Low levels of vitamin D are common in primary antiphospholipid syndrome with thrombotic disease

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SUMMARY

The aim of this study was to assess vitamin D (vit. D) levels in patients with primary antiphospholipid syndrome (PAPS), the association between hypovitaminosis D and clinical manifestations, and the effect of vit. D supplementation on serum levels. Vit. D serum levels of 115 PAPS patients, classified according to the 2006 revised criteria at the Rheumatology Department, Brescia, and of 128 voluntary healthy donors (NHD) were tested in collaboration with DiaSorin (Saluggia, Italy) using the LIAISON® chemiluminescent immunoassay. Clinical data were derived from clinical charts.

Vit. D deficiency was more prevalent in PAPS than NHD (17% vs 5%). During the summer, vit. D levels were lower in PAPS than NHD (median 28 vs 40.1 ng/mL, P<0.01). PAPS were subdivided according to clinical characteristics (thrombotic vs obstetric). Both groups had lower vit. D levels compared to NHD. Thrombotic PAPS had significantly lower levels than obstetric PAPS (median 20.8 vs 33.3, P<0.01). Sixteen patients (14%) received oral 25-OH vit. D supplementation (average 400 UI/die), but 63% of them did not reach serum levels above 30 ng/mL. PAPS showed significantly lower levels of vit. D than NHD. Hypovitaminosis D was seen to cluster in patients with thrombosis which may suggest that the lack of vit. D could be one of the many factors involved in the thrombotic process. Low-dose supplementation did not seem to be effective in a small group of patients.

Key words: *Vitamin D, primary antiphospholipid syndrome, thrombosis.*

Reumatismo, 2012; 64 (5): 307-313

Work awarded at XLVIII Congress of Italian Society of Rheumatology (SIR), Rimini 2011

INTRODUCTION

Research interest in vitamin D has increased over the past few years. In addition to the classic role of vitamin D on bone metabolism, leading to its use in the treatment of osteoporosis, many experimental data have underlined its pleiotropic effects on different tissues and cellular types, including those of the immune system (1). Furthermore, several clinical studies report low vitamin D levels in association with an increase in cardiovascular risk (2), development of cancer (3, 4), and onset of autoimmune diseases (5). As far as the immune system is concerned, many studies have shown the anti-inflammatory role of vitamin D through the modulation of both cellular proliferation and differentiation (6). Recent studies focused on the association between hypovitaminosis D and the development of autoimmune diseases, both organ-specific (e.g. multiple sclerosis, diabetes mellitus type 1), and systemic diseases, e.g. systemic lupus erythematosus (SLE), rheumatoid arthritis (RA). Clinical studies have shown that hypovitaminosis D is very common in SLE patients (7) and low levels of vitamin D seem to be correlated with disease activity in RA (8). Another study showed that patients with undifferentiated connective tissue disease (UCTD) and low levels of vitamin D were more likely to develop a well-defined autoimmune disease (9).

In our study, we considered patients with primary antiphospholipid syndrome (PAPS), a disease characterized by venous, arterial and small vessel thrombosis and/or obstetric disease (fetal losses, prema-

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ture births and recurrent abortions) that are mediated by antiphospholipid antibodies (10). Patients with PAPS do not usually have any severe inflammatory organ involvement resembling SLE, so they do not generally need any corticosteroids or immunosuppressive treatment. Furthermore, these patients do not generally have to limit their exposure to the sun nor do they have any specific dietary restrictions concerning foods that are naturally rich in vitamin D. Therefore, PAPS may be a model of an autoimmune disease in which the impact of factors influencing vitamin D levels can be limited.

The few studies that analyzed the relationship between PAPS and hypovitaminosis D found lower vitamin D levels in PAPS patients than in healthy controls (NHD) (11.9±5.4 ng/mL on 160 European patients (11); 21.64±11.26 ng/mL on 23 Brazilian pre-menopausal patients (12); 23.3±10.5 ng/mL on 46 Brazilian patients (13); 18±9 ng/mL on 113 European patients (14)). In all studies, the average value for PAPS patients was considered to be insufficient according to international guidelines (15) being below 30 ng/mL. Some of the limits of these studies include the lack of data on ethnicity, seasonality, nutrition and sun exposure; however, overall they do suggest that PAPS patients are affected by hypovitaminosis D just like patients with other systemic autoimmune diseases.

In the current study, we retrospectively analyzed a large cohort of PAPS patients for serum vitamin D levels, taking into consideration the factors that may influence vitamin D status. We also analyzed vitamin D levels according to different disease manifestations and the supplementation of patients with oral compound contain-

Table I - Clinical and serological features of 115 PAPS patients enrolled in the study.

	PAPS n=115 (%)	Thrombotic PAPS with/without obstetric complications n=74 (%)	Obstetric PAPS only n=41 (%)
Demographic data			
Female	96 (83)	55 (74)	41 (100)
Median age, [range] years	46 [18-79]	51 [18-79]	41 [24-62]
Caucasians	110/115 (96)	74/74 (100)	36/41 (88)
Intake of foods naturally rich in vitamin D	105/115 (91)	69/74 (93)	36/41 (88)
Avoidance of sun exposure	16/115 (14)	9/74 (12)	7/41 (17)
Cigarette smoking	27/115 (23)	19/74 (26)	8/41 (20)
Clinical and laboratory features			
Venous thrombosis	47/115 (41)	47/74 (64)	0
Arterial thrombosis	40/115 (35)	40/74 (54)	0
LA	53/103 (51)	34/66 (52)	19/37 (51)
IgG AcI	78/112 (70)	53/72 (74)	25/40 (63)
IgM AcI	14/112 (12)	13/72 (18)	1/40 (3)
IgG anti-β2GPI	84/113 (73)	54/72 (75)	30/41 (73)
IgM anti-β2GPI	86/113 (76)	53/72 (74)	33/41 (80)
Drug therapy and Vit.D supplementation			
Use of oral vitamin K antagonists	31/115 (27)	31/74 (42)	0
Use of low molecular weight heparin	9/115 (8)	4/74 (5)	5/41 (12)
Use of antiplatelet drugs	82/115 (71)	45/74 (61)	37/41 (90)
Use of steroids*	19/115 (17)	17/74 (23)	2/41 (5)
Use of hydroxychloroquine	24/115 (21)	19/74 (26)	5/41 (12)
Supplementation with 25-OH vit D	16/115 (14)	8/74 (11)	8/41 (20)

*The use of steroids was limited to short-term treatment; LAC, Lupus Anticoagulant, aCL, anti-cardiolipin antibody; anti-β2GPI, anti-β2glycoprotein I antibody.

ing vitamin D. This study is an update of a previous report from the 8th Meeting of the European Forum on Antiphospholipid Antibodies, held in Padua in September 2011 (16).

MATERIALS AND METHODS

One-hundred and fifteen patients with PAPS, living in Northern Italy and classified according to the revised criteria (10) at our Centre of Rheumatology and Clinical Immunology, were included in the study. Table I shows patients' clinical and laboratory characteristics and information about vitamin D supplementation. Data were drawn from clinical charts.

One-hundred and twenty-eight healthy blood donors (NHD) from the same geographical area were enrolled as controls (55 male, 73 female, median age 34 years, range 18-64). The levels of 25-OH vitamin D were determined by a direct competitive test based on chemiluminescence (Liaison® 25-OH vitamin D Assay; DiaSorin S.p.A., Saluggia, Italy). According to international guidelines (15), vitamin D levels above 30 ng/mL were considered sufficient, values 10-30 ng/mL were insufficient and values below 10 ng/mL were deficient. Samples were subgrouped at three different time points in order to capture vitamin D seasonal variations: summer (July-October), fall-winter (November-February), spring (March-June).

Analysis by the Shapiro-Wilk test showed that normal distribution of vitamin D values was not seen in either of the two groups. Therefore, the non-parametric Wilcoxon's or Mann-Whitney test and the Fisher's exact test were used for comparisons between groups. For all tests, P≤0.05 was considered statistically significant.

RESULTS

For NHD, our study showed that only 36% of patients had sufficient vitamin D levels. There was no difference between women and men when data were divided according to different seasons (38.9 vs 40.5 ng/mL in summer, 26.7 vs 18.4 ng/mL in fall-winter, 21.2 vs 19.8 ng/mL in spring; Wilcoxon's test P>0.05).

A comparison between PAPS and NHD showed seasonal variations in both groups

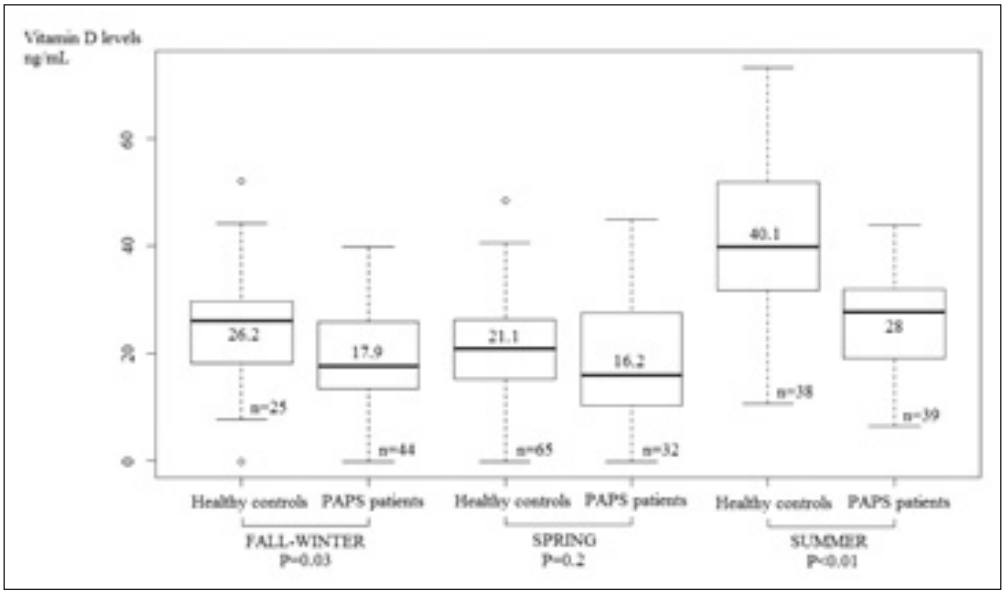

Figure 1 - Vitamin D levels in healthy controls and PAPS patients at three different time points of the year (fall-winter, spring and summer). Bold line shows median value. Wilcoxon's test, P=0.03 in fall-winter; P=0.2 in spring and P<0.01 in summer.

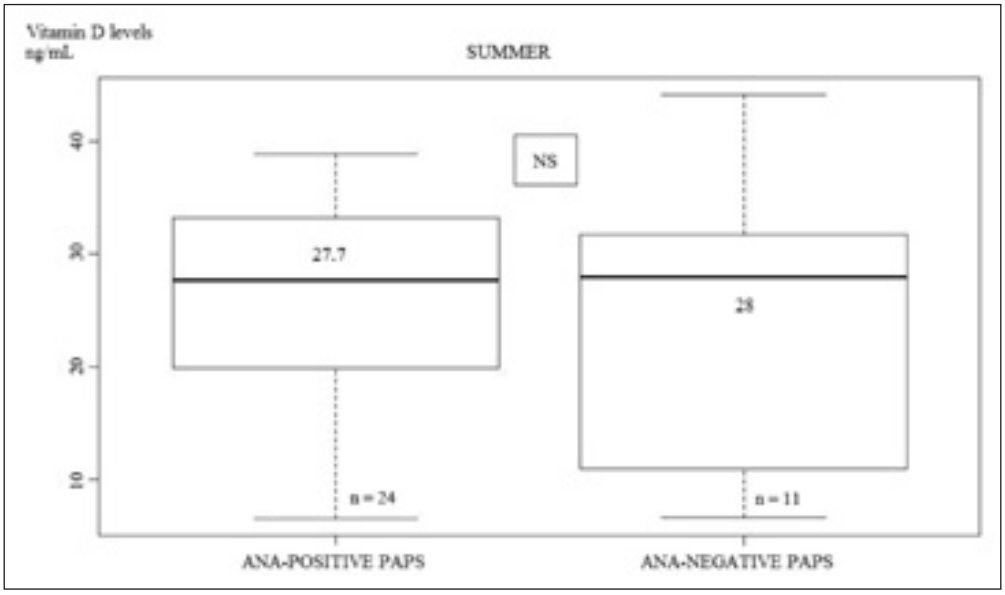

Figure 2 - Vitamin D levels in ANA-positive PAPS and ANA-negative PAPS in summer. Bold line shows median value. No statistically difference was observed between the two patients groups. Wilcoxon's Test P=0.8.

(i.e. higher levels during summer) and significantly lower levels of vitamin D in PAPS (Fig. 1). Likewise, the prevalence of deficiency (<10 ng/mL) was higher in the first group (17% vs 5%, Fisher's exact test P<0.05). A statistically significant differ-

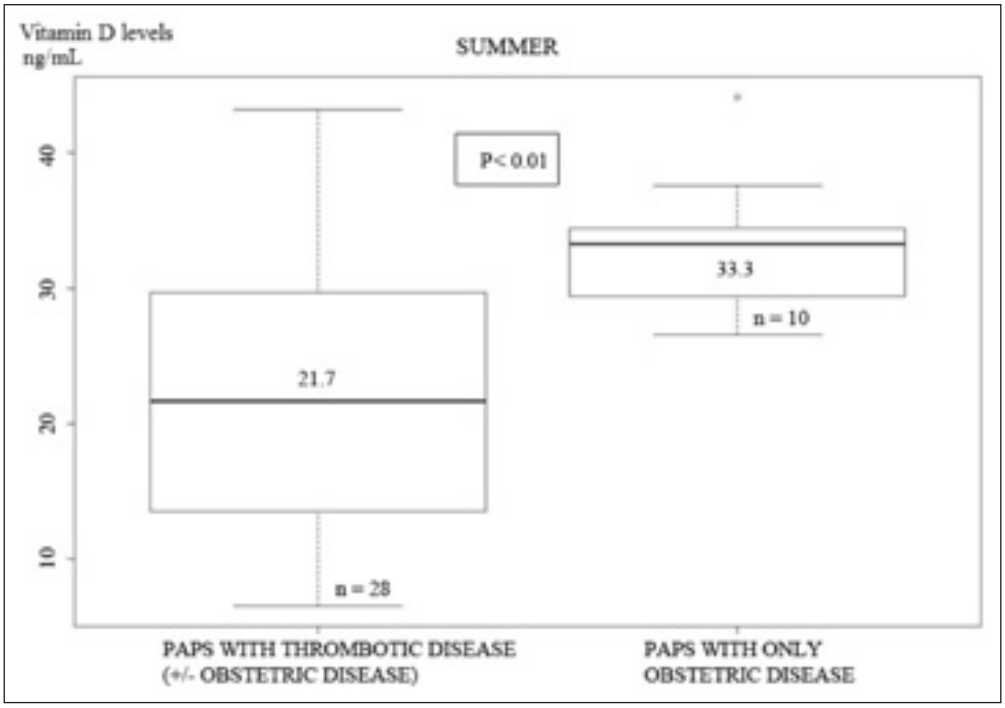

Figure 3 - Vitamin D levels during summertime in PAPS patients according to clinical manifestations (thrombosis, obstetric disease). Bold line shows median value. Wilcoxon's Test P<0.01.

ence between PAPS and NHD was observed during summer and fall/winter (Wilcoxon's test P<0.01 and P=0.03, respectively) so we looked for any possible influence of sun exposure. In our cohort, PAPS patients may have been instructed to use sunscreens if they carried positive antinuclear antibodies (ANA). We considered 103 patients (89%) in which the detection of ANA was available (63 ANA-positive patients, 40 ANA-negative patients). None of the ANA-positive patients displayed any clinical feature of SLE. As shown in Figure 2, there was no statistical difference in vitamin D levels of 24 ANA-positive and 11 ANA-negative patients during summer (27.7 vs 28.0 ng/mL, Wilcoxon's test P=0.8).

In comparison to NHD, PAPS with thrombosis (± obstetric disease) and PAPS with pure obstetric disease showed lower vitamin D levels when the blood was collected in summer (21.7 vs 40.1 ng/mL, Wilcoxon's test P<0.01 for thrombotic PAPS; 33.3 vs 38.9 ng/mL, Wilcoxon's test P=0.04 for women with obstetric complications compared to healthy women). In addition, PAPS with thrombotic manifestations had significantly lower levels of vitamin D than women with pure obstetric disease (21.7 vs 33.3 ng/mL, Wilcoxon's test P<0.01) (Fig. 3).

A comparison was made of 16 PAPS (14%) who were taking oral supplementation (25-OH vitamin D at an average dose of 400 UI per day) with the rest of the patients; no difference was found in vitamin D levels (26.5 vs 20.3 ng/mL, Mann-Whitney test P=0.07). When patients were compared according to subgroups (thrombosis vs pure obstetric disease), still no difference was found. Furthermore 63% and 13% of the PAPS who received vitamin D supplementation did not reach levels of vitamin D above 30 ng/mL or above 10 ng/mL, respectively.

DISCUSSION

Environmental factors that influence vitamin D metabolism should be considered in the evaluation of serum levels of vitamin D. Eighty percent of vitamin D is produced in the skin by UVB radiations; therefore, sun exposure (in terms of season, latitude and habits) (17) and color of the skin (18) are crucial factors. The other 20% of vitamin D comes from the diet (i.e. dietary products, eggs, fish and mushrooms are rich in vitamin D). Obese individuals and older subjects (19) are more predisposed to hypovitaminosis D.

This is due to vitamin D sequestration into body fat and to the reduction in cutaneous 7-dehydrocholesterol, a vitamin D precursor, respectively. As already reported by our group (16), these environmental factors were taken into consideration in the evaluation of vitamin D levels in PAPS patients. Ethnicity and dietary habits were not confounding factors, since 96% of the patients were Caucasian and 97% were on an unrestricted diet. Furthermore, patients with PAPS did not require any long-term corticosteroid treatment or immunosuppressive therapy that could influence vitamin D levels (7) because they did not have any inflammatory organ involvement.

We subdivided patients according to the season during which vitamin D levels were tested and compared them with healthy controls living in the same geographical area at different time points through the year. As controls, we considered 128 NHD with a median age that was lower than that of PAPS (34 vs 46 years) because blood donors are generally younger people. We found a great prevalence (64%) of vitamin D levels below 30 ng/mL among this healthy population, in line with epidemiological data in the literature (20). Our data also showed that there was a seasonal variability in vitamin D levels (that are at their highest during the summer) and that there was no difference between women and men in vitamin D status. We showed that PAPS had lower levels than NHD, in particular during the summer. Therefore, we hypothesized that vitamin D deficiency in PAPS may be related to environmental factors, summer being the season when most of the vitamin D is produced on sun exposure. In fact, patients with PAPS do not receive particular indications about use of sunscreens and limitating sun exposure

(in contrast to patients with SLE for whom sun exposure is forbidden). In our cohort, however, PAPS with positive ANA might have been instructed to use sun protection. Therefore, we used ANA as a surrogate marker of sun exposure; however, no difference in vitamin D levels between ANA-positive and ANA-negative PAPS patients was observed. This led us to speculate that avoiding sun exposure may not be the main reason for lower vitamin D levels in PAPS compared to NHD.

Instead, hypovitaminosis D may be a part of the mosaic of mechanisms that contribute to autoimmunity in PAPS. This assumption was supported by subdividing patients with PAPS according to the main features of the syndrome: PAPS with thrombotic manifestations (± obstetric complications) and PAPS with only obstetric complications. We found that patients with thrombotic manifestations had significantly lower vitamin D levels than those with pure obstetric complications, although there was a difference in median age between the two groups.

A relationship between lower vitamin D levels and thrombotic manifestations of the Syndrome has already been found in a large multicenter cohort of European patients (14). The same authors were able to show that vitamin D inhibits in vitro the expression of tissue factor by monocytes stimulated by anti-beta2glycoprotein I antibodies derived from APS patients.

Furthermore, a Swedish study (21) showed that the risk of venous and arterial thrombosis was lower in patients who sunbathed and another study reported a reduction in the incidence of thrombotic events in cancer patients who were supplemented with calcitriol (22). The type of compound (1,25-OH vitamin D vs 25-OH vitamin D) and the optimal dosage are the subject of discussion.

In our cohort, 14% of PAPS were supplemented over the short-term with treatment with steroid or heparin as a support to bone metabolism. In most of the patients, the low dose of vitamin D (average 400 UI/day) was not sufficient to enable levels to rise above 30 ng/mL. A similar observation was made in SLE patients who received an even higher dosage (800 UI/day): 70% of subjects still had insufficient levels of vitamin D after supplementation (23). According to these observations, it is plausible that higher doses of vitamin D may be required to significantly raise serum levels and possibly exert an immunological effect.

In conclusion, patients with PAPS have lower vitamin D levels than NHD, even if they do not have a full-blown disease such as SLE (therefore not requiring high steroid regimens or immunosuppressive therapies) and they do not have to limit their sun exposure in any particular way. The high prevalence of vitamin D insufficiency means that subjects with PAPS have more in common with patients with other systemic autoimmune diseases than with healthy subjects. These epidemiological data may suggest that vitamin D insufficiency can be one of the several factors that determine autoimmunity, rather than being an outcome of chronic disease and its treatment. In particular, this hypothesis may be supported by the observation that patients with thrombotic PAPS, a more aggressive phenotype, have greater vitamin D deficiency than those with pure obstetric disease.

ACKNOWLEDGMENTS

The authors would like to thank DiaSorin S.p.A. (Saluggia, Vercelli, Italy) for kindly supporting the study with vitamin D measurement; the patients who agreed to participate in the study; the medical staff of the Department of Rheumatology and Clinical Immunology (Spedali Civili, Brescia) for their help in enrolling patients.

FUNDINGS

L. Andreoli is supported by a research grant financed by an agreement between the provincial government of Lombardy and the University of Brescia (Dote Ricercatore e Dote Ricerca Applicata ai sensi dell'accordo regionale per lo sviluppo del capitale umano nel sistema universitario Lombardo sottoscritto tra Regione Lombardia e Università Lombarde il 20-10-2009, Università degli Studi di Brescia).

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