Original Article

VITAMIN D LEVELS AND AUTOIMMUNE DISEASES: A CROSS-SECTIONAL STUDY

Antonia Mourtzikou [1], Marilena Stamouli [2], Perseas Houtopoulos [3], Athanasios Akalestos [4], Anastasios Skliris [2], Georgia Kontelia [5], Christos Kroupis [5], Paraskevi Moutsatsou [5], Emmanouil Mournianakis [2] and Christiana Demetriou [6,7]

[1]*Department of Cytopathology, Attikon University Hospital, Athens, Greece*

[2]*Department of Biochemistry, Naval and Veterans Hospital, Athens, Greece*

[3]*Laboratory Diagnosis S.A., Nea Smyrni, Greece*

[4]*Roche Hellas S.A., Athens, Greece*

[5]*Department of Clinical Biochemistry, National and Kapodestrian University of Athens, Medical School, "Attikon" University Hospital, Athens, Greece*

[6]*Neurology Clinic D, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus*

[7]*Department of Health Sciences, School of Sciences, European University Cyprus*

Background: Autoimmune diseases are a group of disorders with heterogeneity in epidemiology and in clinical phenotype, characterized by tissue and organ damage as a result of auto attack against self antigens. Vitamin D receptors have not only been identified in tissues that take part in calcium homeostasis, but also in a variety of cell groups that mainly interfere in immune regulation. Epidemiological evidence has shown that low levels of vitamin D are related with various autoimmune diseases. The aim of our study was to compare vitamin D levels in patients with autoimmune diseases and in healthy controls. Materials and Methods: The sample group consisted of 100 patients with a confirmed diagnosis of an autoimmune disease and 40 healthy volunteers. Use of medications or treatments that decrease vitamin D levels or interfere with mass bone loss was a strict exclusion criterion. Results: Study results demonstrated statistically significant differences in vitamin D levels between patients and healthy controls, regardless of possible confounders such as gender, as well as complement C3, complement C4 and serum calcium levels. Since these factors presented statistically significant differences between patients and healthy controls, they were selected as potential confounders. Other clinical or demographic characteristics which significantly affect vitamin D levels in patients were not observed. Conclusions: Overall, this study highlights the significant role of Vitamin D in autoimmune disease development for the first time in the Greek population. A statistically significant difference in vitamin D levels between patients and healthy controls was observed.

Key words: vitamin D, autoimmunity, autoimmune diseases, confounders

Received: June 25, 2017 Revised: July 11, 2017 Accepted: October 23, 2017

*Corresponding Author: Assoc. Antonia Mourtzikou, 8 Alex. Ypsilantou street, Peania 19002, Greece
Email: antoniamour@med.uoa.gr

Introduction

Autoimmune diseases constitute a leading cause of death worldwide and they are characterized by the targeted destruction of self-tissue by the immune system. In the European Union, 1 out of 10 citizens suffer from an autoimmune disease. Moreover, 50 million Americans are patients with autoimmune diseases [1, 2]. In Greece, according to an epidemiological study conducted by the Greek Rheumatology Research Foundation, the overall age and sex adjusted prevalence of rheumatic diseases in the total target adult population is 26.9%, being significantly higher among women than in men [3]. Despite their relatively high prevalence rate, the aetiology and pathogenesis of most autoimmune diseases remain unknown. Bacteria, viruses, genetic factors, such as ageing, sex hormones and pregnancy, as well as environmental factors, such as chemical substances, stress and anxiety, affect autoimmune disease prevalence and correlate with their appearance or outbreak [4, 5].

Vitamin D is a steroidal hormone whose main function is the regulation of calcium homeostasis, and bone formation and reabsorption, through the interaction with the parathyroid glands, kidneys and bowel. The main source of vitamin D comes from the endogenous production in the skin after exposure to ultraviolet B light. Diet is a less effective source and is responsible only for 20% of the body vitamin D needs. During the last decade, scientific data link vitamin D deficiency status as a potential environmental factor which affects the prevalence of many autoimmune diseases [6,7]. Many studies concerning the role of vitamin D in immunopathogenesis, have shown that vitamin D receptors are present in the tissues that take part in calcium homeostasis, as well as in a variety of cell groups that interfere in immune regulation (monocytes-macrophages, dendritic cells, activated B lymphocytes, CD4+ T lymphocytes) [6, 7, 8, 9]. Due to its capability to bind to vitamin D receptors and serve as a transcriptional factor, vitamin D can regulate gene expression and further exert its immunomodulatory effects on immune cells. Thus, vitamin D presents many biological functions; it not only affects the bone and musculoskeletal system, but has a pleiotropic effect in the human organism [9, 10]. The aim of our study is a) to measure serum vitamin D levels in patients with autoimmune diseases and in healthy controls, b) to correlate serum vitamin D levels with gender, age and type of autoimmune disease, c) to correlate serum vitamin D levels with the presence of antinuclear antibodies (ANA), ANA titer, ANA fluorescence pattern, the presence of extractable nuclear antigen antibodies (ENA) and the presence of anti-dsDNA antibodies (anti-DNA), d) to correlate serum vitamin D levels with serum calcium levels (Ca), serum complement C3 and C4 levels in patients with autoimmune diseases and in healthy controls, and e) to compare serum vitamin D levels between patients and healthy controls.

MATERIALS AND METHODS

The present study was conducted at the Laboratory of Clinical Biochemistry, "Attikon" University General Hospital, Athens, Greece. The patient group consisted of 100 patients with autoimmune diseases, 40 male and 60 female, aged between 30 and 65 years. The control group consisted of 40 healthy volunteers, 10 male and 30 female aged between 30 and 65 years. The first 100 patients who visited the outpatient clinic of Rheumatology department were included in the study. The control group constituted of blood donors from the blood bank unit. All participants in this study had consented to the use of their personal health information. The collection of information about participants was limited to the details necessary to achieve the aims of this study. Computer-based files were available only to authorized personnel and patient identifiers were removed. The study was approved by the ethics committee of "Attikon" University General Hospital. Exclusion criteria for participating patients were HBV, HCV and HIV infection, cancer and chemotherapy, steroid drug therapy, anti-depressive drug therapy, history of myocardial infarction and history of stroke [11, 12]. In the patients' group, autoimmune diseases were distributed as follows: Hashimoto's disease 16 cases, systemic lupus erythematosus 28 cases, scleroderma 20 cases, Sjögren's syndrome 7 cases, Crest syndrome 5 cases, rheumatoid arthritis 3 cases, primary billiary cirrhosis 3 cases, autoimmune vasculitides 9 cases and miscelanea 9 cases (sarcoidosis, primary sclerosing cholangitis, Adamantiades-Behcet disease, polymyositis, diabetes mellitus type 1, Chrohn's disease, ankylosing spondylitis and Churg-Strauss syndrome, 1 case each). Vitamin D measurements were performed with the Elecsys® Vitamin D total assay by Roche Hellas S.A., based on the electro chemiluminescence (ECLIA) measurement method, on the Roche ELECSYS fully automated analyzer. Method detection

limit is 3.00 ng/mL and measuring range is 3.00 – 70.0 ng/mL. Vitamin D levels <20 ng/mL are characterized as vitamin D deficiency, levels between 20 and 30 ng/mL as insufficiency and levels >30 ng/mL as sufficiency. Method within-run precision is <15 ng/mL:SD≤1.7 ng/mL, >15 ng/mL: 6.5% and intermediate precision is <15 ng/mL: SD≤1.7 ng/mL, >15 ng/mL: 11.5%, according to the manufacturer's analytical characteristics inrofmation.

ANA and anti-DNA were evaluated by indirect immunofluorescence on Hep-2 and Crithidia luciliae substrate slides, according to the manufacturer's instructions (INOVA Diagnostics Inc., San Diego, CA, USA). Antibodies to ENAs (Sm, RNP, SS-A/Ro, SS-B/La, Jo-1, and Scl-70), were determined by the immunodot qualitative test ENA-dot (Generic Assays GmbH, Germany).

Serum Calcium, Complement C3 and Complement C4 levels were measured on the Olympus AU640 fully automated analyzer with the PN 1418-200, PN 1418-760 and PN 1418-0750 respectively, by Medicon Hellas S.A. All statistical analyses were performed using MINITAB 17 statistical package (MINITAB Inc). Between-group comparison was undertaken using Mann-Whitney test and Kruskal–Wallis test. Linear correlation between two variables was evaluated using Pearson correlation coefficient. Categorical variables were compared using the chi-square test. *P* values of less than 0.05 were considered to be statistically significant [13].

RESULTS

Demographic characteristics of patients and controls are outlined in Table 1. Only gender is significantly different between cases and controls, with a greater female predominance in the control group (patients: 63% female, controls: 75% female). Vitamin D levels in patients ranged from 2.5 to 67.59 ng/mL (mean 23.02 ng/mL) and in controls from 28.8 to 75.70 ng/mL (mean 38.20 ng/mL). Mean vitamin D is significantly different between patients and healthy controls (p-value<0.0001). These data are summarized in Table 2. Differences in vitamin D levels between males and females were not observed in any of the two groups (Mann-Whitney test p-value 0.177 and 0.984 respectively). Serum Ca levels in patients ranged from 8.00 to 10.93 mg/dL (mean 9.67 mg/dL) and in controls from 8.30 to 10.30 mg/dL (mean 9.33 mg/dL). Mean Ca levels are significantly different between patients and healthy controls (2-sample T-test p-value<0.001). Serum C3 levels in patients ranged from 66.00 to 151.20 mg/dL (mean 102.38 mg/dL) and in controls from 90.00 to 166.00 mg/dL (mean 118.13 mg/dL). Mean C3 levels are significantly different between patients and healthy controls (Mann-Whitney test p-value<0.0001). Serum C4 levels in patients ranged from 9.70 to 43.60 mg/dL (mean 24.32 mg/dL) and in controls from 22.00 to 71.00 mg/dL (mean 53.15 mg/dL). Mean C4 levels are significantly different between patients and healthycontrols (2-sample T-test p-value<0.0001). These data are summarized in Table 2. In this case we used two different statistical methods in the comparison analysis: Mann-Whitney test was used for comparing the Vitamin D and C3 between patients and controls because the data does not follow the normal distribution and 2-sample T test was used for comparing calcium and C4 between patient and controls, because these data follow the normal distribution. Test for normality was performed for all data by MINITAB 17 statistical package. Correlation between vitamin D levels and serum Ca levels, C3, C4 and participant age was not observed (Pearson correlation coefficients are -0.023, -0.268, -0.122 and -0.118 respectively). Out of the 100 patients with autoimmune diseases 69 presented positive ANA. Positive ANA titres were as follows: 1:1280 in 29 cases, 1:640 in 17 cases, 1:320 in 13 cases and 1:160 in 10 cases. Fluorescence pattern was speckled in 33 cases, nucleolar in 7 cases, homogeneous in 24 cases and anti-centromere in 5 cases. Differences in vitamin D levels between ANA positive and ANA negative patients (Mann-Whitney test p-value 0.7414), between patients with different ANA titres (Kruskal Wallis test p-value 0.865), and between patients with different fluorescence patterns (Kruskal Wallis test p-value 0.920) were not observed. These data are summarized in Table 3. Out of the 100 patients with autoimmune diseases 9 presented positive anti-DNA and 20 positive ENAs. Differences in vitamin D levels between anti-DNA negative and anti-DNA positive patients (Mann-Whitney test p-value 0.238), between ENA negative and ENA positive patients (Mann-Whitney test pvalue 0.480) and between different types of autoimmune diseases were not observed (Kruskal-Wallis test p-value 0.854) were not observed. These data are summarized in Table 3. In this case we used two different non parametric statistical methods in the comparison analysis: Mann-Whitney test was used for comparing the data between two groups, while Kruskal Wallis test was used for the comparison of more than two groups. Correlations between gender and age and ANA, titre, fluorescence pattern or type of autoimmune disease were not observed. These data are summarized in Table 4.

Table 1 Demographic Characteristics of patients and controls

	Patients (n=100)	Controls (n=40)	Test P-value
Age	48.27 ± 11.47*	48.48 ± 19.04*	Mann Whitney p=0.77948
Gender			
--Male	37 (37%)**	10 (25%)**	Chi-Square
--Female	63 (63%)	30 (75%)	p=0.014

*:Mean±SD, **:N(%)

Table 2 Vitamin D, Calcium, Complement C3 and Complement C4, comparison between cases and controls

	Vitamin D		*CALCIUM*		*C3*		*C4*	
	patients	*controls*	*patients*	*control*	*patients*	*controls*	*patients*	*controls*
	(N=100)	*(N=40)*	*(N=95)*	*(N=40)*	*(N=92)*	*(N=40)*	*(N=93)*	*(N=40)*
	*23.02±14.84**	*38.20±8.12*	*9.67±0.62*	*9.33±0.45*	*102.38±16.90*	*118.13±18.20*	*24.32±6.41*	*53.15±12.15*
	*1.48***	*1.28*	*8.00*	*0.07*	*1.76*	*2.88*	*0.66*	*1.92*
	*2.50****	*28.80*	*0.06*	*8.30*	*66.00*	*90.00*	*9.70*	*22.00*
	*67.59*****	*75.70*	*10.93*	*10.30*	*151.20*	*166.00*	*43.60*	*71.00*
	*10.28******	*33.23*	*9.20*	*9.03*	*93.25*	*102.25*	*21.00*	*45.00*
	*21.96*******	*36.75*	*9.68*	*9.25*	*99.00*	*116.00*	*24.50*	*55.00*
	*33.86********	*41.38*	*10.00*	*9.60*	*111.15*	*132.00*	*27.65*	*64.00*
Test p-value	*Mann-Whitney <0,0001*		*2-sample T test 0,001*		*Mann-Whitney <0,0001*		*2-sample T test <0,0001*	

*:Mean±SD, **: Standard error of Mean, ***: Minimum, ****: Maximum, *****:1st quartile, ******: Median, *******: 3rd quartile

DISCUSSION

Vitamin D, beyond its role in calcium homeostasis, has a potential influence in the development of autoimmune diseases. In the literature, vitamin D levels have been studied in different autoimmune diseases and the relationship between vitamin D deficiency and their prevalence has been demonstrated [4, 6, 7, 8]. The vitamin D regulatory system is very complex and interactions between genes may affect autoimmune disease susceptibility [8, 9, 10]. Autoimmune diseases are characterized by a loss of immune homeostasis resulting in the destruction of body tissue by immune cells, as a result of genetic, epidemiological and environmental factors. The availability of sufficient vitamin D levels is an important environmental factor as various epidemiological studies suggest [7, 8]. The aim of the present study was to investigate the prevalence of vitamin D insufficiency or deficiency in Greek patients with autoimmune diseases, as well as in healthy controls. Moreover, in this study we examined all possible correlations between vitamin D levels and serum Ca, serum C3 and C4, age, gender, ANA presence, ANA titer, ANA fluorescence pattern, anti-DNA and ENA presence.

According to our results, vitamin D levels were significantly lower in patients with autoimmune diseases than in the control group, and this difference was statistically significant. In particular, 42 patients have vitamin D deficiency and 25 patients have vitamin D insufficiency, compared with none in the control group. These findings are in accordance with other studies, which have established, an association between autoimmune disease and vitamin D deficiency [14-18]. In our study we did not observe any correlation between vitamin D and gender, or between vitamin D and patient age.

Table 3 Differences in VitD between ANA (-), ANA(+), ANA titter, fluorescence pattern, anti-dsDNA(+), anti-dsDNA(-), ENA (-), ENA (+) and autoimmune disease

		Vitamin D									
		N						1st quartile		3rd quartile	Test p-value
ANA	Negative	31	25.56*	2.95**	16.44***	2.90****	66.41*****	11.96	22.29******	37.34	Mann-Whitney 0.7414
	Positive	69	21.87	1.69	14.03	2.50	67.59	9.51	21.72	32.31	
ANA TITTER	1:1280	29	21.33	2.81	15.15	3.02	67.59	8.21	21.29	30.27	Kruskal Wallis 0.865
	1:160	10	23.88	3.31	10.45	10.84	48.47	18.37	21.56	27.37	
	1:320	13	23.01	3.58	12.91	12.91	39.50	10.50	23.08	35.89	
	1:640	17	20.75	3.78	15.60	15.60	49.24	6.13	20.62	24.72	
	n∝	31	25.56	2.95	16.44	16.44	66.41	11.96	22.29	37.34	
Fluorescence pattern	C	5	26.10	12.7	28.3	2.50	67.6	2.80	14.9	55.10	Kruskal Wallis 0.920
	H	24	20.57	2.47	12.08	3.68	49.24	8.94	22.34	28.33	
	n∝	31	25.56	2.95	16.44	2.90	66.41	11.96	22.29	37.34	
	NU	7	21.36	3.97	10.50	6.59	33.96	10.09	22.20	33.55	
	S	33	22.29	2.39	13.73	3.02	48.47	10.13	21.39	35.86	
anti ds-DNA	Negative	91	22.40	1.56	14.86	2.50	67.59	9.13	21.59	32.18	Mann Whitney 0.238
	Positive	9	29.27	4.62	13.86	8.10	45.37	46.06	35.84	40.14	
ENA	Negative	80	22.81	1.72	15.41	2.50	67.59	9.37	21.49	33.86	Mann Whitney 0.940
	Positive	20	23.86	2.81	14.58	6.00	47.37	12.42	22.67	34.95	
Autoimmune disease	1 Hashimoto thyroiditis	16	27.37	3.62	14.48	3.92	50.34	17.70	24.37	39.64	Kruskal Wallis 0.854
	2 SLE	28	23.36	2.53	13.41	3.10	49.24	11.00	22.96	35.00	
	3 scleroderma	20	19.39	2.70	12.05	4.92	48.47	8.46	19.02	26.16	
	4 Sjogren's syndrome	7	23.31	4.80	12.69	7.50	45.37	13.61	20.62	33.96	
	5 Crest Syndrome	5	26.10	12.70	28.30	2.50	67.6	2.80	14.90	55.10	
	6 Rheumatoid arthritis	3	23.69	5.47	9.48	14.65	33.55	14.65	22.87	33.55	
	7 primary biliary cirrhosis	3	17.61	8.42	14.58	3.02	32.18	3.02	17.64	32.18	
	8 autoimmune vasculitides	9	22.11	6.63	19.88	3.00	66.41	5.83	20.44	31.66	
	9 miscelanea	9	22.84	5.74	17.23	2.90	53.36	7.01	21.59	35.50	

*:Mean, **:Standard error of Mean, ***:Standard Deviation, ****:Minimum, *****:Maximum, ******:Median

Table 4 Correlation analyses between gender and age and ANA TITRE, Fluorescence pattern and Autoimmune disease

	P-value		
	ANA TITRE	FLUORESCENCE PATTERN	AUTOIMMUNE DISEASE
Gender	0.770	0.496	0.972
Age	0.372	0.651	0.118

Mean Calcium levels were higher in patients than in the control group (9.67 vs 9.33) and this difference was statistically significant (p=0,001). This can be attributed to secondary hyperparathyroidism and chronicity observed in patients with autoimmune disorders, [19, 20, 21]. Moreover, given that our sample is characterized by female predominance, it can be attributed to calcium supplement administration for the prevention of osteoporosis. However, this study is performed with data from computer-based files; information about calcium supplement was not available.

Complement C3 was observed to be lower in patients compared to controls, due to disorder of renal function in patients with autoimmune diseases. Moreover, complement C4 was observed to be lower in patients compared to healthy controls [22, 23]. C3 and C4 differences between patients and healthy controls were statistically significant.

Vitamin D levels between patients with ANA positivity/negativity, different ANA titres, different ANA fluorescence pattern, anti-ds DNA positivity/negativity and ENA positivity/negativity were not observed. Moreover, differences in vitamin D levels between patients with different types of autoimmune diseases were not observed. These results are not in accordance with some studies which have highlightened the association between vitamin D deficiency and positivity for ANA [25-30]. However, each of these studies addresses a particular type of autoimmune disease, while the results of our study are applicable to a variety of autoimmune diseases.

Our study is an original one concerning the Greek population and it highlights the importance of vitamin D in autoimmune disease. In our study we observed that vitamin D levels were lower compared to healthy controls and this difference was statistically significant. However, there are some limitations in our study. The size of the study population is small, due to difficulties in performing expensive biochemical tests and spending resources during recession times. The capacity for extrapolation of results from this study is limited by the lack of diversity in ethnicity and gender within the sample population (female predominance in both patient and control groups). Moreover, due to the aforementioned limitations many autoimmune diseases are represented in the population under study only by one or very few cases. Due to the study limitations our results should be viewed as of a pilot nature. A larger confirmatory study with vitamin D measurements in a larger sample is needed in order to validate these findings.

References

1. Lerner A, Jeremias P and Torsten M. The World Incidence and Prevalence of Autoimmune Diseases is Increasing. International Journal of Celiac Disease. 2015;(4):151-155
2. Ramos-Casals M, Brito-Zerón P, Kostov B, et al. Google-driven search for big data in autoimmune geoepidemiology: analysis of 394,827 patients with systemic autoimmune diseases. Autoimmun Rev. 2015;14:670-679
3. Andrianakos A, Trontzas P, Christoyannis F, Dantis P, Voudouris C, Georgountzos A, et al. Prevalence of rheumatic diseases in Greece: a crosssectional population based epidemiological study. The ESORDIG Study. J Rheumatol. 2003;30:1589–1601
4. Alzabin S and Venables PJ Etiology of autoimmune disease: past, present and future. Expert Review Of Clinical Immunology 2012;8(2):111-113
5. Costenbader KH. Gay S. Alarcon-Riquelme ME. Iaccarino L. Doria A. Genes, epigenetic regulation and environmental factors: Which is the most relevant in developing autoimmune diseases? Autoimmun Rev. 2012;11:604–609
6. Agmon-Levin N, Theodor E, Segal RM, Schoenfeld Y: Vitamin D in systemic and organ- specific autoimmune diseases. Clin Rev Allergy Immunol 2013; 45:256-266
7. Lepri G, Stagi S, Casalini E, Matucci-Cerinic M and Falcini F.Vitamin D in Rheumatic Diseases: State of the Art Austin J Nutri Food Sci. 2015; 3(4): 1070
8. Yang CY, Leung PS, Adamopoulos IE and Gershwin ME. The Implication of Vitamin D and Autoimmunity: a Comprehensive Review. Clinic Rev Allerg Immunol 2013;45(2): 217-226
9. Yu-Hsien Lai and Te-Chao Fang. The Pleiotropic Effect of Vitamin DHindawi Publishing Corporation ISRN Nephrology 2013, Article ID 898125, available at http://dx.doi.org/10.5402/2013/898125
10. Prieti B, Treiber G, Pieber TR and Amrein K. Vitamin D and Immune Function Nutrients 2013;5(7):2502-2521
11. Demille DM, Piscitelli M, Ocker A, Vuong C, Hartner L, Lynch MP: Vitamin D deficiency in the oncology setting. J Community Support Oncol 2014;12: 13-19
12. Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. Arch Intern Med 2008; 168:1340-1349
13. Lang TA, Altman DG. Statistical analyses and methods in published literature: The SAMPL guidelines. In: Moher D, Altman DG, Schulz KF, Simera I, Wager E, editors. Guidelines for reporting health research: A user's manual. Oxford, UK: John Wiley & Sons; 2014. p. 264–274
14. Evliyaoğlu O, Acar M, Ozcabı B, Erginoz E, Bucak F, Ercan O, Kucur M. Vitamin D Deficiency and Hashimoto's Thyroiditis in Children and Adolescents: a

Critical Vitamin D Level for This Association? J Clin Res Pediatr Endocrinol 2015;7(2):128-133
15. Yazmalar L, Ediz L, Alpayci M, Hiz O, Toprak M, Tekeoglu I. Seasonal disease activity and serum vitamin D levels in rheumatoid arthritis, ankylosing spondylitis and osteoarthritis. Afr Health Sci. 2013; 13(1):47-55
16. Cutillas-Marco E, Morales-Suárez-Varela M, Marquina-Vila A, Grant W. Serum 25-hydroxyvitamin D levels in patients with cutaneous lupus erythematosus in a Mediterranean region. Lupus. 2010; 19(7):810-4
17. Handono K, Marisa D, Kalim H. Association between the low levels of vitamin D and Treg function in systemic lupus erythematosus patients. Acta Med Indones. 2013;45(1):26-31
18. Pokhai GG, Bandagi S, Abrudescu A. Vitamin D levels in ankylosing spondylitis: does deficiency correspond to disease activity? Rev Bras Reumatol. 2014; 54(4):330-334
19. Michels, T. C. & Kelly, K. M. Parathyroid disorders. Am Fam Physician. 2013; 88**:** 249-57
20. Watad A., Tiosano S., Azrielant S., Whitby A., Comaneshter D., Cohen **A**. D., Shoenfeld Y. and Amital H. Low levels of calcium or vitamin D, which is more important in systemic lupus erythematosus patients? An extensive data analysis. Clin Exp Rheumatol 2017; 35(1):108-112
21. Massironi S, Cavalcoli F, Rossi RE, Conte D, Spampatti MP, Ciafardini C, Verga U, Beck-Peccoz P and Peracchi M. Chronic autoimmune atrophic gastritis associated with primary hyperparathyroidism: a transversal prospective study. European Journal of Endocrinology 2013; 168: 755–761
22. http://Www.Naturanrg.Gr/. Available: http://www.naturanrg.gr/.Idf 2015. IDF Patient & Family Handbook or Primary Immunodeficiency Diseases, 5thEdition. Immune Deficiency Foundation
23. Vergan D., Wells L., Larcher V. F., Nasaruddin B. A., Davies E. T., Mieli-Vergani G. and Mowat A. P. Genetically determined low C4: a predisposing factor to autoimmune chronic active hepatitis. Lancet. 1985 ; 2**:** 294-298
24. Ritterhouse LL, Crowe SR, Niewold TB, Kamen DL, Macwana SR, Roberts VC, Dedeke AB, Harley JB, Scofield RH, Guthridge JM and James JA. 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**:** 1569-1574
25. Santos-Antunes J, Nunes AC, Lopes S and Macedo G. The Relevance of Vitamin D and Antinuclear Antibodies in Patients with Inflammatory Bowel Disease Under Anti-TNF Treatment: A psoepctive Study Inflamm Bowel Dis 2016;22(5):1101-1106
26. Meier HC, Sandler DP, SImonsick EM and Parks CG. Association between Vitamin D Deficiency and Antinuclear antibodies in Middle Aged and Older US Adults Cancer Epidemiol Biomarkers Prev 2016; 25(12):1559-1563.
27. Cláudia Diniz Lopes Marques, Andréa Tavares Dantas, Thiago Sotero Fragoso, Ângela Luzia Branco Pinto Duarte. The importance of vitamin D levels in autoimmune diseases Rev. Bras. Reumatol. 2010;50(1):67-80
28. Zold E., Szodoray P., Gaal J., Kappelmayer J., Csathy L., Gyimesi E., Zeher M., Szegedi G. and Bodolay E. Vitamin D deficiency in undifferentiated connective tissue disease. Arthritis Res Ther. 2008; 10**:** R123
29. Ponsonby AL, Lucas RM. and Van Der Mei IA. UVR, vitamin D and three **a**utoimmune diseases--multiple sclerosis, type 1 diabetes, rheumatoid arthritis. Photochem Photobiol. 2005; 81**:** 1267-1275
30. Falcini F, Stagi S, Lepri G, Casalini E, Rigante D, Matucci-cerinic M. Severe vitamin D deficiency in patients with Kawasaki disease: its possible role in the risk to develop coronary artery damage. Annals of the Rheumatic Diseases 2015; 74 (S2): 843-843