ISSN: 3066-9855
Research Article
Journal of Gynecology Obstetrics and Mother Health

Vitamin D and Physiopathology of Uterine Leiomyomas: Systematic Review in Animal Models, *In Vitro* Studies, And Clinical Observations

PCSG Barillari*, Nunes MG, Patriarca MT, Dardes RCM, Ferreira FP, Gomes MTV & Bonduki CE

Gynecology Department – Sao Paulo Federal University (UNIFESP), Sao Paulo, Brazil

***Corresponding author:** Priscila Barillari, Gynecology Department – Sao Paulo Federal University (UNIFESP), Napoleao de Barros Street, 608. Vila Clementino, Sao Paulo, CEP 04024-002, Brazil.

Submitted: 24 April 2023 **Accepted:** 01 May 2023 **Published:** 07 May 2023

https://doi.org/10.63620/MK.JGOMH.2023.1004

Citation: *Barillari, P. C. S. G., Nunes, M. G., Patriarca, M. T., Dardes, R. C. M., & Ferreira, F. P. Vitamin D and Physiopathology of Uterine Leiomyomas: Systematic Review in Animal Models, In Vitro Studies, And Clinical Observations. J of Gyne Obste & Mother Health 1(2), 01-10.*

Abstract

Background: *Fibroids are benign tumors found in women of reproductive age and are associated with hormonal, genetic, and molecular variables.*

Methods: *A systematic review was elaborated to search for the mechanisms by which vitamin D influences the development of fibroids. Electronic databases were searched from January 2009 to October 2019. The Internet search tool includes the PUBMED, COCHRANE, and EMBASE search engines. Scientific articles, meta-analyses, therapeutic guidelines, reviews, and research articles were consulted, and the most recent guidelines on the subject, according to the Brazilian Society of Gynecology. The inclusion criteria were publications in the last ten years in English, Portuguese, and Spanish; publications that met the proposed objective described in PICO: a. Randomized trials; B. Observational studies (including cohort and case-control studies). Exclusion criteria included: articles published before 2009; languages other than English, Portuguese, and Spanish; articles that did not meet the research objectives; ongoing studies and abstracts. For data collection and analysis, the selected studies were divided into: 1. Newcastle-Ottawa Scale-Case-Control Studies and Cohort Studies; 2. COCHRANE manual for systematic intervention reviews.*

Results: *12 out of 15 studies were non-randomized studies (80%) with Kappa values above six. Kappa agreement was 0.615, suggesting good or substantial agreement.*

Conclusion: *Vitamin D (1,25(OH)2 D3) plays a significant role in cell growth control, programmed cell death, and DNA damage. Low levels of Vitamin D seem to be an essential factor in the etiopathogenesis of uterine fibroids.*

Keywords: Fibroids, Fibroids Therapeutics, Vitamin D Receptor, Leiomyoma, Vitamin D.

Tweetable Abstract

The study evaluated the mechanisms by which vitamin D influences the development of fibroids through a search of electronic databases from January 2009 to October 2019. The search included articles published in full in the last ten years in English, Portuguese, and Spanish and publications that met the proposed objective described in PICO.

Introduction

Currently, uterine fibroids affect approximately 30 to 60% of the world's population and 20 to 40% of women of reproductive age [1]. Fibroids are the most common type of benign tumor of the female genital tract [2-6]. They are the fifth leading cause of hospitalization unrelated to pregnancy in women between 15 and 44 years [3, 7]. The actual incidence of this disease is unknown, as many women are asymptomatic. Usually, fibroids vary significantly in size, location, and symptoms and are associated with hormonal, genetic, and molecular variables [8, 2, 3].

Genetic alterations in these tumors are triggered by myometrium abnormalities, a congenital increase of estrogen receptors, hormonal alterations, or ischemic lesions during menstruation. These changes are usually influenced by promoters (hormones) and effectors (growth factors) [3]. Extensive research has identified several factors related to a higher occurrence of uterine fibroids, but data are still inconsistent and conflicting [9, 10]. A recently published systematic review highlights factors that increase uterine myomas (UM): black race, high body mass index

(BMI), age, premenopausal status, hypertension, family history, and consumption of food additives [10].

Pharmacological treatments are conservative, reducing symptoms through a temporary decrease in the uterus' volume and size of the myoma. However, they are frequently ineffective in definitively eliminating tumors and preventing recurrences [11]. Current treatments act on estrogen and progesterone receptors that are usually increased in fibroids compared to normal myometrium. Ovarian steroids influence tumor growth, causing higher proliferative and mitotic activity in the secretory phase. Therefore, most treatments are hormonal or act on target hormones and associated receptors, interfering with myoma growth [11, 12]. Anti-inflammatory drugs, fibrinolysis inhibitors, contraceptives, and progestogens are intended to alleviate symptoms only.

The role of vitamin D has recently been extended beyond the control of calcium metabolism. The regulation of cell proliferation and differentiation, angiogenesis, and apoptosis, processes that participate in the development of a tumor, has been discussed recently and associated with vitamin D. Consequently, the role of vitamin D has also been a suggested candidate in the development of uterine fibroids. Studies have shown that vitamin D deficiency plays a significant role in the growth of uterine fibroids in women [13-15]. According to several studies, women with low vitamin D levels have severe fibroids cases, while those with adequate vitamin D levels are less likely to develop fibroids [13-15]. Thus, an updated review on the action of vitamin D and associated receptors on the pathophysiology and management of fibroids is highly relevant.

Methods

A systematic review of the role of vitamin D in uterine fibroids was conducted according to the PICO technique, to define the questions and guide search strategies [16, 17].

Eligibility Criteria

Inclusion criteria for the review were: publication in full format in the last ten years in English, Portuguese, and Spanish; publications meeting the proposed objective described in PICO (Supplementary file S1):

a. a. Randomized trials to assess the beneficial effects of vitamin D treatments.
b. b. Observational studies (including cohort and case-control studies) to assess the action of vitamin D and associated receptors on the pathophysiology of uterine fibroids.

Exclusion Criteria Included: articles published before 2010; articles in languages other than English, Portuguese, and Spanish; articles that did not meet the research objectives; ongoing studies; abstract studies.

Search Strategy

The electronic databases were searched between January 2009 to October 2019. The internet search tool included PUBMED, COCHRANE, and EMBASE search engines. Of these, scientific articles, meta-analyses, therapeutic guidelines, reviews, and research articles were consulted, as well as the last guidelines on the subject, prepared by the Brazilian Society of Gynecology (Supplementary file S2).

The keywords used were Fibroids; Leiomyoma; Uterine Fibroma, Vitamin D; Vitamin D Receptors. The descriptors were selected from the database, including all synonyms separated by the terms "AND" and "OR", to increase the sensitivity of the first search, resulting in 304 studies.

Study Selection and Data Collection

The choice of studies was performed by two independent authors who selected the studies meeting the selection criteria. Disagreements were resolved by consensus or, if there was no consensus, by the decision of a third reviewer. In a previously created spreadsheet, the following variables were added for analysis: selection by the first evaluator and selection by the second evaluator; reasons for exclusion; final agreement.

Quality Assessment and Evidence

Together, the selected studies were divided into three tables, according to the type of study (Case-Control, Cohort, and Randomized Studies), and divided into:

1. The Newcastle-Ottawa Scale (NOS) – Case-Control Studies and Cohort Studies (Supplementary file S3);
2. COCHRANE Handbook for Systematic Reviews of Intervention (Supplementary file S4).

The methodological quality of the cohort and case-control studies using the Newcastle-Ottawa scale was calculated in three components: selection of groups (0 - 4 points), quality of fit (0 - 2 points), and assessment of exposure after outcome (0 - 3 points). Scores above 6 points consider the study of high methodological quality.

To assess the degree of agreement between two or more evaluators, the Kappa index was used (Supplementary file S5) [18].

The COCHRANE Handbook for Systematic Reviews of Intervention was used to assess the risk of bias in clinical trials through a domain-based assessment tool. It is a two-part tool containing seven domains: random sequence generation, allocation concealment, blinding of participants and professionals, blinding of evaluators' outcomes, incomplete outcomes, selective outcome reporting, and other sources of bias. The first part describes what was reported in the study evaluated in sufficient detail to judge the information. The second part is the judgment regarding the risk of bias for each of the analyzed domains, which can be classified into three categories: low risk of bias (green color), high risk of bias (red), or uncertain risk of bias (yellow). Tables were created from existing questions in questionnaires referring to the Case-Control Study, Cohort Study, and Randomized Studies (Supplementary file S6). After analysis, graphs were drawn to assess the relevance of the articles.

Outcomes Evaluated

Outcomes of interest were: (a) genetic variants and vitamin D receptors in the treatment of fibroids, (b) serum vitamin D levels and increased risk of fibroids, (c) the effect of paricalcitol (vitamin D analog) on fibroids' cells (d) genetic relationship of vitamin D metabolism and skin pigmentation.

Results

Articles (304) were found through the search carried out in three selected databases. However, when delimiting the period of the last 10 (ten) years in full format, 295 articles remained, of which

18 (eighteen) were selected to compose this systematic review (Figure 1). Due to the studies' considerable heterogeneity, it was impossible to quantitatively integrate the data through meta-analysis.

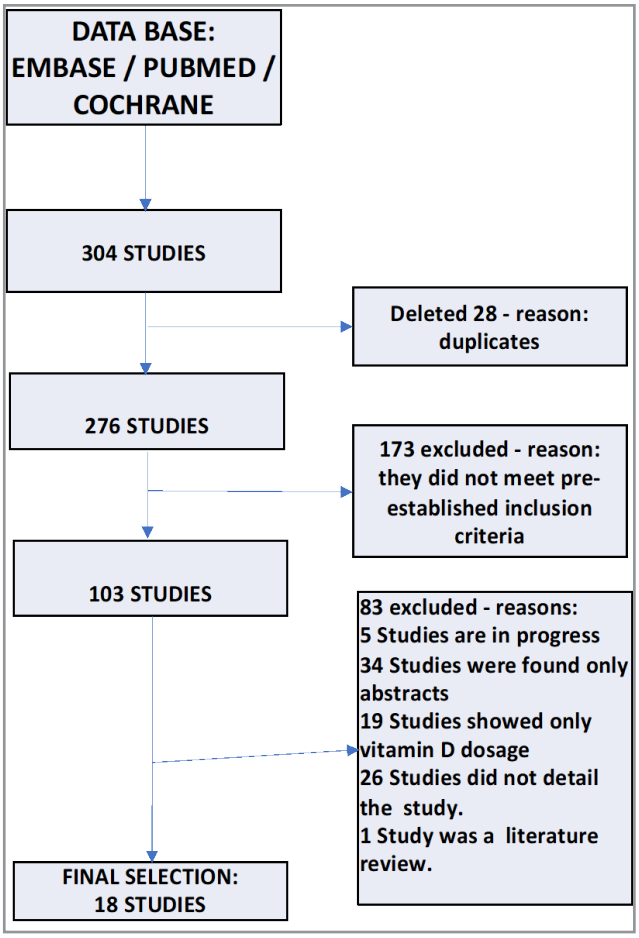

Figure 1: Flowchart of the systematic review and studies selection.

Subsequently, the articles were evaluated for relevance, through the sum of stars, according to the answers presented in the specific questionnaires. The process is represented below (Figures 2 and 3).

Figure 2: Detailed evaluation of Cochrane systematic reviews and the risk of bias.

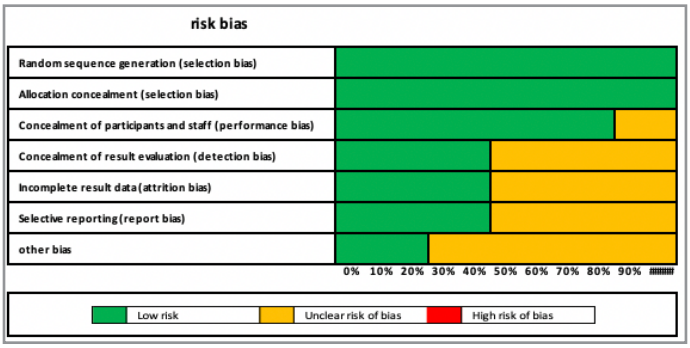

Figure 3: Detailed evaluation of systematic reviews and the risk of bias (%).

The quality of non-randomized studies (case-control and cohort) was assessed using the Newcastle-Ottawa Scale (NOS). In our work, 12 of the 18 studies (70%) had values above six. The Kappa agreement between reviewers for the NOS was 0.615, suggesting good or substantial agreement.

The study's characteristics are detailed in Table 1.

Table 1: Summary of the studies selected for the systematic analysis.

Author (year)	Type of Study	Population Studied	Intervention Group	Control Group	Measured Outcome	Key Results
Halder et al. (2011)[26]	Case-Control Study	HuLM cells	HuLM cells treated with TGF-β3 with vitamin D3	HuLM cells treated with TGF-β3 without vitamin D3	To test the effect of vitamin D3 on TGF-β3-induced protein expression of collagen type 1 proteins, fibronectin and plasminogen activator inhibitor-1.	TGF-β3 induced expression of fibronectin protein and type 1 collagen in HuLM cells. TGF-β3 also induced plasminogen activator inhibitor-1 protein expression. TGF-β3 induced Smad2 phosphorylation as well as Smad2 and Smad3 nuclear translocation in HuLM cells, whereas vitamin D significantly reduced all these TGF-β3 mediated effects

Sharan et al. (2011)[27]	Cohort Study	HuLM cell lineage	HuLM cells were treated with vitamin D for 48 h and cell lysates analyzed by Western blot.	----	The levels of PCNA, BCL-2, BCL-w, CDK-1 and COMT proteins were analyzed by Western blot.	Vitamin D inhibited the growth of HuLM cells compared to control cells. Vitamin D inhibited ERK activation and decreased BCL-2, BCL-w, CDK1 and PCNA expression. It demonstrated inhibitory effects of vitamin D on COMT expression and enzymatic activity. The silencing of endogenous COMT expression abolished vitamin D-mediated inhibition of HuLM cell proliferation.
Halder et al. (2012)[21]	Case-Control Study	Female rats with myoma, between 14-16 months.	6 female rats with fibroids, treated with vitamin D using an infusion pump	6 rats with myoma, treated with ethylene glycol	Evaluate the antitumor and therapeutic effects of 1,25(OH)2 D3 on uterine fibroids.	Treatment with 1,25(OH)2 D3 reduced leiomyoma size by suppressing genes related to cell growth and proliferation (Pcna, cyclin D1 [Ccnd1], Myc, Cdk1, Cdk2 and Cdk4), anti-apoptotic genes (Bcl2 and Bcl2l1 [Bcl-x]) and estrogen and progesterone receptors. Furthermore, immunohistochemistry revealed decreased expression of PCNA and MKI67 (a marker of proliferation) and increased expression of caspase 3 in leiomyomas of Eker rats treated with 1,25(OH)2 D3.
Halder et al. (2013)[28]	Case-Control Study	Cell lines of 40 women aged 35-53 years undergoing hysterectomy for fibroids.	Myoma cell lines were examined by Western blot analysis using anti-VDR and anti-b-actin antibodies.	Standard uterine smooth muscle cell lines were examined by Western blot analysis using anti-VDR and anti-b-actin antibodies.	Evaluate VDR expression levels in uterine fibroids compared to levels in adjacent normal myometrium.	Fibroids express reduced levels of VDR compared to adjacent normal myometrium and 1,25(OH)2 D3 treatment can potentially reduce aberrant expression of ECM-associated proteins in HuLM cells
Halder et al. (2013)[23]	Cohort Study	Cell lines were obtained from an African-American woman who underwent laparoscopic hysterectomy to remove uterine fibroids surgically.	Fibroids cell line.	Standard Uterine Smooth Muscle Cell Lines	To evaluate the expression of various matrix metalloproteinases (MMPs), metalloproteinase inhibitors (TIMP) 1 and 2 and the activities of MMP-2 and MMP-9 after treatment with 1,25(OH)2 D3.	1,25(OH)2 D3 effectively reduced the expression and activities of MMP-2 and MMP-9 in cultured human uterine fibroid cells.
Halder et al. (2014)[21]	Case-Control Study	Tissue from female rats aged 5 - 6 weeks	8 rats received 1.25 (OH)2 D3 administered by micro osmotic pumps, and the final group received treatment with Paricalcitol	8 rats received 95% propylene glycol (PEG) and 5% ethyl alcohol (control)	Evaluate the therapeutic action of Paricalcitol, a 1,25(OH)2 D3 analog against uterine fibroids	It was found that Paricalcitol has the potential to reduce the proliferation of immortalized human uterine fibroid cells. The results suggest that Paricalcitol may be a potential candidate for an effective, safe, and non-invasive medical treatment option for uterine fibroids.
Wise et al (2014)[22]	Cohort Study	Women between 21-69 years old	2,232 premenopausal women with uterine fibroids confirmed by ultrasound or surgery during 1997–2011.	2,432 premenopausal women never diagnosed with uterine myomatosis until 2011.	To assess the incidence of fibroids in relation to polymorphisms in genes involved in vitamin D metabolism and skin pigmentation in premenopausal women.	Three of the twelve polymorphisms were associated with fibroids at the nominal significance level: rs4944957 and rs12800438 next to DHCR7 and rs6058017 in ASIP. Two of the polymorphisms remained significantly associated with myoma (rs12800438 and rs6058017).

Al-Hendy et al. (2015)30	Case-Control Study	Myoma cells and adjacent myometrium	Myoma cells were treated with 1,25(OH)2 D3 and analyzed for expression and localization of receptors.	Adjacent myometrium cells were treated with 1,25(OH)2 D3 and analyzed for receptor expression and location	To study the role of 1,25(OH)2 D3 in the expression of estrogen receptor (ER)-α, progesterone receptor (PR)-A and PR-B, as well as members of the co-activator family of estrogen receptors (SRC) in fibroid cells.	An inverse correlation was observed between estrogen receptor-α (ER-α), progesterone receptor-A (PR-A) and progesterone receptor-B (PR-B) and vitamin D expression (VDR) in fibroids. Treatment with 1,25(OH)2 D3 significantly decreased ER-α, PR-A and PR-B levels, as well as steroid receptor coactivators (SRCs) in human uterine leiomyoma cells (HuLM). In contrast, 11,25(OH)2 D3 self-induced its own VDR, which induced the VDR-retinoid X receptor-α complex in HuLM cells. Together, these results suggest that 1,25(OH)2 D3 antagonizes sex steroid hormone receptors on HuLM cells.
Shahbazi (2016)[23]	Case-Control Study	98 women	45 women with uterine fibroids diagnosed by imaging and laboratory tests	53 healthy women volunteer with no family history of myoma.	To evaluate the distribution of the fok 1 polymorphism in Iranian patients with uterine fibroids.	The results demonstrated a correlation between the VDR TT genotype and myoma. This study supports the increased risk of fibroids associated with VDR polymorphism
Al-Hendy et.al. (2016)[31]	Cohort Study	Women undergoing surgery (hysterectomy or myomectomy) to remove the fibroids.	Myoma cells were treated with increasing doses of vitamin D for 48 hours	HuLM cells were treated with increasing doses of vitamin D for 48 hours.	To evaluate the role of vitamin D3 in modulating Wnt /-catenin and rapamycin (mTOR) signaling in myoma cells.	Fibroids with Med12 somatic mutations showed up-regulation of Wnt4 and alpha-catenin compared to the adjacent myometrium. Vitamin D3 administration reduced Wnt4 and cat-catenin levels in HuLM and PUF cells, reduced mTOR signaling expression/activation in both cell types, increased DNA damage-induced gene expression 4 (an mTOR inhibitor) and tuberous sclerosis (TSC1/2) in a concentration-dependent manner in HuLM cells. There was also a reduction in the concentration of Wisp1 (Wnt 1-induced signaling protein 1) and flap endonuclease 1 protein in HuLM cells.
Ciebiera et al. (2016)[9]	Cohort Study	188 women	105 women admitted for fibroid surgery.	83 healthy women of similar age, with a normal uterus, free of fibroids.	To evaluate the influence of 25(OH) vitamin D and serum TGF-3 concentrations, weight, and family history on the risk of developing uterine fibroids.	The study found higher BMI, positive family history, and lower serum concentrations of vitamin D and TGF-β3 as risk factors for uterine fibroids.
Ali et al. (2019)[32]	Case-Control Study	Women of reproductive age (22-55 years) undergoing hysterectomy or myomectomy for symptomatic fibroids	Myoma cells were treated with 100nM vitamin D for 3 days	Primary cells isolated from corresponding adjacent myometrium treated with vitamin D and ethanol vehicle added.	To evaluate the role of vitamin D in fibroids, through the recovery of the damaged DNA repair system, in inhibiting the progression of the fibroid.	Myoma cells accumulate unrepaired DSB concomitant with reduced expression of a vast network of DNA repair proteins. Vitamin D3/VDR is functionally linked to DNA damage and instability in uterine fibroid cells.

Elhusseini H et al. (2018)[20]	Case-Control Study	Female rats with fibroids, 4-6 weeks old.	Female rats with a vitamin D deficient diet for 8 weeks.	Female rats fed a regular diet containing 4 IU/g vitamin D for 8 weeks.	To evaluate, in in vivo mice with a vitamin D-deficient diet, the effect of vitamin D deficiency on inflammation, genomic instability, and myometrium proliferation.	Vitamin D deficiency was associated with increased expression of sex steroid receptors in the myometrium of fibroids, increased expression of genes related to proliferation, promotion of fibrosis and increased inflammation, and promotion of immunosuppression. Also, a diet deficient in vitamin D increased DNA damage in the myometrium.
Liu F. et al. (2018)[33]	Case-Control Study	Women who underwent hysterectomy or myomectomy for fibroids, aged 31 to 53 years	Uterine fibroid tissue	Normal adjacent tissue	To evaluate the expression of vitamin D receptor (VDR) and cyclooxygenase-2 (COX-2) in healthy fibroids and smooth muscle.	VDR expression decreased, and COX-2 expression increased in fibroids compared to healthy uterine smooth muscle.
Othman et al. (2018)[4]	Case-Control Study	Women undergoing hysterectomy for symptomatic uterine fibroids, diagnosed through clinical and ultrasonographic examinations and confirmed postoperatively by histopathological diagnostic	Uterine Fibroids Tissue	Adjacent myometrium tissue and normal myometrium tissue.	To evaluate the tissue concentration of 1,25 (OH)2 D3 and the expression level of the gene CYP27B1, which encodes 1-α hydroxylase (vitamin D activating enzyme). Moreover, to evaluate CYP24A1, which encodes 24-hydroxylase (a catabolic vitamin D enzyme) in fibroids, adjacent myometrium, and normal myometrium.	Fibroids contain significantly lower concentration of 1.25 dihydroxyvitamin D3 than Myo-F. ULM, Myo-F and Myo-N express CYP27B1, which codes for 1-hydroxylase. Fibroids express a significantly higher level of CYP24A1 encoding 24-hydroxylase than Myo-N, indicating that 24-hydroxylase overexpression is a mechanism by which fibroids sustain a hypovitaminosis D state.
Yilmaz G et al (2018)[24]	Case-Control Study	Women with or without uterine fibroids	Peripheral blood samples from women with uterine fibroids (n = 27)	Peripheral blood samples from healthy women (n = 33)	Evaluate the association between the fok1 polymorphism and uterine fibroids.	There was a statistically significant difference in the CC genotype frequency between the fibroid and control groups. The frequencies of the T allele in the fibroid group were significantly higher than in the control group.
Corachan et al (2019)[34]	Case-Control Study	Women between 35 and 52 years old submitted to myomectomy or hysterectomy due to symptomatic fibroids and with no previous hormonal treatment	HULP cells treated with 100 nM vitamin D for 144 hours	Untreated HULP cells	Evaluate HULP cells treated with or without vitamin D to assess the effect on cell proliferation, cell cycle, Wnt/-catenin genes, Wnt-related proteins (protein matrix), and apoptosis.	The fibroid tissues compared with adjacent myometrium showed higher proliferation and altered the Wnt / b-catenin pathway, while no differences were observed in apoptosis. 1,25(OH)2 D3 induced cell growth arrest and decreased proliferation in HULP cells. Furthermore, Vitamin D reduced the expression of the Wnt pathway in HULP cells at gene and protein levels. However, 1,25(OH)2 D3 did not induce apoptosis expression.
Hajhashemi et al. (2019)[25]	Case-Control Study	Women between 35 and 49 years old with fibroids and vitamin D deficiency	Group treated (n = 35) with vitamin D 50,000 IU every 2 weeks for 10 weeks	Control group (n = 34) received a placebo in the same color and shape.	To evaluate the effect of vitamin D supplementation on fibroid size offered to women with vitamin D deficiency.	After a 10-week intervention, 1,25(OH)2 D3 levels were significantly higher in the vitamin D receiving supplementation. The size of fibroids in the 1,25(OH)2 D3 group significantly decreased compared to the placebo group.

Discussion

The systematic review included 18 studies with vitamin D; of these, three studies used animal models, ten studies were performed in vitro, and five were clinical observations. Studies in rats showed that genes related to cell growth and proliferation, anti-apoptotic, estrogen, and progesterone receptors, as well as caspase expression, decreased the size of fibroids in rats treated with 1,25(OH)2D3 [19, 20]. Furthermore, vitamin D deficiency in rats led to fibrosis growth, increased inflammation, and immunosuppression. The vitamin D-deficient diet increased DNA damage in the myometrium [20]. In addition, paricalcitol (a vitamin D analog) and vitamin D administered to female rats reduced the size of fibroids. Both activated the enzyme caspase, demonstrating that paricalcitol reduces fibroids' cell proliferation more efficiently than vitamin D itself [21].

Wise et al., 2014 demonstrated that genes involved in vitamin D metabolism and skin pigmentation are associated with fibroids (rs12800438 and rs6058017), fok1 genotype and Vitamin D Receptor (VDR) TT genotype (T allele) and are risk factors for developing fibroids [22-24]. For two other clinical studies, higher BMI, positive family history, low concentrations of vitamin D, and higher serum concentration of transforming growth factor (TGF-3) increased the probability of developing fibroids while higher concentrations of serum vitamin D reduced fibroids' risk [9]. In addition, the fibroids' size significantly decreased in women treated with vitamin D at 50,000 IU every two weeks, for ten weeks, compared to the placebo group [25].

The in vitro studies reported here involved immortalized human uterine leiomyoma (HuLM) cells, isolated myoma cells and HULP cells [26, 27, 4, 28-34]. The variables studied in vitro were estrogen receptors (VDR), TGF-3 induced fibrosis, extracellular matrix metalloproteinases (MMPs), sex steroid receptors, the Wnt / -catenin system, damaged DNA repair system recovery, proteins involved in apoptosis and cell proliferation, gene expression of 1-α hydroxylase (vitamin D activating enzyme) and 24-hydroxylase (vitamin D catabolic enzyme).

Next, the studies were grouped according to the different roles of vitamin D3.

Chromosomal Abnormalities

About 40% of fibroids have chromosomal abnormalities related to tumor development. The CYP24A1 gene, which encodes 24-hydroxylase, has altered expression in several types of cancer. The high activity of 24-hydroxylase in malignant tissues causes tumor cells to degrade 1,25(OH)2 D3 and limit its antiproliferative and apoptotic effects4. Othman et al. confirmed that uterine fibroids, similar to other tumors, express relatively high levels of CYP24A1, thus suppressing the antitumor effects of 1,25(OH)2 D3 [4]. Typical fibroids and myometrium express 1-α hydroxylase, indicating that they can use 25-hydroxyvitamin D3 to produce their own 1,25(OH)2 D3. This active and locally produced vitamin D3 can modulate cell functions, including proliferation, differentiation, apoptosis, and immune response by paracrine and autocrine secretion, or both [4].

Interestingly, vitamin D activates its cellular receptor, the VDR, a nuclear receptor that functions as a transcription factor. It plays an essential role in gene expression modulation and interacts with the VDR-response element (VDRE) in the promoter region of target genes [14, 24]. Polymorphism of genes involved in vitamin D metabolism and skin pigmentation, including rs12800438 and rs6058017, are associated with the development of fibroids [23]. In addition, vitamin D inhibits the inflammatory process through the vitamin D receptor (VDR). Results from various studies suggest that uterine fibroids express reduced levels of VDR [28, 30, 32, 33]. Moreover, vitamin D3 modulates DNA Damage Response (DDR) via induction of DNA repair gene expression [32]. Ali et al. showed that fibroids accumulate DDR and express other genes related to DNA repair [32, 20, 22].

The Wnt/b-catenin pathway controls cell proliferation, cell maintenance, migration, and differentiation in many types of cancer cells. Two studies evaluated the Wnt / alpha-catenin pathway, specifically in uterine fibroids. Al-Hendy et al. e Corachán et al. showed that fibroids had mutations in the Wnt/b-catenin pathway, similar to cancer cells in general [31, 34].

Two studies evaluated the action of the fok1 polymorphism associated with the VDR gene in fibroids [23, 24]. Shahbazi et al. assessed the distribution of the fok 1 polymorphism in Iranian patients with leiomyomas [23]. They found a correlation between the VDR TT genotype and uterine myoma, supporting an increased risk of fibroids associated with VDR polymorphism. Yilmaz et al. concluded that fok1 genotype might be related to reduced risk for fibroids development [24].

Remodeling of Extracellular Matrix and Fibrosis

Fibroid's growth increases cell proliferation and extracellular matrix (ECM) deposition [28]. Although several types of proteinases are involved in ECM degradation, the central enzymes involve matrix metalloproteinases (MMPs). Halder et al. observed that tissue inhibitors of matrix metalloproteinases (TIMP-2) are down-regulated in myomas, while the activity of MMPs increases [29]. Decreased TIMP-2 expression may induce MMP-2 and MMP-9 expression and activity in uterine fibroids, similar to excessive collagen deposition causing MMP activation and imbalance between ECM production and degradation. 1,25 (OH)2 D3 seems to reduce the expression of MMP-2 and MMP-9 proteins in a concentration-dependent mode in fibroid cells. It is known that MMP-2 and MMP-9 decreased protein expressions are correlated with their proteolytic activity when treated with 1,25 (OH)2 D3 [29].

Transforming growth factor β (TGF-β) is a dimeric polypeptide responsible for controlling the differentiation and proliferation of cells. TGF-β3 is a standard isoform found in fibroids in high concentrations, as demonstrated in some studies [9, 26, 35]. It is well known that TGF-β3 is the isoform usually involved in ECM deposition and increases fibroids' size. Interestingly, vitamin D3 is a robust antifibrotic factor, reducing the expression of collagen and other TGF-β3-dependent factors in uterine fibroid cells in a dose-dependent manner [9, 20, 26, 36]. Type 1 collagen and fibronectin are also overexpressed in uterine fibroids [19, 26, 28].

Cell Apoptosis

Vitamin D3 inhibits the cell cycle and induces apoptosis, or reduces tumor metastasis through the regulation of proteases [37, 21]. In addition, vitamin D3 can control the BCL-2 gene family, which is essential for signaling mechanisms in apoptosis. Halder

et al. observed that vitamin D3 reduced the cell proliferation marker Proliferating Cell Nuclear Antigen (PCNA), anti-apoptotic proteins BCL2, and the expression of cell cycle regulatory proteins in HuLM cells [19]. In addition, expression blocking of anti-apoptotic BCL-2 and BCL caspase-3 was observed [20, 38]. In contrast, Corachan et al. did not observe regulation of apoptosis but an antiproliferative action and cell growth arrest in cells treated with vitamin D3 [34].

Cell Proliferation

PCNA is a marker that is involved in cell growth and proliferation, as well as tumorigenicity. Sharan et al. carried out a study with HuLM cells treated with vitamin D for 48 h and observed that vitamin D reduces the expression of PCNA in fibroid cells [27].

A range of receptors modulate fibroids' growth, both positively and negatively. These receptors are associated with sex steroid α receptor, estrogen β receptor, progesterone A and B receptors, growth hormones, prolactin, extracellular matrix genes, and cell apoptosis [3]. Ample evidence supports that estrogen and progesterone are critical for fibroids' growth [21]. Reduced estrogen and progesterone receptors are found in Eker rats fibroids treated with 1,25(OH)2 D3 [19]. Moreover, vitamin D3 is a potential antiestrogen receptor antagonist in rat myoma cells and Eker rats sex steroids receptors in HuLM cells treated with 1,25(OH)2 D3 [20, 30].

Finally, results suggest that vitamin D may be a potent Cathecol-O-methyltransferase (COMT) inhibitor in HuLM cells, both in terms of expression and enzymatic activity [27]. COMT converts 2-hydroxyestrogen, which is an estrogen antagonist in a variety of cells. Vitamin D deficiency can promote higher expression of COMT in fibroids, acting as a modifiable risk factor [27].

Conclusions

Vitamin D hormone (1,25(OH)2 D3) plays an essential role in cell growth, programmed cell death, and DNA damage. Low vitamin D3 appears to be a critical factor, directly or indirectly, in the etiopathogenesis of uterine fibroids. There are numerous attempts to produce a safe, effective, and low-cost drug for treating and preventing fibroids. Identifying modifiable risk factors such as 1,25(OH)2 D3 deficiency is a promising route. Evidence exists supporting the beneficial action of vitamin D supplementation in women with small-volume fibroids. Still, additional randomized studies are necessary to understand deeply the role of vitamin D in the pathophysiology of uterine fibroids.

Disclosure of Interests

This project has no conflicts of interest

Contribution to Authorship

PCSGB: designing, data collection, analysis e manuscript revision; CEB: experimental design, manuscript revision; discussions; MTVG: experimental design, manuscript revision; MGN: discussions e manuscript revision; FPF: discussions e manuscript revision; MTP: manuscript revision and discussions; RCMD: manuscript revision and discussions

Details of Ethics Approval

The research was evaluated and approved by the Research Ethics Committee of Escola Paulista de Medicina. Number CEP6354160419. It was also evaluated and approved by the International prospective register of systematic reviews (PROSPERO), under number CRD42020130665.

Funding

Capes-Brazil

List of Abbreviations

UM - Uterine Myomas
BMI – Body Mass Index
NOS - Newcastle-Ottawa Scale
TGF- Transforming Growth Factor
HuLM - Human Uterine Leiomyoma
MMPs - Matrix Metalloproteinases
VDR - Vitamin D Receptor
VDRE - Vitamin D Response Element
DDR - DNA Damage Response
ECM - Extracellular Matrix
TIMP-2 - Tissue inhibitor of Matrix Metalloproteinases
TGF-β - Transforming Growth Factor β
PCNA – Proliferating Cell Nuclear Antigen
COMT - Cathecol-O-methyltransferase

References

1. Marques, C. (2017). Clinic and diagnosis. In National consensus on uterine fibroids (8–17). Portuguese Society of Gynecology.
2. Okolo, S. (2008). Incidence, aetiology and epidemiology of uterine fibroids. Best Practice & Research Clinical Obstetrics & Gynaecology, 22(4), 571-588. https://doi.org/10.1016/j.bpobgyn.2008.04.002
3. Parker, W. H. (2007). Etiology, symptomatology, and diagnosis of uterine myomas. Fertility and Sterility, 87(4), 725-736. https://doi.org/10.1016/j.fertnstert.2007.01.093
4. Othman, E. R., Ahmed, E., Sayed, A. A., Hussein, M….& Abdelaal, I. I. (2018). Human uterine leiomyoma contains low levels of 1,25 dihydroxyvitamin D3, and shows dysregulated expression of vitamin D metabolizing enzymes. European Journal of Obstetrics & Gynecology and Reproductive Biology, 229, 117-122. https://doi.org/10.1016/j.ejogrb.2018.08.013
5. Nowak, R. A. (2004). Drug therapies for uterine fibroids: A new approach to an old problem. Drug Discovery Today: Therapeutic Strategies, 1(2), 237-242. https://doi.org/10.1016/j.ddstr.2004.10.002
6. Fleischer, R., Weston, G. C., Vollenhoven, B. J., & Rogers, P. A. W. (2008). Pathophysiology of fibroid disease: Angiogenesis and regulation of smooth muscle proliferation. Best Practice & Research Clinical Obstetrics & Gynaecology, 22(4), 603-614. https://doi.org/10.1016/j.bpobgyn.2008.04.001
7. Petraglia, F., Luisi, S., Ciani, V. (2009). Oral contraceptives after myomectomy: A short term trial. International Journal of Endocrinology, 2009, 476897. https://doi.org/10.1155/2009/476897
8. Stewart, E. A., Laughlin-Tommaso, S. K., Catherino, W. H., Lalitkumar, S…& Gupta, D. (2016). Uterine fibroids. Nature Reviews Disease Primers, 2, 16043. https://doi.org/10.1038/nrdp.2016.43.
9. 9. Ciebiera, M., Włodarczyk, M., Słabuszewska-Jóźwiak, A., Nowicka, G… & Jakiel, G. (2016). Influence of vitamin

D and transforming growth factor β3 serum concentrations, obesity, and family history on the risk for uterine fibroids. Fertility and Sterility, 106(1), 1787-1792. https://doi.org/10.1016/j.fertnstert.2016.08.038
10. Stewart, E. A., Cookson, C. L., Gandolfo, R. A., & Schulze-Rath, R. (2017). Epidemiology of uterine fibroids: A systematic review. BJOG: An International Journal of Obstetrics & Gynaecology, 124(10), 1501-1512. https://doi.org/10.1111/1471-0528.14640
11. Costa, A. R. (2009). Pelvic mass. In Manual of Gynecology (147-165).
12. Talaulikar, V. S., & Manyonda, I. (2012). Progesterone and progesterone receptor modulators in the management of symptomatic uterine fibroids. European Journal of Obstetrics & Gynecology and Reproductive Biology, 165(2), 135-140. https://doi.org/10.1016/j.ejogrb.2012.08.014
13. Baird, D. D., Hill, M. C., Schectman, J. M., & Hollis, B. W. (2013). Vitamin D and the risk of uterine fibroids. Epidemiology, 24(3), 447-453. https://doi.org/10.1097/EDE.0b013e31828acca0
14. Sabry, M., Halder, S. K., Ait Allah, A. S. A., Roshdy, E., Rajaratnam, V. (2013). Serum vitamin D3 level inversely correlates with uterine fibroid volume in different ethnic groups: A cross-sectional observational study. International Journal of Women's Health, 5, 93-100. https://doi.org/10.2147/IJWH.S38894
15. Grzechocinska, B., Dabrowski, F. A., Cyganek, A., & Wielgos, M. (2013). The role of vitamin D in impaired fertility treatment. Neuroendocrinology Letters, 34(7), 756-762.
16. Schardt, C., Adams, M. B., Owens, T., Keitz, S., & Fontelo, P. (2007). Utilization of the PICO framework to improve searching PubMed for clinical questions. BMC Medical Informatics and Decision Making, 7, 16. https://doi.org/10.1186/1472-6947-7-16
17. Mamédio, C., Roberto, M., & Nobre, C. (2007). The PICO strategy for the research question. Revista Latino-Americana de Enfermagem, 15(3), 508–511. https://doi.org/10.1590/S0104-11692007000300023
18. Landis, J. R., & Koch, G. G. (1977). The measurement of observer agreement for categorical data. Biometrics, 33(1), 159–174. https://doi.org/10.2307/2529310
19. Halder, S. K., Sharan, C., & Al-Hendy, A. (2012). 1,25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. Biology of Reproduction, 86(4), 114. https://doi.org/10.1095/biolreprod.111.095026
20. ElHusseini, H., Elkafas, H., Abdelaziz, M., Halder, S., Atabiekov, I., Eziba, N., ... & Al-Hendy, A. (2018). Diet-induced vitamin D deficiency triggers inflammation and DNA damage profile in murine myometrium. International journal of women's health, 503-514.
21. Halder, S. K., Sharan, C., Al-Hendy, O., & Al-Hendy, A. (2014). Paricalcitol, a vitamin D receptor activator, inhibits tumor formation in a murine model of uterine fibroids. Reproductive Sciences, 21(9), 1108–1119. https://doi.org/10.1177/1933719114528822
22. Wise, L. A., Ruiz-Narváez, E. A., Haddad, S. A., Rosenberg, L., & Palmer, J. R. (2014). Polymorphisms in vitamin D-related genes and risk of uterine leiomyomata. Fertility and Sterility, 102(2), 503–510.e1. https://doi.org/10.1016/j.fertnstert.2014.04.027
23. Shahbazi, S. (2016). Exploring the link between VDR rs2228570 and uterine leiomyoma in Iranian women. Egyptian Journal of Medical Human Genetics, 17(2), 115-118. https://doi.org/10.1016/j.ejmhg.2015.07.002
24. Güleç Yılmaz, S., Gül, T., Attar, R., Yıldırım, G., & İşbir, T. (2018). Association between Fok1 polymorphism of vitamin D receptor gene with uterine leiomyoma in Turkish populations. Journal of the Turkish-German Gynecological Association, 19(3), 128-131. https://doi.org/10.4274/jtgga.2017.0034.
25. Hajhashemi, M., Ansari, M., Haghollahi, F., & Eslami, B. (2019). The effect of vitamin D supplementation on the size of uterine leiomyoma in women with vitamin D deficiency. Caspian Journal of Internal Medicine, 10(2), 125–131. https://doi.org/10.22088/cjim.10.2.125
26. Halder, S. K., Goodwin, J. S., & Al-Hendy, A. (2011). 1,25-Dihydroxyvitamin D3 reduces TGF-β3-induced fibrosis-related gene expression in human uterine leiomyoma cells. Journal of Clinical Endocrinology & Metabolism, 96(4), 754–762. https://doi.org/10.1210/jc.2010-1724
27. Sharan, C., Halder, S. K., Thota, C., Jaleel, T., Nair, S., et al. (2011). Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-O-methyltransferase. Fertility and Sterility, 95(1), 247–253. https://doi.org/10.1016/j.fertnstert.2010.05.049
28. Halder, S. K., Osteen, K. G., & Al-Hendy, A. (2013). 1,25-Dihydroxyvitamin D3 reduces extracellular matrix-associated protein expression in human uterine fibroid cells. Biology of Reproduction, 89(1), 1–13. https://doi.org/10.1095/biolreprod.112.106062
29. Halder, S. K., Osteen, K. G., & Al-Hendy, A. (2013). Vitamin D3 inhibits expression and activities of matrix metalloproteinase-2 and -9 in human uterine fibroid cells. Human Reproduction, 28(9), 2407–2416. https://doi.org/10.1093/humrep/det221
30. Al-Hendy, A., Diamond, M. P., El-Sohemy, A., & Halder, S. K. (2015). 1,25-Dihydroxyvitamin D3 regulates expression of sex steroid receptors in human uterine fibroid cells. Journal of Clinical Endocrinology & Metabolism, 100(4), E572–E582. https://doi.org/10.1210/jc.2014-3365
31. Al-Hendy, A., Diamond, M. P., Boyer, T. G., & Halder, S. K. (2016). Vitamin D3 inhibits Wnt/β-catenin and mTOR signaling pathways in human uterine fibroid cells. Journal of Clinical Endocrinology & Metabolism, 101(4), 1542–1551. https://doi.org/10.1210/jc.2015-4078
32. Ali, M., Shahin, S. M., Sabri, N. A., Al-Hendy, A., & Yang, Q. (2019). Hypovitaminosis D exacerbates the DNA damage load in human uterine fibroids, which is ameliorated by vitamin D3 treatment. Acta Pharmacologica Sinica, 40, 957–970. https://doi.org/10.1038/s41401-018-0077-6
33. Liu, F., Zhang, X., Yan, K., Liang, J., Deng, L., Feng, D., & Ling, B. (2018). Vitamin D receptor and cyclooxygenase-2 expression in uterine leiomyoma tissues and their correlation. Translational Cancer Research, 7(1), 135-141. https://doi.org/10.21037/tcr.2018.01.17
34. Corachán, A., Ferrero, H., Aguilar, A., Garcia, N., Monleon, J., Faus, A., Cervelló, I., & Pellicer, A. (2019). Inhibition of tumor cell proliferation in human uterine leiomyomas by vitamin D via Wnt/β-catenin pathway. Fertility and Sterility, 111(2), 397–407. https://doi.org/10.1016/j.fertnstert.2018.10.031
35. Faustino, F., Martinho, M., Reis, J., & Águas, F. (2017). Update on medical treatment of uterine fibroids. European Journal of Obstetrics & Gynecology and Reproductive Biology, 216, 61–68. https://doi.org/10.1016/j.ejogrb.2017.07.011

36. 36. Al-Hendy, A., & Badr, M. (2014). Can vitamin D reduce the risk of uterine fibroids? Women's Health, 10(4), 353–358. https://doi.org/10.2217/whe.14.24
37. 37. Ciebiera, M., Włodarczyk, M., Ciebiera, M., Zaręba, K., Łukaszuk, K., & Jakiel, G. (2018). Vitamin D and uterine fibroids-Review of the literature and novel concepts. International Journal of Molecular Sciences, 19(7), 2051. https://doi.org/10.3390/ijms19072051
38. 38. Halder, S. K., Sharan, C., Osteen, K. G., & Al-Hendy, A. (2011). 1,25-Dihydroxyvitamin D3 is a potent anti-tumor agent for non-surgical treatment of uterine leiomyoma in Eker rats. Fertility and Sterility, 96(3 Suppl), S51–S52. https://doi.org/10.1016/j.fertnstert.2011.07.213

Supplementary file S4: COCHRANE Handbook for Systematic Reviews of Intervention.

	STUDIES	COCHRANE Handbook for Systematic Reviews of Intervention						
		Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
1	Thomas J. 2010	LR	LR	LR	LR	LR	UR	UR
2	Brakta S, 2015	LR	UR	UR	UR	UR	UR	UR
3	Ciebiera M, 2018	LR	LR	LR	UR	UR	UR	UR

Supplementary file 1: Eligibility and inclusion criteria for the systematic review.
Supplementary file S2: Search strategy for the systematic review.
Supplementary file S3: The Newcastle-Ottawa Scale (NOS) – Case-Control Studies and Cohort Studies.
Supplementary file S5: The selected studies and the Kappa values for each.
Supplementary file S6: COCHRANE Handbook for Systematic Reviews of Intervention and the risk of bias in clinical trials using a domain-based assessment tool.