Journal of Advances in Medicine and Medical Research

32(20): 19-24, 2020; Article no.JAMMR.61968
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Vitamin D and Vitamin D Receptor in Scleroderma Subtypes

Kocak Ayse[1*]

[1]Kütahya Health Sciences University, Turkey.

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i2030677
Editor(s):
(1) Dr. Sevgul Donmez, Gaziantep University, Turkey.
Reviewers:
(1) Prashant Ashok Punde, Krishna Institute of Medical Sciences (KIMS) (Deemed University), India.
(2) Sunayana Baruah, Assam Agricultural University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61968

Original Research Article

Received 10 August 2020
Accepted 14 October 2020
Published 04 November 2020

ABSTRACT

Vitamin D Receptor (VDR) is a member of the nuclear hormone receptor family. 1,25(OH)2D, a form of metabolically active vitamin D3 form, is the ligand of VDR. When VDR and 1,25(OH)2D are connected, many genes start to molecular interaction reactions that will modulate the transcription. VDR has been shown to be a negative regulator of the transforming growth factor beta-1 / Smad (TGF-β1 / Smad) signalling pathway. TGF-β1 / Smad signalling is important in the pathogenesis of scleroderma (SSc). Vitamin D has pleiotropic effects including immunomodulatory and antifibrotic properties in scleroderma pathogenesis. The aim of this study was to investigate the expression of VDR and the levels of vitamin D in scleroderma subtypes and study the possible correlation between the two parameters. 28 SSc patients and 30 healthy controls were included in the study and they were classified according to the 2013 ACR / EULAR criteria and Rodnan Scores were calculated. 14 were of the limited type and 14 were of the diffuse type of scleroderma. Vitamin D levels were determined in serum. Vitamin D level was measured by chemiluminescence immunometric assay. VDR gene expression was determined by quantitative PCR in isolated RNAs from the blood. Changes in mRNA levels were analysed and beta-actin was used as the housekeeping gene. Also, TGF-β1 gene expressions were determined. VDR gene expressions in diffuse type scleroderma patients were significantly decreased compared to the control. TGF-β1 gene expressions were increased in diffuse type scleroderma. It was found that VDR gene expression in limited type scleroderma patients did not show any significant difference when compared to control. Vitamin D levels and VDR gene expressions showed no correlation in

**Corresponding author: E-mail: kocak.ayse@gmail.com, ayse.kocaksezgin@ksbu.edu.tr;*

scleroderma subtypes. VDR gene expression decreased in patients with diffuse type scleroderma and showed negative correlation with the Rodnan score and TGF-β1 gene expressions. There was no significant difference between vitamin D and VDR levels.

Keywords: Vitamin D; vitamin D receptor; scleroderma; scleroderma subtypes.

1. INTRODUCTION

Systemic sclerosis or scleroderma (SSc) is autoimmune disease which is characterized by fibrosis, dysregulation of immunity and damage of the vascular system [1]. The fibrosis involve skin and internal organs such as kidney, lung, heart and gastrointestinal system [2]. Extracellular matrix (ECM) is increased in this disease. However, intracellular signalling cascades that activate extracellular matrix production are partly known. However, the available information has not yet been converted into antifibrotic therapies [3,4].

Scleroderma patients have heterogeneity of clinical findings, autoantibody profiles, disease progression, treatment response and survival [5]. The disease is basically divided into two according to the skin condition as limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc) subsets [6]. However, some patients with systemic sclerosis do not observe any of these two disease classes. Some of them may change over time. Dermal skin fibrosis is calculated using the modified Rodnan skin score (mRSS) at 17 body surface areas with a scale of 0 (normal) to 3 (severe) and has a maximum total score of 51.

Normally, vitamin D is synthesized from skin. Deficiency of Vitamin D is associated with pathological conditions of many different organs and systems, including the autoimmune disease [7]. In addition, vitamin D has been shown to have immunomodulatory effects and, its role is observed in several clinical manifestations of chronic autoimmune diseases [8]. In SSc patients, vitamin D levels are found to be low [9,10].

1,25(OH)2D is active form of the Vitamin D and it activates vitamin D receptor (VDR). VDR is a member of the superfamily of nuclear receptors [11]. VDR signalling has a role calcium and phosphorus balance in human metabolism. Also, VDR signaling is a key modulator of cell differentiation, immunomodulation and proliferation [12].

Here, we present the results of VDR and TGF-β1 expressions and Vitamin D levels in limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc).

2. PATIENTS AND METHODS

All patients were made examined by the rheumatologist and diagnosis made according to the American College of Rheumatology (ACR) criteria for scleroderma. 14 limited type and 14 diffuse type of scleroderma patients' sample were collected. Patients' Rodnan scores were calculated. 30 control patients were included in present study.

1 mL blood samples were collected from the patients. The blood samples were centrifuged and the serum was separated. Serum concentrations of 1,25 (OH) 2D in patients were determined using the chemiluminescence immunometric assay [13].

Total RNA was isolated from blood using the RNeasy kit (Qiagen, Basel, Switzerland) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 µg total RNA in 20 µL by reverse transcription using high capacity cDNA kit (Applied Biosystems, CA, USA) according to the manufacturer's instructions. Reverse transcription reaction consisted of 2 µL Oligo-dT (50 µM), 2 µL of 10x reverse transcriptase buffer, 0.8 µL of deoxynucleoside triphosphate (25 mM), 1 µL of RNase inhibitor (40 U/µL), 1 µL of MultiScribe Reverse Transcriptase (50 U/µL), and RNase free dH2O, up to a final volume of 20 µL. The cDNA was then stored at −20°C for the gene expression study.

Real time quantitative PCR was performed to detect the gene expression of VDR in blood using SYBR master mix (Thermo Fisher Scientific, USA) at 95℃ for 10 minutes, followed by 40 cycles of 95℃ for 15 s and 60℃ for 30s and the reaction was performed on Roche Lightcycler (Roche, USA)., β-Actin human sequence forward 5'-AGAGCTACGAGCTGCCTGAC-3' and reverse 5'-AGCACTGTGTTGGCGTACAG-3', was used as an internal control. The primers that were used are VDR human sequence forward 5'-CCTTCACCATGGACGACATG-3′, reverse 5'-

CGGCTTTGGTCACGTCACT-3', TGF-β1 forward primer 5'-TCCTGGCGATACCTCAGCAA-3', reverse primer 5'- AGGTAGCGCTCTGCAAACTGG-3'.

A relative quantification was performed using the $2^{\Delta\Delta}$Ct method. The experiments were performed in triplicate and were repeated twice.

2.1 Statistical Analysis

Data were presented as means ±SD and for comparisons between the groups, ANOVA Sidak analysis of variance and, for dual comparisons, Mann–Whitney U-test were used. Statistical evaluations were performed using the SPSS package program, version 20.0. A p-value of <0.05 was considered to be statistically significant.

3. RESULTS

3.1 Patients Demography

28 patients with scleroderma and 30 controls were included in this study. Patients and controls demographic information were given in Table 1. The SSc patients had no other immunologic disorder.

Table 1. SSc patients and controls overview

Description	SSc	Control
Age (Mean ± SD)	52.01± 18.3	42.19±7.1
Women (n)	25	28
Men (n)	3	2

3.2 Vitamin D Status

In the diffuse cutaneous systemic sclerosis (dcSSc) (n=14) the mean of vitamin D level was 16.54 ng/mL and in limited cutaneous systemic sclerosis (lcSSc) (n=14) vitamin D mean was 20.50 ng/mL. Control patients vitamin D levels were 21.06 ng/mL (n=30). No patients showed toxic concentrations of vitamin D. Regarding the 25(OH)D serum concentration of the two groups, no statistical differences were observed as assessed by Student's t-test (Fig. 1) (P>0.05).

3.3 VDR Expression

VDR expression was decreased in diffuse cutaneous systemic sclerosis (dcSSc) compared to limited cutaneous systemic sclerosis (lcSSc) (Fig. 2), *P<0.01.

3.4 TGF-B1 Expression

TGF-β1 expression was increased in diffuse cutaneous systemic sclerosis (dcSSc) compared to limited cutaneous systemic sclerosis (lcSSc) (Fig. 3), *P<0.01, ** P<0.05.

There is positive correlation between Rodnan Skin Score, VDR and TGF-β1 expressions (Fig. 4), p<0.05.

4. DISCUSSION

We demonstrate in the present study the VDR signalling, and Vitamin D levels in SSc subtypes.

Scleroderma is a multisystem disease mediated by autoimmunity and results in tissue fibrosis [14]. Excessive ECM is synthesized. Several studies from different countries reported significantly reduced levels of vitamin D3 in patients with SSc as compared with healthy individuals [15]. This study has not shown statistically significant differences in Vitamin D levels between SSc patients and healthy controls.

Vitamin D is synthesized—in the skin and hydroxylated in two steps in the liver and kidneys. Vitamin D is generated into its active form, 1,25 dihydroxyvitamin D (1,25(OH)2D) [16]. 1,25(OH)2D activates vitamin D receptor (VDR). VDR is member of the superfamily of nuclear receptors [12]. VDR is best known for its role in calcium and phosphorus metabolism [16]. Besides, VDR signalling is the key regulator of cell proliferation, differentiation and immunomodulation [17]. Cutolo stated that vitamin D deficiency is important in various autoimmune diseases such as scleroderma [18]. Giovannucci in his review [19] pointed out to the fact that Vitamin D deficiency may increase the risk of cancer.

In scleroderma, several researchers suggest that vitamin D3 is significantly reduced [20-27]. Arnson suggested that vitamin D deficiency and VDR signalling may contribute to SSc [20]. In our results showed that vitamin D reduced in parallel to SSc progression, but the results have no significance statistically. This study offers to the scientific literature that VDR expression is decreased in diffuse cutaneous systemic sclerosis (dcSSc) compared to limited cutaneous systemic sclerosis, which is expected to be of value in the design of research on the therapeutic approaches to the different types of SSc.

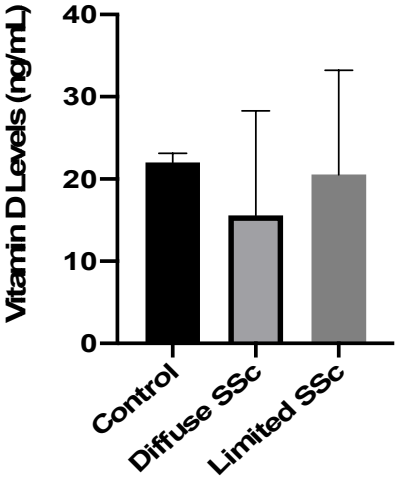

Fig. 1. Vitamin D levels in Diffuse SSc, Limited SSc and Controls

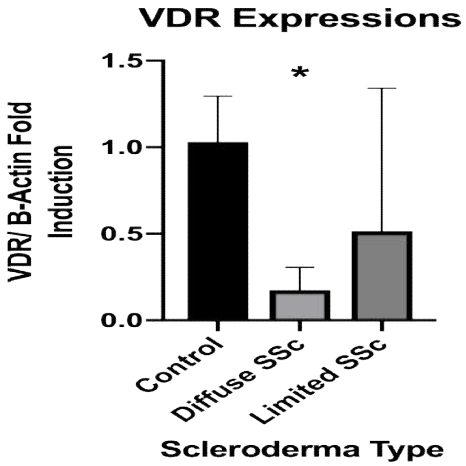

Fig. 2. Expression of Vitamin D Receptor (VDR) in diffuse and limited Systemic Sclerosis (SSc). *p<0.01

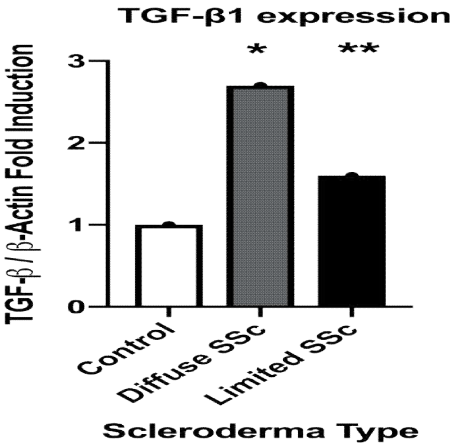

Fig. 3. Expression of TGF-β1 expression in diffuse and limited Systemic Sclerosis (SSc) *p<0.01, ** p<0.05

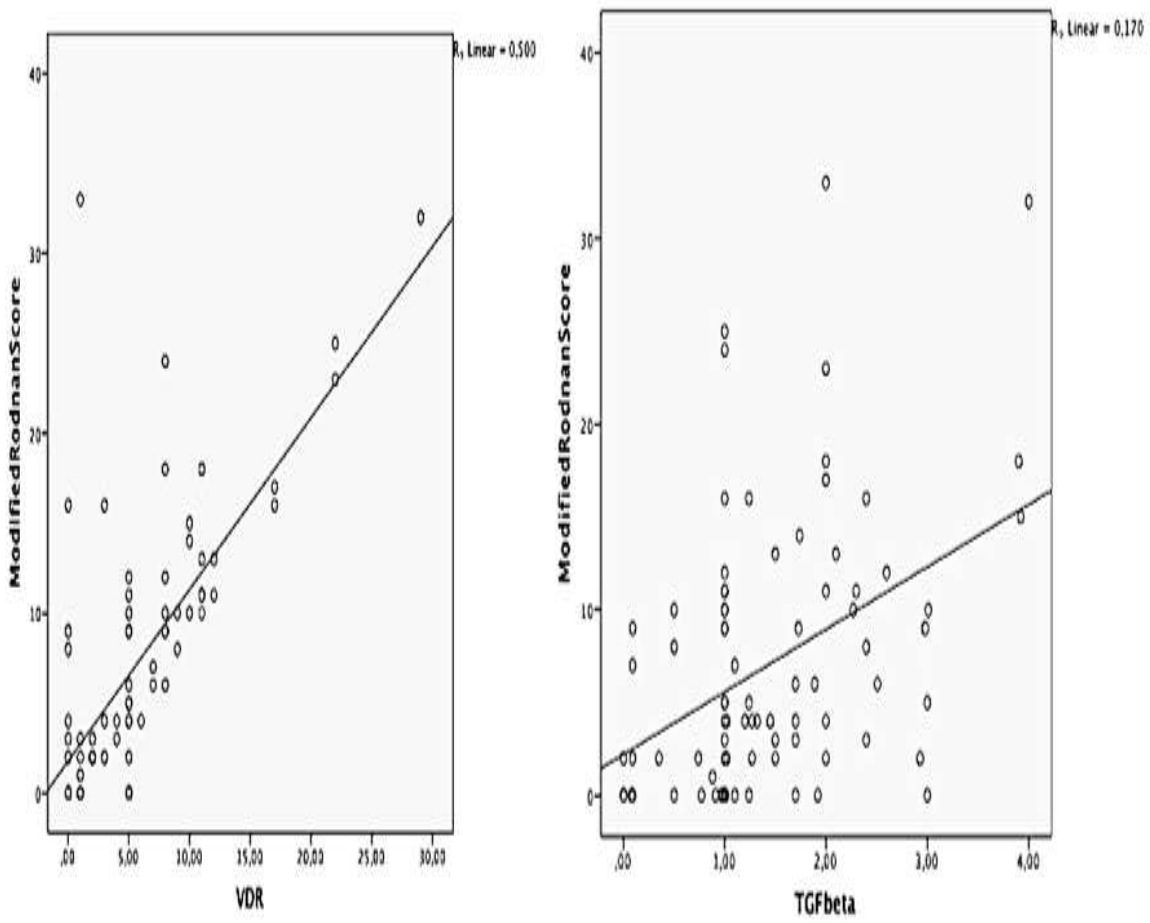

Fig. 4. Modified Rodnan score and VDR, TGF-Beta gene expression correlation, p<0.05

5. CONCLUSION

Vitamin D levels and vitamin D reseptor may play a role SSc pathogenesis.

CONSENT AND ETHICAL APPROVAL

The study was approved by the Ethical Committee of the University of Dokuz Eylul. Informed consent was obtained from all participants.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Nihtyanova SI, et al. Prediction of pulmonary complications and long-term survival in systemic sclerosis. Arthritis Rheumatol. 2014;66(6):1625-35.
2. Bhattacharyya S, Wei J, Varga J. Understanding fibrosis in systemic sclerosis: Shifting paradigms, emerging opportunities. Nat Rev Rheumatol. 2011; 8(1):42-54.
3. Beyer C, Distler O, Distler JH. Innovative antifibrotic therapies in systemic sclerosis. Curr Opin Rheumatol. 2012; 24(3):274-80.
4. Kowal-Bielecka O, et al. EULAR recommendations for the treatment of systemic sclerosis: A report from the EULAR Scleroderma Trials and Research group (EUSTAR). Ann Rheum Dis. 2009; 68(5):620-8.
5. Allanore Y, et al. Systemic sclerosis. Nat Rev Dis Primers. 2015;1:15002.
6. Le Roy EC, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol. 1988;15(2): 202-5.
7. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81.

8. Corrado A, et al. Relationship between Body Mass Composition, Bone Mineral Density, Skin Fibrosis and 25(OH) Vitamin D Serum Levels in Systemic Sclerosis. PLoS One. 2015;10(9):e0137912.
9. An L, et al. Vitamin D levels in systemic sclerosis patients: A meta-analysis. Drug Des Devel Ther. 2017;11:3119-3125.
10. Belloli L, Ughi N, Marasini B. Vitamin D in systemic sclerosis. Clin Rheumatol. 2011; 30(1):145-6.
11. Saccone D, Asani F, Bornman L. Regulation of the vitamin D receptor gene by environment, genetics and epigenetics. Gene. 2015;561(2):171-80.
12. Messa P, Alfieri C, Rastaldi MP. Recent insights into vitamin D and its receptor. J Nephrol, 2011;24(Suppl 18):S30-7.
13. Stagi S, Rigante D, Lepri G, et al. Severe vitamin D deficiency in patients with Kawasaki disease: a potential role in the risk to develop heart vascular abnormalities? Clin Rheumatol. 2016;35: 1865.
14. Mulligan-Kehoe MJ, Simons M. Vascular disease in scleroderma: angiogenesis and vascular repair. Rheum Dis Clin North Am. 2008;34(1):73-9.
15. Arnson Y, et al., Serum 25-OH vitamin D concentrations are linked with various clinical aspects in patients with systemic sclerosis: a retrospective cohort study and review of the literature. Autoimmun Rev. 2011;10(8):490-4.
16. Haroon M, Fitzgerald O. Vitamin D and its emerging role in immunopathology. Clin Rheumatol. 2012;31(2):199-202.
17. Yee YK, et al., Vitamin D receptor modulators for inflammation and cancer. Mini Rev Med Chem. 2005;5(8):761-78.
18. Cutolo M. Further emergent evidence for the vitamin D endocrine system involvement in autoimmune rheumatic disease risk and prognosis. Ann Rheum Dis. 2013;72(4):473-5.
19. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control. 2005;16:83-95.
20. Arnson Y, Amital H, Agmon-Levin N, et al. Serum 25-OH vitamin D concentrations are linked with various clinical aspects in patients with systemic sclerosis: a retrospective cohort study and review of the literature. Autoimmun Rev. 2011;10: 490–4.
21. Belloli L, Ughi N, Marasini B. Vitamin D in systemic sclerosis. Clin Rheumatol. 2011; 30:145–6.
22. Calzolari G, Data V, Carignola R, et al. Hypovitaminosis D in systemic sclerosis. J Rheumatol. 2009;36:2844; author reply 2845.
23. Caramaschi P, Dalla Gassa A, Ruzzenente O, et al. Very low levels of vitamin D in systemic sclerosis patients. Clin Rheumatol. 2010;29:1419–25.
24. Gambichler T, Chrobok I, Hoxtermann S, et al. Significantly decreased serum 25-hydroxyvitamin d levels in a large German systemic sclerosis cohort. J Rheumatol. 2011;38:2492–3; author reply 2494.
25. Rios Fernandez R, Fernandez Roldan C, Callejas Rubio JL, et al. Vitamin D deficiency in a cohort of patients with systemic scleroderma from the south of Spain. J Rheumatol. 2010;37:1355; author reply 1356.
26. Rios-Fernandez R, Callejas-Rubio JL, Fernandez-Roldan C, et al. Bone mass and vitamin D in patients with systemic sclerosis from two Spanish regions. Clin Exp Rheumatol. 2012;30:905–11.
27. Vacca A, Cormier C, Piras M, et al. Vitamin D deficiency and insufficiency in 2 independent cohorts of patients with systemic sclerosis. J Rheumatol. 2009; 36:1924–9.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61968