were prevalent among both RA patients and controls.

Two previous Egyptian studies found no significant difference between RA patients and controls; yet they included patients with a wide diversity and also used a different level of Vit D assessment than ours [13, 14]. On the other hand, a Canadian study was in agreement with our study with considerably higher risk of disease activity in individuals with low vit D [15]. This was also confirmed by Hiraki et al. and Raczkiewicz et al. [16, 23]. These conflicting results could be attributed to different methods of serum vit D assay; inter individual variation and absence of universal definition of adequate vit D levels between different associations [24].

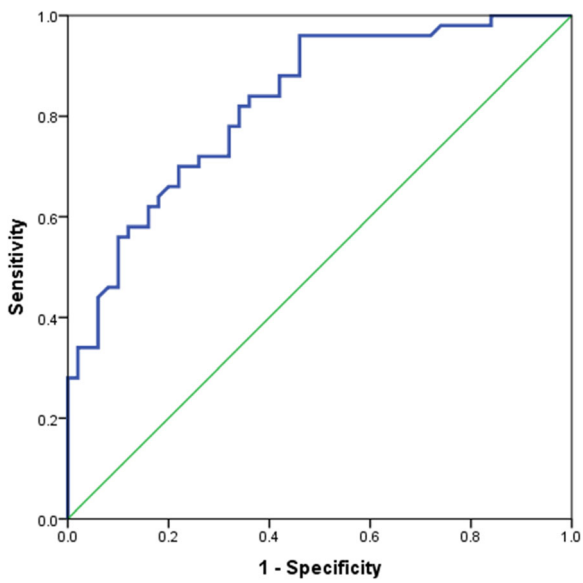

Fig. 1 ROC curve for VDR gene expression as a predictor of RA

In addition to seasonal variation in serum vit D concentrations with lowest in winter could be another explanation cause of conflicting results [25]. Until more solid evidence is available of the pleotropic effects (extra-osseous benefits) of higher vit D levels, the Spanish Society of Endocrinology proposed a serum vit D levels ranging from 30 to 50 ng/mL to ensure benefits for bone while maintaining an adequate safety level and minimizing the inaccuracy seen with the different commercial tests [26]. In fact, it is now well recognized that vit D deficiency is associated with many AID and RA is one of them [27]. There is a debate regarding vit D status is one of predisposing factors or a result of disease. One of the proposed explanations is altered vit D metabolism: reduced activation and/or increased catabolism or the sequestration of vit D in adipose tissue [28]. Regular physical activity correlated with higher vit D titers and better quality of life (QoL) in RA [23].

The discovery that VDR is expressed in almost all human cells has further increased attention for the pleotropic effects of vit D. The importance of VDR lies in the fact that not only they are expressed in almost all human cells; including T-lymphocytes and bone marrow macrophages, but also, that vit D has a unique capability to bind to this widely distributed VDR and serve as a transcriptional factor, so can regulate gene expression and exert its immunomodulatory effects [29].

In line with previous data of vit D level, the current study demonstrated that VDR gene expression in RA patients was significantly decreased compared to controls. Additionally, low VDR expression was associated with a lower mean of vit D level ($p = 0.008$), and this could be explained by the fact that proper amount of vit D is crucial for VDR to work and thus to produce their genomic and non-genomic effect, the role of VDR polymorphisms which may be altered gene expression or gene function through physiologic and pathologic phenotypes [30].

VDR polymorphisms in RA had been extensively studied with controversial results due to ethnicities, extensive geographic variations and possibly study designs [8]. In the COMORA database from 15 countries, prevalence of low vit D levels was common in RA patients and varied between countries. They stated that it could also be related to the well-known VDR gene polymorphism and its expression across different populations. Differential

Table 4 Difference between RA groups with low and high VDR gene expression regarding clinical data

Clinical data	Low VDR ≤ 1.05 (*n* = 33)		High VDR > 1.05 (*n* = 17)		*p*
	No.	%	No.	%	
Age at diagnosis (years)					0.167
Min.–max.	20–55		17–51		
Mean ± SD	35.05 ± 8.94		30.34 ± 8.82		
Disease duration (years)					0.517
Min.–max.	0.25–21		0.25–30		
Mean ± SD	6.61 ± 6.19		5.49 ± 6.62		
Disease activity (DAS 28 score)					< 0.001*
Min.–max.	2.38–6.88		2.1–4.47		
Mean ± SD	4.72 ± 1.23		3.41 ± 0.78		
Remission (< 2.6)	2	6.1	4	23.5	$^{MC}p = 0.006^*$
Low (< 2.6 and ≤ 3.2)	2	6.1	3	17.6	
Moderate (3.2 and ≤ 5.1)	15	45.4	10	58.8	
Severe (> 5.1)	14	42.4	0	0.0	
Disease-related disability (HAQ score)					< 0.001*
Min.–max.	0.8–2.6		0.6–2.1		
Mean ± SD	1.82 ± 0.58		1.22 ± 0.41		
Mild	8	24.2	11	64.7	$^{MC}p = 0.001^*$
Moderate	6	18.2	5	29.4	
High	19	57.6	1	5.9	
Drugs					0.465
▪ **Corticosteroids + DMARDS**	21	36.4	9	52.9	
▪ **DMARDS only**	12	63.6	8	47.1	
Prescribed DMARDS					$^{MC}p = 0.642$
• One drug	4	12.1	3	17.6	
• Two drugs	19	57.6	7	41.2	
• Three drugs	10	30.3	7	41.2	

t calculated value for Student *t* test, *Z* *Z*-score of Mann-Whitney test, χ^2 calculated value for chi-square test, ^{MC}p *p* value of Monte Carlo test
*$p < 0.05$ is significance

VDR expression is related to ethnicity and may affects the genetic associations in RA [31].

RA patient with normal level of Vit D and has no polymorphism in VDR may have low expression of VDR on various cells [32]. So, it is speculated that higher levels of vit D may be needed to bind to the fewer number of available receptors to express its biological effects [33].

From this work, it was observed that low VDR expression was associated with higher ESR, CRP, RF, and anti-CCP in comparison to higher VDR group, but not with gender, age at diagnosis, or disease duration. It was also associated with DAS 28 (higher RA activity) or HAQ (higher functional limitations), and these results were concordant with Cavalcanti et al., who confirmed lower VDR mRNA levels in RA patients with high disease activity when compared to patients in remission [34]. Low VDR expression was not associated with medications, whether corticosteroid intake or not, or the number of DMARDs prescribed; so, a ROC curve analysis was performed for VDR gene expression studying it role as a predictor of RA.

The association between low VDR expression and high disease activity and functional disability may be explained at cellular level, as virtually all immune cells express VDR, making them susceptible to vit D-mediated modulation, so, after binding to VDR, active vit D has a direct immunosuppressive effect on DC, reduces CD4+ cells proliferation, and differentiation into Th1 and Th17 [35]. VDR agonists were proposed to be selective inhibitors of Th1 cell development and found to inhibit Th1-type cytokines such as IL-2 and TNF-α directly. So, Adorini presumed that direct T cell targeting by VDR agonists could contribute in the treatment of AIDs [36]. It also increases production of the immunosuppressive

Table 5 Comparison between RA groups with low and high VDR gene expression VDR gene expressions regarding biochemical data

Biochemical data	Low VDR ≤ 1.05 (*n* = 33)		High VDR > 1.05 (*n* = 17)		*p*
	No.	%	No.	%	
RF (IU/ml)					0.04*
Min.–max.	5.4–860		8–112		
Mean ± SD	158.94 ± 223.87		45.17 ± 29.67		
Positive	30	90.9	14	82.4	$^{FE}p = 0.396$
Negative	3	9.1	3	17.6	
Anti-CCP (IU/ml)					< 0.001*
Min.–max.	18–558		5.9–118		
Mean ± SD	226.46 ± 167.52		35.26 ± 29.61		
Positive	32	97.0	12	70.6	$^{FE}p = 0.014$*
Negative	1	3.0	5	29.4	
RF and anti-CCP (IU/ml)					$^{FE}p = 0.012$*
Positive	29	87.9	9	52.9	
Negative	4	12.1	8	47.1	
Hemoglobin (g/dl)					0.167
Min.–max.	7.1–14.0		9.6–13.1		
Mean ± SD	11.16 ± 1.48		11.54 ± 1.16		
WBC count					0.814
Min.–max.	3180–12000		2800–11000		
Mean ± SD	6666.0 ± 2288.9		6796.7 ± 2156.9		
Platelet count (× 10^3)					0.024*
Min.–max.	70–650		113–356		
Mean ± SD	332.79 ± 143.2		257.93 ± 59.74		
SGPT (U/L)					0.545
Min.–max.	10–48		10–42		
Mean ± SD	24.69 ± 9.95		27.13 ± 7.51		
SGOT (U/L)					0.207
Min.–max.	10–46		14–46		
Mean ± SD	24.79 ± 10.32		30.47 ± 7.97		
Urea (mg/dl)					0.486
Min.–max.	11–60		10–43		
Mean ± SD	29.06 ± 10.21		27.27 ± 9.28		
Creatinine (mg/dl)					0.749
Min.–max.	0.44–2.0		0.58–1.0		
Mean ± SD	0.86 ± 0.30		0.83 ± 0.11		
Uric acid (mg/dl)					0.487
Min.–max.	2.9–9.0		3.5–6.1		
Mean ± SD	4.74 ± 1.65		4.75 ± 0.89		
ESR (mm/h)					< 0.001*
Min.–max.	5–110		16–38		
Mean ± SD	48.76 ± 27.32		22.27 ± 6.25		
CRP (mg/dl)					0.001*
Min.–max.	1–96		3–12		

Table 5 Comparison between RA groups with low and high VDR gene expression VDR gene expressions regarding biochemical data *(Continued)*

Biochemical data	Low VDR ≤ 1.05 (*n* = 33)		High VDR > 1.05 (*n* = 17)		*p*
	No.	%	No.	%	
Mean ± SD	17.48 ± 19.28		5.72 ± 2.34		
Vitamin D (ng/ml)					$^{MC}p = 0.156$
< 20 (deficiency)	27	81.8	12	70.6	
20–< 30 (insufficiency)	6	18.2	3	17.6	
≥ 30 (sufficiency)	0	0.0	2	11.8	
Min.–max.	8.1–29.0		8.1–36.0		0.008*
Mean ± SD	16.41 ± 5.72		18.75 ± 8.49		

t calculated value for Student *t* test, *Z* *Z*-score of Mann-Whitney test, χ^2 calculated value for chi-square test *FEp* *p* value of Fisher's exact test, ^{MC}p *p* value of Monte Carlo test
*$p < 0.05$ is significance

Th2 and Treg cells, so its deficiency or low VDR expression may alter this protective role and induce disease activity [37]. Vit D can stimulate a Treg-like phenotype even under Th17 polarizing conditions due to binding of VDR to three vit D responsive elements in DNA (VDREs) in the conserved non-coding sequence of the Forkhead box p3 (FoxP3) promoter, thus controlling FoxP3 transcription. It also induces the expression of indoleamine 2, 3-dioxygenase (IDO), which increases the number of Tregs and has the capability of reversing the inhibitory effect of Th17 polarizing cytokines on cytotoxic T lymphocytes associated protein 4 (CTLA4), leading to upregulation of CTLA4 [38].

In addition, B cell that plays a vital role in the milieu of autoimmunity by many mechanisms among which is the production of antibodies may be decreased by active vit D. Remarkably, the VDR binds to the promoter region of genes involved in the immune system in lymphoblastoid B cell lines, suggesting a role of vit D in AID by inhibiting the pathogenic function of B cells in autoimmunity [39]. Moreover, it was discovered that vit D supplementation could prevent the initiation and progression of collagen-induced arthritis in experimental models of RA [40]. In addition, VDR deficiency or deletion aggravates arthritis severity, inflammation, and then bone loss in mouse model of RA [41].

Classically, the effects of 1,25D are thought to be mediated by its interaction with a nuclear vitamin D receptor (VDRn), a member of the nuclear receptor superfamily of ligand-activated transcription factors. Liganded VDRn forms a heterodimeric complex with retinoid-X-receptor (RXR) and either upregulates or downregulates the expression of target genes through binding to promoter sequences termed a vitamin D3 response element (genomic action) [30].

It is recognized that 1,25D also exerts non-genomic actions that are manifested, in the main, as the activation of signaling molecules, such as phospholipase C and phospholipase A2 (PLA2), phosphatidylinositol-3 kinase (PI3K) and p21ras, and the rapid generation of second messengers (Ca2+, cyclic AMP, fatty acids and 3-phosphoinositides such as phosphatidylinositol 3,4,5 trisphosphate), accompanied by the activation of protein kinases, such as protein kinase A, src, mitogen-activated protein (MAP) kinases, protein kinase C (PKC), and Ca2+-calmodulin kinase II. The non-genomic actions also include the opening of Ca2+ and Cl– channels [42].

Limitations

The current study has some limitations. Firstly, the study was a single center not involving different geographical distribution. Secondly, our sample size was small. Thirdly, the effect of sun exposure was not studied.

Conclusions

Low VDR expression is significantly associated with higher values regarding ESR, CRP, double-positive RF+ anti-CCP+ antibodies and DAS 28 reflecting disease activity as well as MHAQ representing functional limitation in RA patients. This indicates that apart from low vit D levels, low VDR expression is associated with inflammatory process and it has a potential role in RA pathogenesis and prognosis. Further studies are required to clarify therapeutic effect of vit D as add on supplements on controlling the disease activity being potentially safe and inexpensive.

Abbreviations

RA: Rheumatoid arthritis; AIDS: Autoimmune diseases; Vit D: Vitamin D; CVD: Cardiovascular diseases; VDR: Vitamin D receptor; DC: Dendritic cells; Treg: Regulatory T cells; DAS28: Disease activity score in 28 joints; ESR: Erythrocyte sedimentation rate; ELFA: Enzyme-linked fluorescent assay; IOF: International Osteoporosis Foundation; ELISA: Enzyme-linked immunosorbent assay; CRP: C-reactive protein; RF: Rheumatoid factor; Anti-CCP: Anti-cyclic citrullinated peptide; MHAQ: Mean Health Assessment Questionnaire; DMARDS: Disease modified anti-rheumatic drugs; BMI: Body mass index; VDR: Vitamin D receptor; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic oxaloacetic transaminase; QOL: Quality

of life; FoxP3: Forkhead box p3; CTLA-4: Cytotoxic T lymphocytes associated protein 4

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Authors' contributions
Authors have all made important contributions. ESN and NM contributed to conception of the study; MM and EAS contributed to design of the study; RE and NM collected the data; EAS and MM contributed to data curation; NM and RE carried out patients' clinical assessment; ESN carried out acquisition of the samples; ESN executed the laboratory, immunological, and PCR for gene expression analyses, and ESN and MM acquired the data; RE and ESN provided key reagents and resources; EAS contributed to software formal analysis; EAS and RE performed the data analysis; MM and NM contributed to validation and visualization of data; NM wrote the first draft of the manuscript; MM and ESN provided intellectual input and edited the manuscript; EAS and RE revised the manuscript. All authors contribute equally to the work, read, and approved the submitted version.

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Availability of data and materials
All authors confirm that the data and materials are available upon reasonable request.

Ethics approval and consent to participate
Every subject enrolled in this study provided a written informed consent after explaining the nature, steps and aim of the study. Details that might disclose the identity of the subjects under study were omitted. The study received approval from the Medical Ethics Committee of the Faculty of Medicine, Alexandria University, and the practical work has been carried out in accordance with the code of Ethics of the World Medical Association (1964 Declaration of Helsinki and its later amendments) [IBR NO: 00007555-FWA NO: 00015712, Serial number: 0303546].

Consent for publication
All enrolled subjects gave their written consent for publication and all authors read and approved the submitted version.

Competing interests
All the authors declare that they have no conflict of interest in relation to the current manuscript.

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