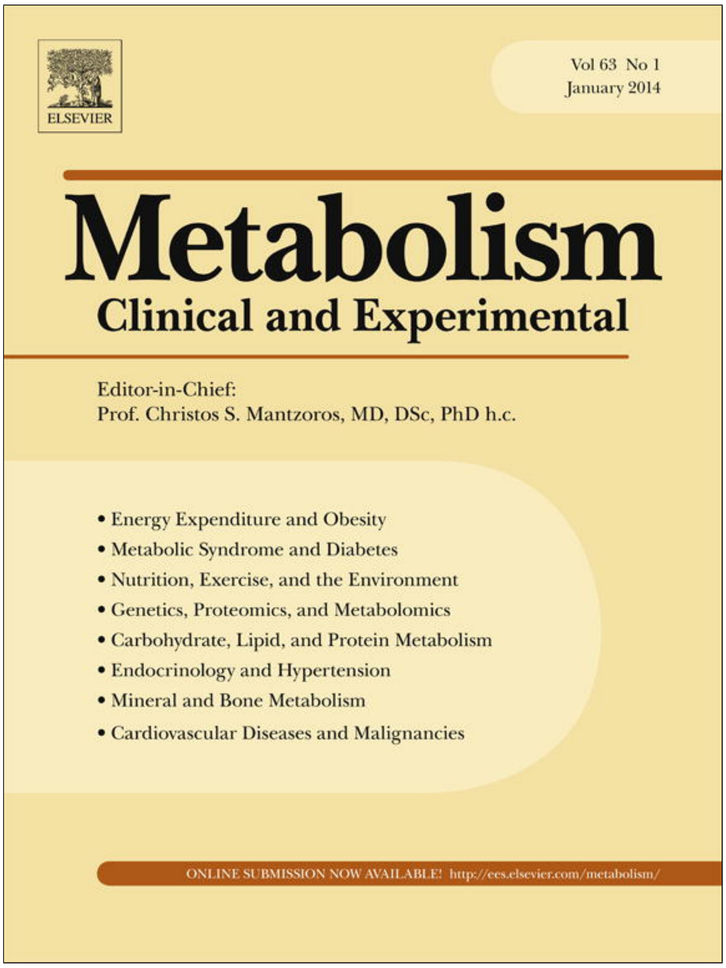
ELSEVIER
Vol 63 No 1
January 2014
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METABOLISM CLINICAL AND EXPERIMENTAL 63 (2014) 32–41

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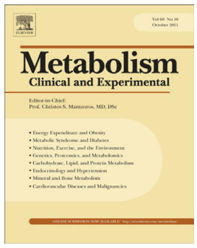

Vitamin D in endometriosis: A causative or confounding factor?

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ARTICLE INFO

Article history:
Received 17 April 2013
Accepted 16 September 2013

Keywords:
Vitamin D binding protein
Endometrium
HOXA 10 gene
Elocalcitol

ABSTRACT

Objective. The aim of this paper is to review the evidence from studies that evaluated the relationship between vitamin D and endometriosis.

Design. Comprehensive review.

Materials and Methods. Systematic literature search in Medline for relevant publications from 1946 until June 2013.

Results. Endometriosis risk may be influenced by dietary vitamin D intake and plasma hydroxyvitamin D concentration. Vitamin D receptor and vitamin D metabolizing enzymes, 24-hydroxylase and 1-α hydroxylase, are found in the normal cycling endometrium and also in the eutopic and ectopic endometrium of women with endometriosis. The endometrium is a target of 1, 25 dihydroxyvitamin D actions through regulation of specific genes and via immunomodulation. The endometrium in endometriosis expresses dysregulation of some vitamin D enzymes and receptors. If vitamin D and its metabolites are implicated in endometriosis-associated infertility, it is likely through interference with HOXA10 gene expression. The Gc2 phenotype of vitamin D binding protein is prevalent in women with endometriosis and may be implicated in its pathogenesis. In a mouse model, Elocalcitol, a VDR-agonist was shown to reduce the development of endometriotic lesions and recurrence.

Conclusion. A biological plausibility for a role of vitamin D, as an immunomodulator and anti-inflammatory agent, in the pathogenesis and treatment of endometriosis is suggested in this article, but is difficult to illustrate due to sparse evidence from human studies limited primarily to case–control studies. A significant knowledge gap precludes the establishment of a clear cause–effect relationship. The intriguing leads presented herein need to be investigated further with placebo-controlled supplementation trials.

1. Introduction

Endometriosis, defined as the presence of endometrial glands and stroma in ectopic locations, affects 6%–10% of reproductive-age women. It is associated with dysmenorrhea, dyspareunia, chronic pelvic pain, irregular uterine bleeding and/or infertility [1].

A unifying theory regarding the origin of endometriosis has remained elusive. Several theories have been presented to account for the heterogeneity of this disease [2–4], the

Abbreviations: VD, vitamin D; DBP, VD binding protein; Gc, group-specific component; GcMAF, Gc-protein derived macrophage-activating factor; HOXA10, homeobox A10; IBD, inflammatory bowel disease; 25-OHD, 25-hydroxyvitamin-D3; MMP, matrix metalloproteinase; 1,25(OH)2D, 1, 25 dihydroxyvitamin D; VDRE, VD responsive element; OPN, Osteopontin.

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0026-0495/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.metabol.2013.09.012

most widely accepted being Sampson's theory of reflux menstruation that assumed that endometriosis is caused by the seeding of endometrial cells by trans-tubal regurgitation during menstruation. However, whatever the pathophysiology of this disease is, endometriosis is considered currently as a chronic, estrogen-dependent, and inflammatory disease [2].

When oxidative stress was thought to be incriminated in endometriosis pathophysiology, antioxidants like Vitamin A and E were evaluated, but were not found to be associated with endometriosis in adjusted analysis [5].The association between vitamin D and endometriosis seems more complex. Indeed increased levels of serum 25-hydroxyvitamin-D_3 (25-OHD) in the sera of women with endometriosis, independent of season, age, and therapy were reported by Somigliana et al. [6] with a biological gradient showing more striking differences in women with advanced stages, however, other studies have failed to demonstrate this association [7,8]. Recently, in a large prospective study a significantly lower rate of laparoscopically confirmed endometriosis was observed among women with greater predicted plasma 25-OHD levels and higher intake of dairy foods [9].

The pleotropic effects of vitamin D (VD), extending beyond the regulation of plasma calcium concentration and skeleton mineralization, have emerged in recent years. VD plays an important role in several other physiological pathways, and has been shown to be endowed with anti-inflammatory properties, and thus may play a central role in the pathogenesis/treatment of this complex inflammatory disease [10,11].

The exact nature of the relationship between endometriosis and the VD endocrine system remains to be clarified. In order to gain insight into this topic we performed a systematic literature search and used references from selected pertinent articles retrieved.

2. Materials and methods

We conducted a review of the literature using the Medline OVID database searching for clinical and experimental in vitro studies on Vitamin D and endometriosis, from January 1946 to June, 2013. The Boolean model created consisted of the 2 major concepts and their related MESHS terms ultimately combined with the AND term. The first concept included the terms vitamin D, Calcitriol, vitamin D deficiency, with each term searched singly, and captured whether included in title, abstract, subject heading, unique identifier, then all were then merged through the OR term leading to 64,832 titles. The second concept included the terms endometriosis, adenomyosis, endometrium, infertility, sterility entered as full terms or truncated term, and captured whether included in title, abstract, subject heading, unique identifier, all were then merged through the OR term leading to 201,728 titles. The above two concepts merged through the use of the AND term, leading to 294 titles. All articles were scanned by title and abstract to select relevant publications, and N = 87 were identified for full review and an additional N = 24 retrieved from selected references and/or author's libraries, were also used after deleting 10 duplicates.

2.1. *Vitamin D metabolism*

Cholecalciferol (Vitamin D_3) is the result of the conversion of 7-dehydrocholesterol, a VD precursor present in the skin, under Ultraviolet B radiation. To become biologically active, two hydroxylation steps of vitamin D_3 are necessary: a 25-hydroxylation mainly in the liver, leading to 25-OHD, and a 1α-hydroxylation mainly in the proximal tubules cells, leading to 1, 25 dihydroxyvitamin D, (1,25(OH)2D) the metabolite that best reflects VD nutritional status [9,12]. However, because several extrarenal tissues also express the 1α-hydroxylase, the biologically active secosteroid, 1,25(OH)2D can be locally made in tissues such as bone, bone marrow, prostate, and macrophages [13,14]. For transport, VD compounds are complexed to plasma proteins, of which the most important is the liver-produced VD binding protein (DBP) [9].

Biological actions of VD are mediated through the VD receptor (VDR), a nuclear receptor that affects transcription of over 900 genes, and that is expressed in many tissues and organs including skeleton, parathyroid glands, and the reproductive tissues [9,15]. Catabolism of 1,25(OH)2D and 25-OHD to biologically inactive calcitroic acid is mediated by the 24-hydroxylase, in the kidney and liver [16].

2.2. *Vitamin D and endometriosis: A plausible link?*

2.2.1. *Diet, dairy products, Serum 25-OHD levels and endometriosis*

Diet may influence endometriosis through effects on inflammation, smooth muscle contractility, immune functions and estrogenic effects. In the largest prospective cohort study performed over a 14 year period and published recently by Harris et al., which included 1385 cases of incident laparoscopically confirmed endometriosis, women consuming more than 3 servings of total dairy foods per day were 18% less likely to be diagnosed with endometriosis than those reporting two servings per day [RR = 0.82, 95% CI (0.71–0.95)]. In addition, predicted plasma 25-OHD level was inversely associated with endometriosis; women in the highest quintile of predicted vitamin D level had a 24% lower risk of endometriosis than those in the lowest quintile [RR = 0.76, 95% CI (0.6–0.97)] [9]. These findings are in contrast with reports of increased vitamin D reserve [6] or comparable 25-OHD levels between women with and without endometriosis [7,8]. This could be explained by the small sample size, heterogeneity, and case–control nature of these previous studies (Tables 1 and 2).

2.2.2. *Endometriosis as autoimmune disease and VD as an immunomodulator*

Endometriosis fulfills most of the classification criteria for an autoimmune disease, including female preponderance, familial occurrence with possible genetic preference, increased likelihood of other autoimmune disease as inflammatory bowel disease (IBD), polyclonal B cell activation, and immunological abnormalities in T and B cell functions [17–20].

An immune-mediated defect in recognition and elimination of endometrial fragments refluxed in the peritoneal cavity has been proposed to play a crucial role in endometriosis development [17]. Activated CD4+ CD8+ lymphocytes,

Table 1 – Overview of the dysregulation of vitamin D metabolites, enzymes, and carrier protein in body fluids and tissues in Endometriosis. [a]

Serum	
Increase in 25-OHD	Somigliana et al. [6]
No change in 25-OHD	Agic et al. [7]
Decrease in 25-OHD	Harris et al. [9]
No change in DBP	Borkowski et al. [8]
Increase three fold in DBP and three fold in GC2 allele expression	Faserl et al. [79]
Endometrium	
Increase in VDR	Agic et al. [7]
Increase in 1-αhydroxylase	
Increase in 24-hydroxylase expression	
Peritoneal fluid	
No change in DBP	Borkowski et al. [8]
Urine	
Increase in DBP	Cho et al. [89]

[a] Additional details of the studies mentioned in Table 2.

macrophages and dendritic cells, express widely VDR and both the activating and metabolizing enzymes, 1-α hydroxylase and 24- hydroxylase [21,22]. This suggests that 1,25(OH)2D can be produced locally in the immune system and plays an autocrine–paracrine role [23].

The relation between VD and autoimmune diseases has been strengthened by two main observations: an association between VD deficiency and the increased risk of IBD, rheumatoid arthritis (RA), systemic lupus erythematosus, multiple sclerosis and type 1 diabetes, all known to have an autoimmune component [24]. The second is the seasonal variation in the onset and exacerbation of some autoimmune diseases, such as RA, with a peak in late winter, correlating with serum VD levels [25].

Despite the evidence presented in favor of viewing endometriosis as an autoimmune disease and VD as an immunomodulator, the relationship between these two entities remains complex. This is mainly because endometriosis is associated with normal [7,8] or high 25-OHD reserve [6], rather than insufficiency/or deficiency, as would have been expected. Furthermore, the manifestations of endometriosis do not exhibit seasonal flares or exacerbations that potentially could correlate with the well described seasonal changes in 25-OHD levels. It is plausible that the immunomodulatory role of VD in this disease, if existent, is local, autocrine and/or paracrine, at the level of endometriotic foci or lesions. If so, it would be missed by correlating disease manifestations with circulating serum 25-OHD levels, and could only be identified by targeted in vitro studies, which to the best of our knowledge, are lacking.

2.2.3. *Endometriosis mimicking malignancy and vitamin D as anti-neoplastic agent*

Endometriosis also shares several similarities with malignant diseases such as reduced apoptosis, invasion of endometrial cells into adjacent organs (bowel, bladder), increased angiogenesis, recurrence, and the need of repeated surgical interventions [7,26,27]. Women with endometriosis have twice the risk of developing ovarian cancer, compared to controls, a risk that is 4-fold increased if they also suffer from infertility [28].

In preclinical models, 1,25(OH)2D has been shown to exert significant antineoplastic activity acting like a transcription factor that influences central mechanisms of tumorigenesis: growth, cell differentiation, and apoptosis [29]. Inhibition of tumor invasion by 1,25(OH)2D includes inhibition of serine proteinases, metalloproteinases, and angiogenesis [30]. Moreover, a double-blind randomized placebo-controlled trial on 1179 women revealed that improved calcium and VD status substantially reduces all cancer risk in postmenopausal women [31]. However, the potential antineoplastic/antiproliferative effect of 1,25(OH)2D on endometrial lesions is currently speculative and remains to be pursued on experimental endometriosis models. Elucidation of such role will have a significant impact on our understanding of the pathogenesis of endometriosis and its treatment.

2.2.4. *Chronic pelvic pain in endometriosis and vitamin D*

Endometriosis is the most common cause of chronic pelvic pain in women of child-bearing age, resulting in significant physical and social debility [32,33]. VD has been inconsistently implicated in chronic pain conditions, such as musculoskeletal pain, pain perception in elderly, premenstrual syndrome, fibromyalgia, and dysmenorrhea [34–37].

Recently, a randomized double-blind study, investigated the role of VD in primary dysmenorrhea [38]. Women received a single oral dose of cholecalciferol (300,000 IU/ml) 5 days before their expected menses ($n = 20$) or placebo ($n = 20$). A 41% reduction in the mean pain score was noticed in the VD-treated group, over the 60-day study period ($p < 0.01$). The greatest reduction of pain scores ($r = -0.76$; $p < 0.01$) was noted in women with severe pain at baseline.

The pain reduction could be attributed to the action of 1,25(OH)2D on the endometrium with a decrease in prostaglandin synthesis and an increase in prostaglandin inactivation by suppression of cyclo-oxygenase2 and up-regulation of 15-hydroxyprostaglandin dehydrogenase, respectively. 1,25(OH)2D may also exert anti-inflammatory effects through other pathways, such as inhibiting nuclear factor-β signaling and increasing mitogen-activated protein kinase phosphatase 5 activity, thus blocking cytokine production via p38 activation [39]. Use of cholecalciferol in these patients, especially when exhibiting low plasmatic levels of 25(OH)D, may allow these women to limit the use of non-steroidal anti-inflammatory drugs [38].

In endometriosis, the leading cause of secondary dysmenorrhea [32], the etiology of pain could be related to sprouting of nerve fibers by a process of neuroangiogenesis initiated in local endometriotic foci and lesions [40]. These nerve fibers will lead to a peripheral sensitization followed by a central sensitization involving the neurons of the dorsal root of the spinal cord and affecting their peripherally dependent or peripherally independent sensitization. This will result in propagation of the painful stimuli to multiple spinal cord segments and generation of other pain syndromes in women with endometriosis, such as painful bladder syndrome, and irritable bowel syndrome [40].

This is way more complex than the simple theory of prostaglandins dysregulation related to primary dysmenorrhea and relieved by VD supplementation, however taking into consideration the role $1,25(OH)_2D$ as a reducer of angiogenesis in vivo [41], and the neuroangiogenesis

Table 2 – Specific aspects of studies investigating vitamin D system dysregulation in Endometriosis.

Authors	Study Date	Study Characteristics	Study Size	No. of cases with endometriosis/ Controls	Endometriosis Definition	Vitamin D-Related Compounds Measured	Adjustment for confounders [a]	Results	Conclusion
Somigliana et al.	2007	Prospective cross sectional study	140	85/73	rASRM, 1996	Serum 25-OHD levels	Adjustment for season of the year and for medications known to affect bone metabolism only.	24.9 ± 14.8 ng/ml and 20.4 ± 11.8, (P = 0.05) [b]	Endometriosis is associated with higher serum levels of vitamin D
Agic et al.	2007	Case control	79	46/33	rASRM, 1996	Serum 25-OHD levels	Adjustment for season of the year only	25.7 ± 2.1 ng/mL and 22.6 ± 2.0 ng/mL, (P = 0.31) [b]	No differences in 25-OH vitamin D levels between the serum of patients with endometriosis and those without
Borkowski et al.	2008	Case control Arm 1	43	26/17	rASRM, 1996	Serum vitamin D-binding protein (DBP)	No adjustment for confounders	449.4 ± 24.4 μg/ml and 424.5 ± 23.5, (P = 0.491) [b]	Serum and peritoneal DBP concentrations are not affected in women with endometriosis
		Case control Arm 2	28	21/7	rASRM, 1996	Peritoneal DBP		387 ± 24.7 μg/ml and 408 ± 23.1 μg/ml (P = 0.6390) [b]	
Faserl et al.	2011	Cross sectional	76	56/20	rASRM, 1996	Serum DBP	No adjustment for confounders	The abundance of DBP was higher in all endometriosis pools by a factor of ~3 compared with the control pool (P < 0.02)	Serum DBP levels are higher in patients with endometriosis compared with controls
Cho et al.	2012	Case Control	95	57/38	rASRM, 1996	Urinary DBP	Adjustment for medications (vitamin D and calcium supplements intake) only	111.96 ± 74.59 versus 69.90 ± 43.76 ng/mg Cr (P = 0.001) [b]	Urinary DBP levels are elevated in patients with endometriosis
Harris et al.	2013	Prospective Cohort	70,556	1385/69171	Not specified	Predicted 25-OHD levels	Adjustment for age, season, race, geographical region, vitamin D intake, alcohol, physical activity	Women in the highest quintile of predicted vitamin D level had a 24% lower risk of endometriosis than those in the lowest quintile [RR = 0.76, 95% CI (0.6–0.99)]	Inverse association between predicted plasma 25-OHD levels and endometriosis

rASRM: revised American Society for Reproductive Medicine classification.

[a] Confounders affecting vitamin D metabolism: obesity, season of the year, diet type, vitamin D supplements intake, activity.

[b] For women with and without endometriosis, respectively.

hypothesis by Stratton et al. detailed above, 1,25(OH)2D may have a role in endometriosis-related pain which merits further investigation. Furthermore, the strong benefit of vitamin D on dysmenorrhea observed by Lasco and colleagues provides important support for larger, long-duration randomized trials of vitamin D as a therapeutic agent, in the treatment of menstrual pain and other pain related conditions in women, such as endometriosis.

2.2.5. *Endometriosis associated infertility and vitamin D*

Among infertile women, 25%–50% have endometriosis and 30%–50% of women with endometriosis are infertile [42]. Biologic mechanisms linking endometriosis and infertility are distorted pelvic anatomy, altered peritoneal function, ovulatory abnormalities and impaired implantation [43]. The latter could be related to the fact that the eutopic endometrium has reduced expression of biological markers of endometrial receptivity such as $\alpha v\beta 3$ integrin, glycodelin A, osteopontin, and HOXA10 [44,45].

VD metabolites have been implicated in implantation in both animal models and humans. Treatment of rat uteri with 1,25(OH)2D induced decidualization, a crucial step in the process of blastocyst implantation [46]. Patients with pseudo-vitamin D deficient rickets, a condition with inability to convert 25-OHD to 1,25(OH)2D, have been found to have defective decidualization [47,48]. The role of 1,25(OH)2D in implantation likely involves the direct transcriptional activation of HOXA10 gene, implicated in the implantation process as a potent stimulator of the $\alpha\nu\beta 3$, which is a known biomarker of the window of implantation [49]. HOXA10's crucial role in implantation is illustrated by its high RNA message expression at the tubal mucosa during ectopic pregnancy, specifically at the implantation site, whereas its expression is low in the presence of hydrosalpinx in normoovulatory women and is restored to normal after salpingectomy [50,51]. In an experimental endometriosis model, there was an alteration in the methylation pattern and expression of the HOXA10 gene in the eutopic endometrium, which may lead to lack of endometrial $\alpha\nu\beta 3$ expression [52]. Low $\alpha\nu\beta 3$ expression has been described in half of women with endometriosis and could explain the high failure rate of assisted reproductive technologies noted in this population [53]. The potential role of 1,25(OH)2D in endometriosis-associated infertility may thus be via an altered VD metabolism at the endometrial level, which may reduce HOXA10 and $\alpha\nu\beta 3$ expression, thus jeopardizing implantation and human fertility.

2.2.6. *The endometrium: A target for vitamin D action*

The human endometrium is a steroid hormone-dependent tissue displaying complex cellular regulation mediated by nuclear receptors [54]. Stromal endometrial cells were shown to express VDR and the active form of 1α-hydroxylase gene and protein, independently of the menstrual cycle phase, but these are up-regulated in early pregnant versus cycling endometrium [55]. The endometrium is also a site of 1,25(OH)2D extra renal synthesis and a target of 1,25(OH)2D actions through gene regulation and immunomodulation [52,56].

2.2.7. *Regulation of specific genes*

HOXA10 gene is a member of the homeotic genes that are highly conserved transcription factors that impart anatomical and functional identities to the various segmental body units during ontogeny [57]. HOXA10 is involved in the embryogenesis of the uterine epithelium, stroma and muscle [58]. It is cyclically expressed in the adult endometrium in response to estrogen and progesterone, regulating endometrial receptivity during the nidation window [59]. 1,25(OH)2D induces HOXA10 transcription through VDR binding to a VD responsive element (VDRE) in the HOXA10 gene 5 region. The direct transcriptional activation of HOXA10 by VD may induce differentiation of diverse tissues including differentiation of endometrial cells to decidual cells [60]. A lower expression of HOXA10 gene in the eutopic and ectopic endometrium of endometriosis has been found by Deng et al. and might be associated with the pathogenesis and infertility of endometriosis [61].

Osteopontin (OPN) gene is a highly phosphorylated sialoprotein, known as a major component of the extracellular matrices of bones and teeth [62]. OPN is expressed by cells in a variety of tissues, including bone, dentin, kidney, brain, vascular tissues, and cytotrophoblasts of the chorionic villus in the uterus and decidua [63,64]. It has been implicated in many biological events, including bone calcification and resorption, wound healing, immunological responses, tumorigenesis, and in implantation and decidualization [65–68]. Cho et al. demonstrated that OPN mRNA expression in eutopic endometrium and plasma OPN levels are higher in patients with endometriosis than those without the disease, moreover, Hapangama et al., in a recent immunohistochemical study with confirmatory immunoblotting and RT-PCR data, suggested an increased expression in OPN in the luteal secretory endometrium of women with endometriosis, which suggests a possible role of OPN in the pathogenesis of endometriosis [69,70].

Taking into consideration that 1,25(OH)2D is a particularly potent stimulator of OPN synthesis by bone cells and epidermal cell lines, and induces the expression of OPN gene in both cycling and early pregnant endometrium [55,71], the perturbation in OPN endometrial expression and serum levels in women with endometriosis, could be secondary to the secondary disturbance in vitamin D metabolism.

2.2.8. *Immunomodulatory effect*

1,25(OH)2D promotes the shift away from Th1-type responses and favors a Th2-type immunity by inhibiting the secretion of IL-12, IL-2, TNF and interferon, by T cells, macrophages, and dendritic cells [72,73]. In normal pregnancies, 1,25(OH)2D levels increase, starting in the second and third trimester maybe in anticipation for such shift in immune tolerance, in addition to ensuring enhanced intestinal calcium reabsorption for fetal calcium bone accretion [74]. This suggests a possible action of 1,25(OH)2D as a natural regulator of the immune system acting locally in the uterus to aid in the establishment of a normal pregnancy [75].

Since 1,25(OH)2D is involved in uterine physiology as immune modulator and regulator of specific endometrial genes, a disturbance in its expression could lead to pathological conditions affecting the uterus and the endometrium milieu.

2.2.9. *Expression of vitamin D metabolites in the endometrium of women with endometriosis*

The endometrium in endometriosis demonstrates dysregulation of DNA methylation and transcriptional repression signaling, chromatin remodeling, and gene expression of steroid hormone receptors and transcription factors such as HOXA10, secondary to the influence of epigenetic modifications [76]. The dysregulation of the VD pathway in the eutopic endometrium of women affected by endometriosis was studied by Agic et al. [7]. Endometrial biopsies from 10 women with laparoscopy-documented endometriosis and 5 healthy controls were studied. VDR mRNA levels in epithelial and endometrial cells in cases were greater than controls (71.9 ± 23 versus 20.8 ± 7.9, $p < 0.01$ and 31.3 ± 9.8 versus 7.4 ± 2.6, $p < 0.01$, respectively). An increase in 1 α-hydroxylase mRNA expression and a tendency for elevated 24-hydroxylase expression in the endometrium of women with endometriosis compared with controls were also documented. This elevation in VDR, 1 α-hydroxylase, and 24-hydroxylase mRNA expression in the endometrium of women with endometriosis, suggests an active production and deactivation of 1,25(OH)2D, and thus points to an acceleration in VD metabolism at the endometrial level, decreasing its potential to enhance immune tolerance [7].

2.2.10. *Serum DBP polymorphism in endometriosis pathogenesis*

DBP or group-specific component (Gc) is recognized as major plasma protein carrier of VD and its metabolites and is the precursor of Gc-protein derived macrophage-activating factor, (GcMAF) that can activate the scavenger function of macrophages without initiating the macrophage-induced inflammatory response [77,78].

Borkowski et al. have shown no difference in the total concentration of DBP in the serum and peritoneal fluid of women with laparoscopy-documented endometriosis (n = 26) compared to those with benign gynecological conditions (n = 17) [8]. However, this study was unable to differentiate between the different Gc allele products of DBP. On the other hand, significantly higher levels of DBP in women with stage I–II endometriosis (n = 20), stage III endometriosis (n = 20) and stage IV endometriosis (n = 20) compared to controls (n = 20) were noted by Faserl et al. In addition, in this study, the expression of Gc2 allele product was 3 fold higher the combined endometriosis groups compared with controls ($p = 0.006$) [79].

The DBP form encoded by Gc2 allele is the least glycosylated, the least converted to GcMAF and thus the least activating of the macrophage scavenger function [80]. The inability to sufficiently activate phagocytic function in women with endometriosis, due to specific polymorphisms in DBP (Gc2), may allow endometriotic tissue implantation in the peritoneal cavity [79]. This may explain the higher macrophage and cytokine levels (IL-1β, TNF-α and vascular endothelial growth factor) in peritoneal fluid of women with endometriosis compared to controls, without the ability to inhibit endometriotic tissue implantation [81,82].

2.2.11. *Urinary DBP: A novel marker for endometriosis?*

The search for a potential biomarker for endometriosis has involved the study of a variety of clinical specimens including serum, peritoneal fluid, endometrial fluid, endometrial tissue and even urine [83–85]. Recent studies suggest that urinary proteomic analysis, such as two-dimensional electrophoresis, liquid chromatography and/or mass spectrometry, may provide a novel method of diagnosing and staging endometriosis [86,87]. In a prospective, blinded study, urinary matrix metalloproteinase (MMP-2, MMP-9, and MMP-9/neutrophil gelatinase-associated lipocalin) were significantly more likely to be detected in the urine of women with endometriosis than in controls [88]. The urinary presence of any of these three gelatinases increased the odds of endometriosis by eight times [OR = 8.3, 95% CI (3–22.7)] [88]. Tokushige et al. showed that all urine samples from women with proven endometriosis (n = 11) were positive for anticytokeratin-19 antibody (CK-19), while those from women without endometriosis (n = 6) were all negative [85]. A prospective randomized pilot study comparing the urinary peptide profiles of women with moderate/severe endometriosis (n = 23) and controls (n = 30), detected the differential expression of a peri-ovulatory peptide mass and a luteal peptide mass with a sensitivity of 75% and a specificity of 85% and 71%, respectively in detecting endometriosis [86]. A Korean study, using proteomic techniques on urine samples of women with and without endometriosis revealed the differential expression of 22 protein spots, one which was identified as urinary DBP. Urinary DBP corrected for creatinine expression (DBP-Cr) was significantly greater in women with endometriosis [89].

Although urinary DBP-Cr had limited value as a diagnostic marker for endometrioisis (sensitivity 58%, specificity 76%) [89] and the tendency currently is toward evaluating a panel of serum biomarkers to aid in the definitive diagnosis of endometriosis [86], elevated urinary DBP levels in these women strengthen the suggested association between DBP and endometriosis pathogenesis.

2.2.12. *Treatment of endometriosis: An eye on VDR agonists*

Multiple pharmacological treatments for endometriosis have been suggested based on presumptive pathogenic mechanisms or hypothesized hormonal selectiveness [90]. Aromatase inhibitors, gonadotropin-releasing hormone antagonists, selective estrogen receptor modulators, immunomodulators (Rapamycin, Guanosine analogue, Loxoribine), anti-angiogenetic agents (Cabergoline, Sirolimus), statins (Atorvastatin, Lovostatin), and antioxidants (Vitamin E succinate) have been tried [81,91,92].

According to experts, and although the current medical treatment of endometriosis has almost reached pharmacological extravagance, it is still not satisfactory, and there is a constant need to find novel drugs with better efficacy and tolerability.

2.2.13. *VDR agonists: Potential candidates?*

VDR agonists are being evaluated for potential therapeutic applications in RA, systemic lupus erythematosus, and autoimmune prostatitis [93]. The pleiotropic effects exerted by 1,25(OH)2D and its analogues, and their immune-regulatory and anti-inflammatory properties may be beneficial in proliferative conditions such as psoriasis, and in other pathological conditions characterized by chronic inflammation [94].

To overcome hypercalcemic liability associated with 1,25(OH)2D, Elocalcitol (1-α-fluoro-25-hydroxy-16,23E-diene-26,27-bishomo-20-epi-cholecalciferol), a VDR agonist with low calcemic liability and well-defined anti-proliferative and anti-inflammatory properties has been studied in chronic inflammatory conditions [95]. In preclinical studies, its efficacy was established in benign prostatic hyperplasia. In a phase IIb multicenter trial, its safety but not efficacy was proven in women with detrusor instability [96,97].

2.2.14. *Elocalcitol and endometriosis*

Research progress in endometriosis faces the difficulty of finding an available model of the disease, since endometriosis occurs spontaneously only in primates [98]. Using non-human primates to study endometriosis is limited by the low incidence and slow progression of the disease, ethical issues especially for studies targeting the use of a new candidate drug, and high costs associated with primate manipulation [99].

Elocalcitol has been found to inhibit the inflammatory response by targeting the nuclear factor-Kappa beta pathway, that it is constitutionally activated in endometriotic cells. This pathway has also been implicated in IL-8 production, resulting in the recruitment of macrophages and natural killer cells [100]. Recently, a validated mouse model of endometriosis by injection of syngeneic endometrial tissue fragments into adult female mice was developed [99]. In this model, Elocalcitol administrated at a dose of 100 μg/kg once daily was able to reduce, total lesion weight by up to 70%, upon treatment for 1 week before and two weeks after disease induction, and it also inhibited macrophages recruitment in the peritoneum [99].

Further testing of this drug and potential other VDR agonists in primate models and eventually in women affected by endometriosis may find the "Waited Godot" which would eliminate endometriotic lesions, prevent recurrence and not impede ovulation [90].

3. Discussion

It seems justified to search for an association between endometriosis, a disease that mimics malignancy and fulfills most of the criteria of an autoimmune disease, and VD, an agent with anti-proliferative, anti-inflammatory and immunomodulatory properties [7,29,72].

Vitamin D supplementation has been associated with reduction in pain scores in primary dysmenorrhea through interference with prostaglandins synthesis [36]. The effectiveness of vitamin D in relieving secondary dysmenorrhea or endometriosis chronic pelvic pain, related to a neuroangiogenesis process, needs to be investigated.

The expression of VD receptors and 1α-hydroxylase in the normal cycling endometrium, the up-regulation of VD enzymes and receptors in the eutopic endometrium of women affected by endometriosis, the characteristic DBP polymorphism reflected by the differential expression of GC-2 allele in the endometriosis pool, and the inverse relation reported recently, between endometriosis and 25-OHD level combined with evidence of increase in urinary binding protein in women with endometriosis, are all in favor of a potential role of VD and its metabolites as local autocrine/paracrine agents incriminated in endometriosis etiology/pathology [6,55,81].

Endometriosis is conceptualized as a pelvic inflammatory condition and an estrogen-dependent chronic inflammatory disease. Therefore, the disturbance in VD metabolites and receptors, discussed above could be induced secondary to the unfavorable and hostile inflammatory milieu instead of being the primary inciting event [7]. In order to avoid dealing with this egg–chicken theory, investigating the complex pathophysiology of endometriosis by creating primate experimental models of the disease is warranted. Moreover, future studies evaluating VDB polymorphism as a risk factor for endometriosis in large populations of reproductive age women are needed.

Despite the fact that urinary DBP lacks power as diagnostic marker for endometriosis, it opens the window to the possibility of finding other urinary protein or a panel of urinary protein powered enough to diagnose endometriosis.

VDR agonists, such as Elocalcitol, have attained the objective of limiting growth and recurrence of endometriotic lesions in a mouse model [99]. Future experiments using primate models as well as clinical trials will be helpful in evaluating the therapeutic benefit of VDR agonists in women with endometriosis and may be added to the armamentarium of endometriosis therapy.

4. Conclusion

The purpose of this review was to elucidate the role of vitamin D in endometriosis, in a translational approach linking basic research findings to observations in clinical studies and trials. Although no placebo-controlled supplementation trials are currently available, recent observational data suggest that vitamin D regulatory network is involved in the pathogenesis of endometriosis. In a recent large prospective cohort study, a greater predicted plasma 25-OHD level was associated with a lower risk of endometriosis, and in a randomized double-blind study, dysmenorrhea was reduced with vitamin D supplementation. This highlights the role of vitamin D as a possible modifiable risk factor for endometriosis, and underscores the significant knowledge gap that precludes the establishment of a cause–effect relationship. Larger, placebo studies taking into consideration parameters such as seasonal variations, dietary intake of vitamin D, skin phototype, ultraviolet exposure, are needed to clarify the possible favorable effects of vitamin D supplementation in women with endometriosis.

Conflict of interest

Authors have no conflict of interest.

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