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ORIGINAL ARTICLE

Evaluation of serum 25-hydroxyvitamin D levels in vitiligo patients with and without autoimmune diseases

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None declared.

SUMMARY

Background

Vitiligo is an autoimmune depigmentation disorder, commonly associated with systemic autoimmune diseases. Deficient serum 25-hydroxyvitamin D (25(OH)D) levels have been noted in some patients with autoimmune diseases.

Aim

To evaluate serum 25(OH)D levels in vitiligo patients with and without systemic autoimmune diseases.

Methods

A case-control study was conducted on 40 vitiligo patients (20 patients with systemic autoimmune diseases and 20 patients without autoimmune diseases) and 40 age-, gender- and skin phototype-matched healthy controls. Serum 25(OH)D was measured in all subjects, divided into: normal or sufficient ($\geq$ 30 ng/ml), insufficient ($<$ 30–$>$ 20 ng/ml) and deficient ($\leq$ 20 ng/ml) levels.

Results

One patient with vitiligo (2.5%) versus 33 healthy controls (82.5%) have sufficient serum 25(OH)D levels while 39 patients (97.5%) versus 5 controls (12.5%) have deficient 25(OH)D levels with significantly lower serum 25(OH)D levels in patients compared to controls (P-value < 0.001). The other 2 healthy controls have insufficient 25(OH)D levels. Patients with vitiligo and autoimmune diseases have lower serum 25(OH)D levels than vitiligo patients without autoimmune diseases but with no significant difference. No significant correlations existed between age of the patients, duration of vitiligo, duration of associated autoimmune diseases, affected body surface area and serum 25(OH)D levels of patients.

Conclusion

Deficient serum 25(OH)D levels are present in vitiligo patients with and without systemic autoimmune diseases. Accordingly, screening for vitamin D deficiency seems of value in vitiligo patients for the possibility of vitamin D supplementation.

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Vitiligo is an autoimmune depigmentation disorder (1), commonly associated with systemic autoimmune conditions, including hypo and hyperthyroidism, autoimmune thyroiditis (2–5), type 1 diabetes mellitus, pernicious anaemia, rheumatoid arthritis and systemic lupus erythromatosus (3–5).

Recently, a connection between some autoimmune diseases and vitamin D deficiency has been reported, and vitamin D deficiency was suggested to act as an environmental trigger for the induction of autoimmunity (6). Vitamin D is a secosteroid hormone, and besides its known metabolic function has recently shown to have non-calciotropic immunomodulatory role through its varied effects on T and B lymphocytes, macrophages, and dendritic cells, which express nuclear vitamin D receptors (6, 7).

Vitamin D is obtained by the body from three sources. Endogenous synthesis of vitamin D is the main source. It occurs in the skin and is induced by ultraviolet (UV) B radiation. It may be also obtained exogenously through dietary intake and dietary supplements (7–9). 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) is the biologically active form of vitamin D, but its half-life is less than 4 h and it may remain normal in vitamin D-deficient states. 25-hydroxyvitamin D (25(OH)D), with a half-life of about 2 weeks, is the routinely assessed form of vitamin D levels (9). It reflects all sources of vitamin D exposure and represents a comprehensive and stable indicator of vitamin D status (7).

Regarding vitiligo and vitamin D, Silverberg *et al.* (10) in their pilot study assessed serum 25(OH)D levels in 45 vitiligo patients. More than 50% (55.6%) of patients were found to have insufficient levels of 25(OH)D. Moreover, the authors identify a subset of vitiligo patients (13.3%) with very low (deficient) levels of 25(OH)D that has comorbid autoimmunity. They suggested that vitamin D deficiency may be a risk factor for vitiligo development and that monitoring vitamin D levels in vitiligo patients may identify individuals at greater risk for secondary autoimmune disorder. However, the study of Silverberg and his colleagues (10) is a non-controlled cohort stud, that assessed the point prevalence of 25(OH)D deficiency in vitiligo patients.

Given the deficiency of studies on 25(OH)D levels in vitiligo patients, the effectiveness of topical vitamin D compounds on repigmenting vitiligo (11–13), the association of low vitamin D levels with systemic autoimmune diseases (6), and the results of the pilot cohort study of Silverberg *et al.* (10), the present study aimed to assess serum 25(OH)D levels in vitiligo patients with and without autoimmune diseases in comparison to healthy controls matched for age, gender, skin phototype and time of blood sampling.

METHODS

Study design and population

The present study represents a case-control study. Cases included two groups of equally distributed adult patients, Fitzpatrick skin phototypes III-IV, with nonsegmental vitiligo: 20 patients with systemic autoimmune diseases (group 1) and 20 patients without autoimmune diseases (group 2). They were randomly selected from patients attending the outpatient Dermatology and Rheumatology clinics of Ain Shams University Hospitals, Cairo, Egypt. To limit the effects of the seasonal fluctuation in vitamin D photosynthesis, patients were recruited during the same season, from April 2011 to mid-June 2011. Controls were 40 vitiligo-free healthy volunteers; with no family history of vitiligo or systemic autoimmune diseases in their first-degree relatives, matched for age ($\pm$ 2 years), gender, Fitzpatrick skin phototype (nearly the same level based on skin colour and response of individual's skin to sun exposure) and time of blood sampling (same day) to avoid any potential sources of bias.

Exclusion criteria included the use of any drugs that could alter the outcome of the study as vitamin D or calcium supplements, systemic steroids, hepatic enzyme inducers, weight loss drugs, cholesterol lowering drugs and thiazide diuretics. Patients with malabsorption disorders, kidney or liver diseases, and patients receiving phototherapy or photo-chemotherapy in the last 6 months, were also excluded from the study. Moreover, pregnant and lactating females, smokers, obese subjects and subjects applying sunscreen were not included in the present study. The study protocol conformed to the ethical guidelines of Ain Shams University and was approved by the local ethical committee of scientific research. Prior to initiation, every subject was informed about the aim of the study and gave consent.

Every subject was subjected to history taking including occupation (indoor or outdoor), course and duration of vitiligo in the patients, presence of systemic autoimmune disease(s), family history of vitiligo and/or autoimmune disease(s). The approximate daily amount of vitamin D intake from dietary source was calculated through the 24-h dietary recall method (7). They were also subjected to general examination searching for clinical manifestation(s) suggestive of autoimmune disease(s), and dermatological examination for assessment of extent of vitiligo in

the patients according to the affected body surface area and searching for the presence of skin manifestations suggestive of autoimmune disease(s).

Venous blood samples (5 ml) were then taken from all studied subjects to assess serum 25(OH)D levels in all subjects and to confirm the association of autoimmune disease(s) in group 1 and to exclude the presence of autoimmune disease(s) in group 2 and controls (thyroid disorders, type 1 diabetes mellitus, pernicious anaemia, rheumatoid arthritis and systemic lupus erythromatosus). Other appropriate laboratory tests were done when needed. Samples were collected in three sterile tubes, one containing anticoagulant EDTA for complete blood count, fasting blood sugar, fasting insulin and C-peptide and the second tube with separator gel for thyroid stimulating hormone, anti-thyroid peroxidase antibodies, rheumatoid factor and antinuclear antibody. All methods were performed according to the manufacturer's instructions. The third tube with separator gel was used for assessment of 25(OH)D levels. Blood was allowed to clot, centrifuged and separated into serum in sterile tubes and stored at −20°C until analysis. Quantitative determination of 25(OH)D was performed using reagents supplied by Diasorin (Stillwater, MN, USA) employing a radioimmunoassay technique. The values were interpreted as follows (7, 14): $\leq$ 20 ng/ml: deficient or very low, < 30–> 20 ng/ml: insufficient or low and $\geq$ 30 ng/ml: sufficient or normal.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 15. Quantitative data were presented as mean ± standard deviation (SD) and median while qualitative data were presented as number (n) and percentage (%). Comparison of quantitative and qualitative data between two groups was made using Student's t-test and Chi-square (χ^2) test respectively. Mann-Whitney test was used to assess the statistical significance of the difference between two study group medians. Analysis of variance (ANOVA) and χ^2 tests were used to compare more than two groups regarding quantitative and qualitative variables, respectively. Pearson correlation test was used to assess the strength of association between two quantitative variables. Linear regression was used to test and estimate the dependence of a quantitative variable based on its relationship to one or more independent variables. Probability (P) values $\leq$ 0.05 were considered statistically significant and $\leq$ 0.001 highly significant.

RESULTS

The present study included 40 vitiligo patients; 18 males and 22 females, 20 to 50 years of age (mean 34.1 ± 11.4) with median disease duration of 3.5 years (range 3 months to 20 years) and median affected body surface area of 20% (range 5–80%). The controls were 40 age-, gender- and Fitzpatrick skin phototype-matched vitiligo-free healthy volunteers. All female patients and controls were veiled. The patients were distributed equally into 20 patients with vitiligo and autoimmune diseases (group 1) and 20 patients with vitiligo and no autoimmune diseases (group 2).

The demographic data, clinical characteristics of vitiligo and vitamin D intake of all vitiligo patients, vitiligo patients with autoimmune diseases (group 1), vitiligo patients without autoimmune diseases (group 2) and controls are summarized in Table 1.

The comparison between all patients and controls revealed statistically non-significant differences as regards age ($P = 0.9689$), gender ($P = 1$), Fitzpatrick skin phototype ($P = 1$) and vitamin D intake ($P = 0.396$). On the other hand, statistically highly significant difference was found regarding their occupation (indoor versus outdoor) ($P = 0.001$). The comparison between vitiligo patients with autoimmune diseases (group 1) and those without autoimmune diseases (group 2) revealed statistically non-significant differences as regards age ($P = 0.44$), gender ($P = 0.204$), Fitzpatrick sin phototype ($P = 1$), occupation ($P = 0.204$), course of vitiligo ($P = 0.752$), vitamin D intake ($P = 0.109$), family history of vitiligo ($P = 0.147$) and family history of autoimmune diseases ($P = 1$). On the other hand, there were statistically significant differences between both groups regarding duration of vitiligo ($P = 0.008$) and affected body surface area ($P = 0.04$).

The serum 25(OH)D levels of all vitiligo patients, vitiligo patients with and without autoimmune diseases and controls are summarized in Table 2. One patient with vitiligo (2.5%) versus 33 healthy controls (82.5%) have sufficient serum levels of 25(OH)D while 39 patients (97.5%) versus 5 controls (12.5%) have deficient 25(OH)D levels with statistically highly significant difference ($P = 0.0001$). Moreover, statistically highly significant lower serum 25(OH)D levels existed in patients compared to controls ($P = 0.0001$). On the other hand, the comparison of serum 25(OH)D levels between patients with vitiligo and autoimmune diseases (group 1) and patients with vitiligo and no autoimmune diseases (group 2) revealed statistically non-significant difference, although lower levels were found in group 1 ($P = 0.095$). Highly significant differences existed on comparing serum 25(OH)D levels of each

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Table 1. Demographic data, clinical characteristics of vitiligo and vitamin D intake of all vitiligo patients, vitiligo patients with autoimmune diseases (group 1) and vitiligo patients without autoimmune diseases (group 2) and controls

			Patients $n = 40$		
		Patients $n = 40$	Group 1 $n = 20$	Group 2 $n = 20$	Controls $n = 40$
Gender	Males n (%)	18 (45)	11 (55)	7 (35)	18 (45)
	Females n (%)	22 (55)	9 (45)	13 (65)	22 (55)
Age (years)	Mean ± SD	34.1 ± 11.4	35.5 ± 12	32.7 ± 10.9	34.2 ± 11.5
Fitzpatrick skin phototype	III	19 (47.5)	10 (50)	9 (45)	19 (47.5)
	IV	21 (52.5)	10 (50)	11 (55)	21 (52.5)
Occupation	Outdoor n (%)	18 (45)	7 (35)	11 (55)	35 (87.5)
	Indoor n (%)	22 (55)	13 (65)	9 (45)	5 (12.5)
Course of vitiligo	Stable n (%)	19 (47.5)	9 (45)	10 (50)	–
	Progressive n (%)	21 (52.5)	11 (55)	10 (50)	–
Duration of vitiligo (years)	Median	3.5	2	5.5	–
Vitamin D intake	50–100 IU/day n (%)	14 (35)	5 (25)	9 (45)	15 (37.5)
	100–150 IU/day n (%)	14 (35)	6 (30)	8 (40)	18 (45)
	150–200 IU/day n (%)	12 (30)	9 (45)	3 (15)	7 (17.5)
Family history of vitiligo	Negative n (%)	38 (95)	20 (100)	18 (90)	40 (100)
	Positive n (%)	2 (5)	0 (0)	2 (10)	0
Family history of autoimmune diseases	Negative n (%)	32 (80)	16 (80)	16 (80)	40 (100)
	Positive n (%)	8 (20)	4 (20)	4 (20)	0
Affected body surface area (%)	Median	20	15	22.5	–

IU, international units; n, number of patients; %, percentage; SD, standard deviation.

Table 2. Serum 25-hydroxyvitamin D levels of all vitiligo patients, vitiligo patients with autoimmune diseases (group 1), vitiligo patients without autoimmune diseases (group 2) and controls

			Patients $n = 40$		
		Patients $n = 40$	Group 1 $n = 20$	Group 2 $n = 20$	Controls $n = 40$
25-hydroxyvitamin D levels	Deficient n (%)	39 (97.5)	20 (100)	19 (95)	5 (12.5)
	Insufficient n (%)	0 (0)	0 (0)	0 (0)	2 (5)
	Sufficient n (%)	1 (2.5)	0 (0)	1 (5)	33 (82.5)
25-hydroxyvitamin D levels/ ng/ml	Mean ± SD	12 ± 4.1	10.9 ± 3.7	13.1 ± 4.3	48.6 ± 23.3

n, number; %, percentage; SD, standard deviation.

patient's group with controls ($P = 0.001$ and $P = 0.001$, respectively), of indoor patients (10.7 ± 3.9) with indoor controls (48.42 ± 21.5) ($P = 0.001$) and of outdoor patients (13.5 ± 4) with outdoor controls (50.6 ± 23) ($P = 0.001$).

The serum 25(OH)D levels of the different subgroups of vitiligo patients categorized according to their gender, age (≤ 35 and > 35 years), Fitzpatrick skin phototype, occupation, vitamin D intake, course of vitiligo, associated autoimmune diseases and its type, family history of vitiligo and family history of autoimmune diseases are summarized in Table 3. Linear regression analysis that estimate the dependence of serum 25(OH)D levels on these different assessed variables revealed that occupation was the only independent factor affecting serum 25(OH)D levels, as those with outdoor occupation had significantly higher

Table 3. Serum 25-hydroxyvitamin D levels of the different subgroups of vitiligo patients

		25-hydroxyvitamin D level ng/ml			
		Mean	± SD	*P* value	Sig
Gender	Males ($n = 18$)	12.3	4.2	0.603	NS
	Females ($n = 22$)	11.7	4.1		
Age (years)	≤ 35 ($n = 29$)	12.5	4.2	0.671	NS
	> 35 ($n = 11$)	11.9	3.2		
Fitzpatrick skin phototype	III ($n = 19$)	12.6	3.6	0.22	NS
	IV ($n = 21$)	11.4	4		
Occupation	Indoor ($n = 22$)	10.7	3.9	0.036	S
	Outdoor ($n = 18$)	13.5	4		
Vitamin D intake	50–100 IU/day ($n = 14$)	13.1	4.8	0.413	NS
	100–150 IU/day ($n = 14$)	11.2	3.5		
	150–200 IU/day ($n = 12$)	11.5	3.9		
Course of vitiligo	Stable ($n = 19$)	12.85	4.54	0.197	NS
	Progressive ($n = 21$)	11.16	3.59		
Associated autoimmune diseases	Negative ($n = 20$)	13.1	4.3	0.095	NS
	Positive ($n = 20$)	10.9	3.7		
Type of autoimmune diseases	Diabetes mellitus ($n = 10$)	11.8	3.22	0.191	NS
	Hypothyroidism ($n = 8$)	10.8	4.06		
	Scleroderma ($n = 2$)	6.6	0.85		
Family history of vitiligo	Negative ($n = 38$)	12.12	4.15	0.301	NS
	Positive ($n = 2$)	9	0		
Family history of autoimmune diseases	Negative ($n = 32$)	12.39	4.23	0.190	NS
	Positive ($n = 8$)	10.25	3.2		

n, number of patients; NS, non-significant; P, probability; SD, standard deviation; S, significant; Sig, significance.

serum 25(OH)D levels compared to those with indoor occupation ($P = 0.036$).

There were no significant correlations between age of the patients ($r = 0.043$, $P = 0.791$), duration of vitiligo ($r = 0.040$, $P = 0.806$), duration of autoimmune diseases ($r = 0.026$, $P = 0.912$), affected body surface area ($r = 0.078$, $P = 0.634$), and serum 25(OH)D levels among patients (mean 12 ng/ml ± 4.1 SD).

DISCUSSION

In the present study, highly significant deficient serum 25(OH)D levels were found in vitiligo patients when compared to their age-, gender- and skin phototype-matched healthy controls. Significantly deficient serum 25(OH)D levels were also found in each group of vitiligo patients (group 1 with autoimmune diseases and group 2 without autoimmune diseases) in comparison to the controls. The possibility of this significantly deficient serum 25(OH)D levels in patients as a result of reduced sunlight exposure is excluded by the presence of significantly lower serum 25(OH)D levels in indoor patients in comparison to indoor controls and in outdoor patients compared to outdoor controls. Moreover, the statistically non-significant difference found between patients and controls regarding the approximate vitamin D daily intake provides further support to the association of vitiligo with vitamin D deficiency regardless of indoor or outdoor occupation or vitamin D daily intake and despite the plentiful exposure to sunlight throughout the year in our community.

The lower serum 25(OH)D levels of vitiligo patients with autoimmune diseases (group 1) than those of patients without autoimmune diseases (group 2), although having shorter disease duration and less affected body surface area, could be attributed to the comorbid association of autoimmune diseases in the first group of patients, resulting in further reduction of serum 25(OH)D levels and not related to disease duration or affected body surface area. The statistically non-significant difference between serum 25(OH)D levels of both groups of patients could be however attributed to the relatively small sample size in each group. Accordingly, further studies on a large scale of patients with and without associated autoimmune diseases are recommended.

In agreement with our results, Silverberg *et al.* (10) in their pilot study reported insufficient levels of serum

25(OH)D levels among vitiligo patients and demonstrated deficient levels in patients with comorbid autoimmune diseases. The key question is whether low 25(OH)D levels in vitiligo patients confer greater risk of developing secondary autoimmunity or autoimmune inflammatory processes consumes excess vitamin D, beyond its endogenous synthesis in the skin under effect of UV radiation, resulting in drops of 25(OH)D levels. Whether low 25(OH)D levels are the consequence or the cause of autoimmune disease, 25(OH)D screening may be a worthwhile screen for vitamin D deficiency and hence vitamin D supplementation to control autoimmunity. Given its relative safety in conjunction with its beneficial immunomodulatory effects (6, 7), there is optimism that correcting vitamin D deficiency will lead to better outcomes for vitiligo patients.

Topical vitamin D compounds were suggested to limit the melanocyte loss in vitiligo by counteracting the skin local immune process, oxidative protection, delayed programmed cell death, correction of the aberrant calcium fluxes and by photo-protecting the epidermal melanin unit (11–13). Also vitamin D analogues, acting mainly through their nuclear vitamin D receptors, restore the melanocyte integrity by controlling the activation, proliferation, and migration of melanocytes and the pigmentation pathways, and modulating the T cell activation that is apparently correlated with the melanocyte disappearance in vitiligo, resulting in reduced autoimmune damage of melanocytes (11). Furthermore, vitamin D analogues enhance tyrosinase activity (13).

Cross-sectional data point to a potential role of vitamin D supplementation in autoimmune disease prevention (6). In experimental animal models, oral vitamin D supplementation was found to prevent autoimmune diseases but prospective interventional evidence in humans is still lacking (15, 16). Accordingly, further studies assessing the role of vitamin D supplementation on vitiligo control and prevention of disease onset in susceptible family members of vitiligo patients are recommended.

Although the exact cause of vitiligo still remains unknown, it is clear that several different pathophysiologic processes may be involved. The autoimmune hypothesis in nonsegmental vitiligo is best supported by the presence of humoral and cellular immune aberrancies (17). Moreover, vitiligo is frequently associated with autoimmune disorders (2–5, 17) and is responsive to treatment with immunosuppressive agents (17). The deficient serum 25(OH)D levels in vitiligo patients of the present study as found in other autoimmune diseases provides further support to the autoimmune hypothesis of vitiligo and to the non-calciotropic immunomodulatory role of vitamin D (7).

In the present study, Although the daily vitamin D intake was below the normal daily requirements in all included subjects (200 IU/day for people aged 19–50 years) (18), deficient serum 25(OH)D levels were found in only 5 controls and insufficient levels in 2 controls, while the remaining 33 controls had sufficient serum levels of 25(OH)D. This could be attributed to the endogenous synthesis of vitamin D, since all 33 controls with sufficient levels had outdoor occupation. The major source of vitamin D is the skin exposed to sunlight, mainly UVB radiation which contributes to more than 90% of the serum concentration of 25(OH)D (7, 19). This is supported by our findings that patients and controls with outdoor occupation had higher serum 25(OH)D levels than their counterpart patients and controls with indoor occupation. Moreover, occupation was the only independent factor affecting serum 25(OH)D levels in patients. This agrees with the study done by Pazaitou-Panayiotou *et al.* (20) who found that working outdoors was an important determinant of serum 25(OH)D levels. All these data add further beneficial role to UVB phototherapy in the treatment of vitiligo as an inducer of endogenous synthesis of vitamin D. However, studies assessing serum 25(O)D levels before and after UVB phototherapy are recommended to prove its validity.

The significantly higher number of vitiligo patients with indoor occupation in comparison to controls could be attributed to feelings of embarrassment, which can lead to a low self-esteem and social isolation (21).

Although sunscreens may significantly reduce the solar-induced production of vitamin D, particularly those with high sun protection factors, their normal usage does not generally result in vitamin D insufficiency; a potential explanation being the well-documented fact that individuals do not apply sunscreens at the concentration that they are tested in the laboratory (2 mg/cm^2) (22). In the present study, we did not include patients using sunscreen to avoid the possibility of false results. Moreover, controls were matched with patients as regards their age, sex, skin phototype and the time of blood sampling to exclude the variations in serum 25(OH)D levels with the variations in any of these parameters between patients and controls. However, the limitations of this study are the absence of matching between patients and controls as regards their occupation, general sun exposure and vitamin D daily intake. Accordingly, further case–control studies are recommended putting in consideration all these variables to avoid potential sources of bias for serum 25(OH)D levels.

Based on the results obtained in the present study, we can conclude that 25(OH)D deficiency is present in vitiligo patients with and without autoimmune diseases. Accordingly, screening for vitamin D deficiency seems of value in vitiligo patients. Future studies are recommended to assess the association of vitamin D receptor gene polymorphisms to vitiligo. Moreover, the growing enthusiasm for vitamin D supplementation in autoimmune diseases emphasizes the need for timely and thorough testing of this hypothesis on a large sample size of vitiligo patients to assess the efficacy of oral vitamin D supplementation on controlling long-term disease activity and the possibility of prevention of disease onset in susceptible family members of vitiligo patients.

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