Review Article

The role of vitamin D in melanogenesis with an emphasis on vitiligo

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ABSTRACT

Vitiligo is a common pigmentary disorder caused by the destruction of functional melanocytes. Vitamin D is an essential hormone synthesized in the skin and is responsible for skin pigmentation. Low levels of vitamin D have been observed in vitiligo patients and in patients with other autoimmune diseases. Therefore, the relationship between vitamin D and vitiligo needs to be investigated more thoroughly. We reviewed the literature to date regarding the role of vitamin D in skin pigmentation. Our review revealed that vitamin D deficiency has been identified in many conditions, including premature and dysmature birth, pigmented skin, obesity, advanced age, and malabsorption. Vitamin D increases melanogenesis and the tyrosinase content of cultured human melanocytes by its antiapoptotic effect. However, a few growth-inhibitory effects on melanocytes were also reported. Vitamin D regulates calcium and bone metabolism, controls cell proliferation and differentiation, and exerts immunoregulatory activities. Vitamin D exerts its effect via a nuclear hormone receptor for vitamin D. The topical application of vitamin D increased the number of L-3,4-dihydroxyphenylalanine-positive melanocytes. The topical application of vitamin D yields significant results when used in combination with phototherapy and ultraviolet exposure to treat vitiligo in humans. Vitamin D decreases the expression of various cytokines that cause vitiligo. In conclusion, application of vitamin D might help in preventing destruction of melanocytes thus causing vitiligo and other autoimmune disorders. The association between low vitamin D levels and the occurrence of vitiligo and other forms of autoimmunity is to be further evaluated.

Key words: Autoimmune diseases, depigmentation, melanocytes, phototherapy, vitamin D, vitamin D receptor, vitiligo

INTRODUCTION

Vitiligo is a common pigmentary disorder characterized by well-demarcated depigmented patches or macules of different shapes and sizes. Vitiligo is caused by the destruction of functional melanocytes in the involved epidermis and the bulb/infundibulum of the hair follicle.[1-3] Vitiligo is an autoimmune disorder that affects 1-4% of the world's population,[4] regardless of gender or basic skin tone.[5] The disorder results in substantial cosmetic disfigurement. In some cultures, patients with vitiligo are regarded as social outcasts and are emotionally and physically affected.[3]

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A variety of therapeutic agents have been described in the literature, and many agents have been used in an attempt to treat vitiligo. However, no agent has been found to be uniformly effective. The most widely prescribed therapies are phototherapy and topical corticosteroids.[1,6]

The active form of vitamin D, calcitriol [1,25-dihydroxyvitamin D_3, $1,25(OH)_2D_3$], and analogues of this hormone (e.g., calcipotriol) are successful treatment options for patients with skin diseases, such as psoriasis and vitiligo,[7] when used topically.

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Although the association between vitamin D and pigmentation and the role of vitamin D deficiency has been established in numerous autoimmune diseases, the association between vitamin D levels and vitiligo still needs to be investigated more thoroughly. In this review, we summarize the existing information on the relationship between vitamin D, autoimmune diseases and pigmentation; we also highlight the knowledge gaps concerning the relationship between vitamin D and vitiligo.

In this review, we aimed to systematically review the published scientific literature till date regarding the role of vitamin D to enhance the pigmentation in human skin. We searched databases including MEDLINE/Pubmed, Embase, and Google Scholar for vitiligo, vitamin D, autoimmune diseases, melanocytes, vitamin D receptor, phototherapy, and depigmentation.

BASIC SCIENCE OF VITAMIN D

Vitamin D

Vitamin D is an essential hormone that is synthesized in the skin via a photochemical reaction, following the exposure of the skin to ultraviolet B (UVB) wavelength present in sunlight. In this reaction, previtamin D is converted by solar UVB-radiation in the skin into vitamin D, especially during the summer months. Limitations of vitamin D synthesis are age, pigmented skin, sunscreen use, and clothing.[8] Skin pigmentation is a known risk factor in patients with hypovitaminosis D because melanin, which is responsible for skin pigmentation, filters UV-radiation.[9]

Vitamin D derivatives

The two main forms of vitamin D are cholecalciferol and ergocalciferol. Both forms of vitamin D can be obtained by nutritional intake; ergocalciferol (vitamin D_2) is present in fungi/yeast, whereas cholecalciferol (vitamin D_3) is found in foods from animal origin[10], especially fatty fish, such as herring and mackerel. Other sources of vitamin D are milk, cheese, eggs, and cereals.

Biochemistry of vitamin D

The active form of vitamin D, 1,25-dihydroxyvitamin D_3 [1,25(OH) $_2D_3$], is a secosteroid (steroid with an opened B-ring) hormone that regulates calcium and bone metabolism, controls cell proliferation and differentiation and exerts immunoregulatory activities. This range of functions has been exploited clinically to treat a variety of conditions, including secondary hyperparathyroidism, osteoporosis, psoriasis, and vitiligo. Recent advances in the understanding of 1,25(OH) $_2D_3$ and its functions and novel insights into the mechanisms of its immunomodulatory properties suggest a wider applicability of this hormone in the treatment of autoimmune diseases and the prevention of allograft rejection.[11]

Physiology of vitamin D

The primary form of vitamin D, cholecalciferol [25(OH) D_3, the form measured to determine the level of vitamin D], is synthesized in the liver. The biologically active form, 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2D_3$], is then synthesized in the kidneys via the hydroxylation of 25(OH)D_3 by 1α-hydroxylase[12] and stimulates calcium absorption from the gut.[13]

The target organs of 1,25(OH) $_2D_3$ include the bone, intestine, and kidney and it stimulates calcium transport from these organs to the blood. The production of 1,25(OH)$_2D_3$ is stimulated by the parathyroid hormone (PTH). There is a negative feedback through calcium that decreases PTH and a direct negative feedback from 1,25(OH)$_2D_3$ to PTH. The active metabolite 1,25(OH)$_2D_3$ also shows rapid activities through a membrane receptor.[8]

Vitamin D receptor

Vitamin D exerts its effect via a nuclear hormone receptor called the vitamin D receptor (VDR). VDR is a member of the superfamily of nuclear receptors for steroid hormones, thyroid hormone, and retinoic acid. The VDR is a type 1 nuclear receptor, a transcription factor that forms homodimers and heterodimers that are active in the transcription and transrepression of approximately 900 genes.[14] VDRs are present not only in cells typically involved in calcium and bone metabolism but also in other cell types, such as keratinocytes, melanocytes, fibroblasts, and immune-system cells of the skin.[15] VDR acts by binding to specific DNA sequences as a heterodimer with a retinoid X receptor and to the basal transcription machinery in a ligand-independent (TFIIB) and -dependent manner (TFIIA). Genes with vitamin D response elements directly and indirectly influence cell cycling and proliferation, differentiation, and apoptosis.[16,17]

Birlea *et al*., found an association between the *VDR-Apa I* polymorphism and vitiligo.[18] This study revealed that *aa* genotype of *Apa-I VDR* was significantly more

frequent in patients with vitiligo; allelic frequencies showed a significant difference between vitiligo with other autoimmune diseases group and controls. *VDR* gene polymorphisms may affect 25(OH) D levels and the risk for the development of vitiligo. The *VDR* variant *BsmI-B* allele, the *ApaI-A* allele, and the *TaqI-t* allele were associated with a decreased risk for vitiligo, and there was also a dose-response relationship between decreased risk and increased 25(OH) D level in individuals with the *ApaI* allele.[19] In another study, Aydingoz *et al*., concluded that VDR TaqI gene polymorphism and the haplotype BsmI/ApaI/TaqI/FokI/Cdx2 GCCCG may be considered as novel risk factors in vitiligo.[20]

VITILIGO, CLINICAL DISORDERS, AND VITAMIN D

Vitamin D deficiency and diseases

Risk factors for vitamin D deficiency are premature and dysmature birth, pigmented skin, low sunshine exposure, obesity, advanced age, and malabsorption. The prevalence of vitamin D deficiency is also higher in elderly people than in adults, and it is especially prevalent in patients with hip fractures and in the residents of homes for the elderly and nursing homes.[21] The low vitamin D levels found in Pemphigus vulgaris and Bullous pemphigoid may suggest a role for this agent in their pathogenesis. The prevalence of fracture was increased in this group.[22] Low levels of vitamin D have also been associated with cardiovascular disease, including myocardial infarction.[23]

The prevalence of vitamin D deficiency is much higher in Europe than in Asia, Australia, or the USA. The prevalence of vitamin D insufficiency is also high in African Americans, whose highly pigmented skin makes the UV-light much less efficacious.[24] A high prevalence of vitamin D deficiency has been reported in nonwestern immigrants in the Netherlands,[25] and similar data was obtained in the Middle East,[26] where life-style factors probably play a role.

Black patients have a higher risk of insufficiency of vitamin D than White patients, and it was observed that prepubescent White girls have higher vitamin D levels than Black girls in the United States.[27] It has been reported that lower vitamin D levels in patients of color may explain the increased rates of peripheral vascular disease and invasive breast cancer.[28] VDR polymorphisms have been associated with breast cancer cases in Caucasian females, but not in African-American females, suggesting that chronic low levels of vitamin D are more at fault.[29]

Low vitamin D levels have also been associated with autoimmune diseases, including systemic lupus, diabetes mellitus, rheumatoid arthritis, and multiple sclerosis.[30-33] The mechanism by which vitamin D affects autoimmunity is unknown, but there is a clear regulation of immune cells by vitamin D *in vitro*.[30] The association of low vitamin D levels with vitiligo and multiple forms of autoimmunity needs to be further evaluated.

IN VITRO STUDIES

Murine B16 melanoma cells treated with vitamin D_3 exhibit an increase in tyrosinase activity and melanogenesis.[34] Tomita *et al.,* showed that vitamin D_3 increased the tyrosinase content of cultured human melanocytes.[35] Watabe *et al.,* provided some important clues to understand the role of vitamin D_3 in melanocyte development and melanogenesis and observed that L-3,4-dihydroxyphenylalanine-positive (DOPA-positive) cells are increased after 1,25(OH) $_2D_3$ treatment in primary neural crest cell cultures.[36] These findings indicate that 1,25(OH) $_2D_3$ may stimulate the differentiation of immature melanocyte precursors. Electron microscopy demonstrates the presence of melanosomes at more advanced stages in 1,25(OH) $_2D_3$-treated cells as compared with untreated cells.[36] In another study, it was observed that vitamin D and UVB irradiation promoted the proliferation of melanocytes, which indicates that this combination might be effective in the treatment of vitiligo.[37]

GROWTH INHIBITORY EFFECTS OF VITAMIN D ON MELANOCYTES

In contrast to its stimulatory effects on melanocyte proliferation, vitamin D was also reported to have an inhibitory effect on melanocyte growth[38] as well as the melanization of cultured human melanocytes.[39] In another study, vitamin D inhibited the proliferation of melanocytes in a dose-dependent manner, though it did not show any adverse effects on the melanization process of melanocytes.[40]

IN VIVO STUDIES

Abdel-Malek *et al.,* showed that the topical application of 100 μg of cholecalciferol to the pinnal epidermis of

DBA/2J mice for 5 or 10 days increased the number of DOPA-positive melanocytes and had a synergistic effect with a low dose of UVB-light.[41] The combination of psoralen and ultraviolet A (PUVA) with calcipotriol in vitiligo works fast, and the duration of PUVA treatment can be reduced to yield more cosmetically acceptable results.[42]

VITAMIN D AND VITILIGO

Topical vitamin D3 analogues are a new addition to the armamentarium of therapeutic modalities for vitiligo. The use of vitamin D analogues in combination with PUVAsol and topical calcipotriol for the treatment of vitiligo was first reported by Parsad *et al.,*[42] Subsequently, a number of studies have been reported the treatment of vitiligo with vitamin D analogues alone or in combination with ultraviolet light or corticosteroids to enhance repigmentation.[43] In a recent review, Birlea *et al*., have shown insight into the main intracellular pathways through which vitamin D3 analogues alone or in different combinations may contribute to repigmentation in vitiligo.[38] Birlea *et al.,* reviewed 22 studies published on calcipotriol/tacalcitol used alone or in combination with other agents for evaluation and concluded that many studies have shown vitamin D3 analogues to be effective in combination with PUVA, NBUVB, or an excimer laser.[38] In another study, Oh *et al*., reported that high concentration of tacalcitol was applied topically with 308-nm xenon chloride excimer laser to lower the energy threshold for significant clinical purpose to treat nonsegmental vitiligo.[44]

In a recent pilot study, serum concentrations of vitamin D in vitiligo patients were estimated and divided into three groups: 31.1% were normal (>30 ng/mL), 55.6% were insufficient (<30 ng/mL), and 13.3% were very low (<15 ng/mL).[45] Insufficient vitamin D levels were associated with an increasing Fitzpatrick phototype. Very low 25-hydroxyvitamin D levels were associated with comorbid autoimmune illnesses, but not with age, gender, race/ethnicity, family history of vitiligo or autoimmune disease, new-onset disease, or body surface area affected. This study was limited, as it assessed point prevalence in a small cohort (total of 45 patients) without assessing the seasonal variations in vitamin D levels and as there was no control group. In a recently published case report, investigators found low levels of vitamin D (12 ng/mL) in a vitiligo patient.[23]

Another study investigated the association between VDR polymorphisms and vitiligo, and it revealed that the *Apa-I* polymorphism of the VDR gene is associated with vitiligo.[18] This suggests that vitamin D or its receptor might play a role in the etiopathogenesis of skin pigmentation.

VITILIGO TREATMENT AND VITAMIN D

Application of vitamin D with phototherapy and UV exposure to treat vitiligo

The occurrence of hyper-pigmentation in psoriatic lesions treated with calcipotriol led to the discovery of a new therapeutic modality in vitiligo.[46] Calcipotriol is effective on immunomodulatory systems, inflammatory mediators, and melanocytes[47] and it may stimulate melanin production by activating melanocytes and keratinocytes.[48] It has been found *in vivo* that melanocytes in the epidermis become swollen with elongated dendrites after UV-irradiation of the skin. The tyrosinase activity in these melanocytes is increased by microphthalmia transcription factor (MITF),[49] resulting in the deposition of the enzyme product, melanin, in the epidermis, several days after irradiation. Tomita *et al.,* found that vitamin D_3-induced features similar to those noted in UV-irradiated skin; specifically, it increased the cell size, the number of dendrites, and the amount of immunoreactive tyrosinase.[35] Ermis *et al.,* also reported that combination treatment with calcipotriol and PUVA seems to be safe and much more effective in initiating and achieving complete repigmentation than a placebo with PUVA.[50]

A marginal type of repigmentation pattern occurred more frequently with these topical agents, and it was observed that the onset of repigmentation induced by calcipotriol was slow.[51] However, in a few cases, treatment failure or no added response to combination therapy with these analogues was also observed at the end of 3 months.[43]

INFLUENCE OF NARROWBAND UVB PHOTOTHERAPY ON VITAMIN D

A recent study investigated the influence of low-dose narrowband UVB phototherapy on serum levels of vitamin D.[52] The results of the study revealed that UVB phototherapy increased vitamin D levels in patients with low initial levels of 25-hydroxyvitamin D (25(OH) D) (the serum marker for vitamin D status),

which indicates that the beneficial effect of UVB depends, at least partially, on the induction of vitamin D.

VITAMIN D REGULATES CA^{2+} FOR PIGMENTATION

Defective calcium (Ca^{2+}) transport has been shown in keratinocytes and melanocytes obtained from vitiliginous skin samples.[53] Ca^{2+} controls the activity of both plasma membrane-associated and cytosolic thioredoxin reductase. Decreased intracellular Ca^{2+} leads to high levels of reduced thioredoxin, the product of thioredoxin, which inhibits tyrosinase activity and results in the inhibition of melanin synthesis. Moreover, it has been shown that melanocytes express 1,25-dihydroxyvitamin D_3 receptors, which take part in the regulation of melanin synthesis.[41,54] It is likely that calcipotriol may play a role in Ca^{2+} regulation by 1,25-dihydroxyvitamin D_3 receptors on melanocytes and/or by the regulation of defective Ca^{2+} homeostasis.[50]

EFFECTS OF VITAMIN D ON VITILIGO BY DECREASING THE EXPRESSION OF CYTOKINES

It has been reported that the increased expression of proinflammatory and proapoptotic cytokines, such as IL-6, IL-8, IL-10, IL-12, INF-α, and TNF-α, cause vitiligo and play a role in the pathogenesis of vitiligo.[2,55] Vitamin D might exert immunomodulatory effects by inhibiting the expression of IL-6, IL-8, TNF-α, and TNF-γ.[56] Vitamin D compounds were shown to have modulatory effects on dendritic cell maturation, differentiation, and activation in both human and murine culture systems,[57] probably via a VDR-dependent pathway.[58] Furthermore, vitamin D compounds are shown to induce the inhibition of antigen presentation.[57,58]

EFFECTS OF ORAL VITAMIN D SUPPLEMENTS ON AUTOIMMUNE DISEASES

In many studies, it was observed that vitamin D supplementation was therapeutically effective in different experimental animal models, such as allergic encephalomyelitis, collagen-induced arthritis, type 1 diabetes mellitus, inflammatory bowel disease, autoimmune thyroiditis, and systemic lupus erythematosus.[59-63] Therefore, the supplementation of vitamin D can possibly be used as a treatment in autoimmune diseases such as vitiligo.

MOLECULAR MECHANISM OF REPIGMENTATION BY VITAMIN D

Vitamin D protects the epidermal melanin unit and restores melanocyte integrity by two main mechanisms: By controlling the activation, proliferation, migration of melanocytes and pigmentation pathways by modulating T cell activation, which is apparently correlated with melanocyte disappearance in vitiligo. The multiple effects of VDR on immune cells lead to the recognition that vitamin D could be a potent immunomodulator. The coordination of T cell activation is exerted mainly by the inhibition of T cell transition from the early to the late G1 phase and by the inhibition of several cytokine genes, such as those encoding TNF-α and IFN-γ.[64]

The mechanism through which vitamin D exerts its effects on melanocytes is not yet fully understood. Vitamin D is believed to be involved in melanocyte physiology by coordinating melanogenic cytokines [most likely endothelin-3 (ET-3)] and the activity of the SCF/c-Kit system, which is one of the most important regulators of melanocyte viability and maturation.[64] Furthermore, a proposed mechanism involving vitamin D in the protection of vitiliginous skin is based on its antioxidant properties and regulatory function towards the reactive oxygen species that are produced in excess in vitiligo epidermis.

VITAMIN D REDUCES APOPTOTIC ACTIVITY IN MELANOCYTES

Vitiligo is characterized by the loss of melanocytes from the epidermis, which causes depigmentation in the skin.[65] Apoptosis has been reported to be a mechanism that removes melanocytes from the skin.[66] The active form of vitamin D reduces the apoptotic activity induced by UVB in keratinocytes[67] and melanocytes[68] by the production of interleukin-6.[67] In another study, it was observed that vitamin D protected melanocytes from apoptosis through the formation of sphingosine-1-phosphate [Table 1], which opposes apoptotic action in diverse melanoma cell lines.[69] A recent study reported that vitamin D protects DNA against oxidative damage, with net tumoristatic and anticarcinogenic effects.[70] The mentioned studies provide evidence that vitamin D can prevent the death of melanocytes, thus preventing the loss of pigment in the skin, which could be a very useful finding in the treatment of vitiligo, if approached correctly.

Table 1: Response of Vitamin D on melanocytes and skin repigmentation

Action of vitamin D	Response	References
Vitamin D	It acts on specific T cell by inhibiting the expression of several proinflammatory cytokines genes, such as tumor necrosis factor alpha and interferon gamma in vitiligo	Birlea *et al.*[64]
1alpha, 25-Dihydroxyvitamin D3	It protects human melanocytes from apoptosis by formation of sphingosine-1-phosphate	Sauer *et al.*[69]
1,25-Dihydroxyvitamin D3 [1,25(OH) 2D3]	It has antiapoptotic effects and decreased cyclobutane pyrimidine dimers damage by up to 60%	Wong *et al.*[72]
Tacalcitol, a vitamin D analogue	Tacalcitol plays a role in Ca^{2+} regulation by vitamin D receptor (VDR) on melanocytes	Lu-yan[73]
Vitamin D	VDR is the nuclear receptor that mediates the effects of vitamin D through regulating the transcription of other genes	Han *et al.*[74]

PATIENT SELECTION CRITERIA FOR TREATMENT OF VITILIGO WITH VITAMIN D ANALOGUES

There are a few important steps that should be followed during the treatment of vitiligo with vitamin D in the clinic. The first step is the selection of patients, as variation in patient features, such as age or duration, extent and type of vitiligo, and affected areas, are important considerations in determining the applicability of treatment and may result in variable responses. As the mechanism of vitamin D action is slow, vitamin D analogues will be effective in patients with stable disease or slow-spreading disease. The second step is to measure the vitiligo affected area by a standard method before and after treatment, as it is an important limiting factor and there is no uniformly accepted scoring system for disease activity. Recently, our group reviewed different vitiligo assessment methods to assess the depigmented and pigmented areas in vitiligo patients before and after treatment.[71]

CONCLUSIONS AND FUTURE DIRECTIONS

Vitiligo is caused by the destruction of functional melanocytes in the epidermis. Vitiligo is generally considered to be an autoimmune disorder. There is preliminary evidence that vitiligo patients, as well as patients with other autoimmune disorders have low levels of vitamin D. Vitamin D is synthesized in the skin in the presence of UVB wavelengths that come from sunlight. Vitamin D and its analogues have been used to successfully treat vitiligo and psoriasis. Vitamin D efficiency is increased when used in combination with UV or corticosteroids. However, in a few *in vitro* studies, vitamin D showed inhibitory effects on the growth of melanocytes, while in some cases, it was not effective for repigmentation. Other effects of vitamin D on melanocytes are summarized in Table 1.

It is still unknown if vitamin D deficiency plays a role in causing vitiligo, as it does in other autoimmune diseases. If vitamin D deficiency does cause vitiligo, then its supplementation could help control the disease. Therefore, the relationship between the level of serum vitamin D and vitiligo should be tested in a large controlled study. Moreover, oral vitamin D intake should be observed to prevent disease onset in susceptible family members of vitiligo patients. More studies are to be performed on this topic to reveal the effect of phototherapy and the application of vitamin D on repigmentation. Additionally, more studies are necessary to determine the association of VDR polymorphisms and disease activity in vitiligo patients.

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Multiple choice questions

1. Vitamin D is synthesized in the skin in the presence of
   a. Ultraviolet B b. Red light
   c. Green light d. Infra-red light

2. The source of ergocalciferol (vitamin D2)
   a. Bacteria b. Fungi/yeast
   c. Algae d. Lichens

3. The active form of vitamin D (1,25-dihydroxyvitamin D3) regulates metabolism of
   a. Calcium c. Iron
   c. Fluoride d. Phosphate

4. The primary form of vitamin D (cholecalciferol) is synthesized in the
   a. Pancreas b. Gall bladder
   c. Intestine d. Liver

5. The production of vitamin D (1, 25(OH) 2D3) is stimulated by
   a. Gonadotropin b. Luteinizing hormone
   c. Parathyroid hormone d. Testosterone

6. What is the normal level of vitamin D in serum?
   a. >30 ng/mL b. from 15 to 30 ng/mL
   c. <15 ng/mL d. <5 ng/mL

7. Which type of radiation is filtered by melanin?
   a. Visible radiation b. Ultraviolet radiation
   c. Electromagnetic radiation d. Infrared radiation

8. Vitamin D receptor is a member of the superfamily of nuclear receptors for
   a. Adrenal hormones b. Gonadotropin hormones
   c. Luteinizing hormones d. Steroid hormones

9. Vitamin D protects melanocytes from apoptosis through the formation of
   a. Sphingosine-1-carbonate b. Sphingosine-1-oxolate
   c. Sphingosine-1-phosphate d. Sphingosine-1-fluorate

10. The active form of vitamin D reduces the apoptotic activity in melanocytes by the production of
   a. Interleukin-6 b. Interleukin-8
   c. Interleukin-10 d. Interleukin-12

Answers:
1. a, 2 b, 3 a, 4 d, 5 c, 6 a, 7 b, 8 d, 9 c, 10 a