Vitamin D and uterine leiomyomata: is it time to let the sunshine in?

In this issue of Fertility and Sterility, Corachál et al. (1) examine one aspect of the accumulating evidence suggesting that the vitamin D signaling pathways are attractive targets for prevention and treatment of uterine leiomyomata (fibroids). Vitamin D is a unique secosteroid prohormone in that it can be obtained through dietary intake and can be synthesized in skin from exposure to sunlight. Vitamin D also requires sequential hydroxylation in the liver and kidney to assume its active form, 1,25-dihydroxy vitamin D. In addition to its well-recognized involvement in calcium and phosphorus homeostasis, vitamin D can also regulate cell proliferation and differentiation, angiogenesis, and apoptosis (2, 3).

Vitamin D has been increasingly implicated in uterine fibroid pathogenesis and pathophysiology. Uterine fibroids, one of the most common disorders of the female reproductive tract, are benign monoclonal tumors of uterine smooth muscle cells. Although multiple risk factors have been identified, among them is insufficient vitamin D. There is a significant inverse relationship between lower serum vitamin D levels and the occurrence and severity of uterine fibroids (3) Moreover, in a small randomized control trial of women with vitamin D deficiency and uterine fibroids, treatment with vitamin D resulted in a modest decrease in fibroid size (4).

The relationship between vitamin D and fibroid pathobiology has been strengthened by in vitro and ex vivo studies and also in animal models (3). Uterine fibroid tissue has a lower expression of the vitamin D receptor, while in vitro vitamin D (or analogue) treatment decreases fibroid cell proliferation and tumor growth and promotes apoptosis. Furthermore, genetic polymorphisms associated with lower serum vitamin D concentrations are also associated with increased fibroid incidence. The effects of vitamin D are thought be mediated through mechanisms that involve cell cycle arrest, inhibition of Wnt/β-catenin pathway, induction of apoptosis, and suppression of TGFβ signaling-induced deposition of extracellular matrix (1, 3).

Genomic analysis of fibroids suggests four distinct molecular subtypes: high-mobility group AT-hook 2 (HMGA2) rearrangements, mediator complex subunit 12 (MED12) mutations, biallelic inactivation of fumarate hydratase, and deletions affecting collagen type IVα-5 and α-6 (COL4A5 and COL4A6). Of those, MED12 mutations are the most commonly found in fibroids, occurring with a population-dependent frequency of 42% to 92%. MED12 mutations have been implicated in the modulation of the Wnt/β-catenin pathway. Additionally, the Wnt/β-catenin pathway is also thought to modulate the TGFβ signaling pathway through stimulation of TGFβ3 (1, 3).

Corachál et al. (1) examine whether the effects of vitamin D on the Wnt/β-catenin pathway and TGβ signaling are dependent on MED12 mutation status. In other words, given that mutations in the MED12 affect these pathways, will the effect of vitamin D be seen only in patients with fibroids that are positive for MED12 mutations? The authors demonstrate that cell proliferation, Wnt/β-catenin, and TGFβ pathways were all upregulated in the MED12 mutated cells, in comparison with normal myometrium. Interestingly, these pathways were also likely upregulated in fibroids without MED12 mutations, though this difference was not statistically validated. Nevertheless, downregulatory effects of vitamin D treatment on these pathways, and a marker of cell proliferation, were seen irrespective of MED12 mutation status (1).

On the basis of prior studies, we know that vitamin D deficiency and risk/severity of uterine fibroids differ by skin color, age, and body mass index, among other factors. Response to vitamin D may also differ on the basis of these characteristics (3). These factors were not specifically addressed or noted in the study and could potentially explain the lack of difference noted between the groups. Moreover, three additional molecular phenotypes are associated with uterine fibroids. Inasmuch as the effects of vitamin D are seen regardless of MED12 mutation status, it may be worthwhile to see whether these additional molecular phenotypes could predict the response to vitamin D.

To classify fibroids based on mutations, the authors used polymerase chain reaction to amplify exon 2 of the MED12 gene from genomic DNA and subsequently used Sanger sequencing to sequence exon 2 (1). Whereas the majority of mutations in the MED12 genes in uterine fibroids are found in exon 2 and the intron 1-exon 2 junction, mutations in exon 1 have also been identified in uterine fibroids, with a similar effect on downstream signaling (5). Although mutations in exon 1 may make up only a small fraction of uterine fibroids, by sequencing only exon 2, the authors could be incorrectly categorizing those fibroids with mutations in exon 1 as negative for the MED12 mutation. Additionally, it would be useful to know the sensitivity of the techniques used to find MED12 mutation in these tissues. The use of polymerase chain reaction to first amplify the genomic DNA introduces the risk of leaving out mutations that occur in only a few cells. If only 10% of the cells had the mutation, would the mutation have been picked up by the methods used? Finally, the studies regarding effects on signaling pathways were done on cultured primary cells, whereas the genetic tests for the presence of MED12 mutations were done on leiomyoma tissue. The assumption is that the MED12 mutation status is congruent between the leiomyoma tissue and cells grown in culture. It may be possible that cells isolated, and selected by growth in vitro, would result in selection for a few cells that already have MED12 mutations. Testing the human leiomyoma primary cells would help answer this question.

This study adds further evidence to support the hypothesis that vitamin D could be a potential novel medical therapy for uterine fibroids. Current treatment options for uterine fibroids, including surgery, artery embolization, or ablating with focused energy, are expensive and can result in a uterus that is less likely to support a healthy pregnancy. Medical treatment options aimed at decreasing the size of uterine fibroids by interrupting estrogen or progesterone production or action are approved only for short-term use owing to safety concerns with long-term use. Thus, a relatively safe

treatment, such as vitamin D or vitamin D analogues, would be an attractive addition to currently available therapies.

One challenge of designing clinical trials will be determining the therapeutic doses. In vitro studies have used a wide range of doses, with the current study using 1000 nM of 1,25-dihydroxyvitamin D (1). It is difficult to translate these doses clinically, given that 1,25-dihydroxyvitamin D is not the supplement traditionally prescribed, nor is it clinically measured (2). Moreover, the two small clinical studies so far have been done only in women with vitamin D deficiency. Repleting vitamin D to normal levels resulted in modest changes in uterine fibroid size (<10 mm) (4). The questions that then arise are these: whether vitamin D therapy would be beneficial in women without deficiency and whether treatment of uterine fibroids would require supraphysiologic levels of vitamin D that might otherwise be toxic. Hypervitaminosis D can lead to hypercalcemia and its associated manifestations. Moreover, vitamin D toxicity can tip the balance in regulation of bone metabolism to favor increased bone resorption (2). Paricalcitol, a 1,25-dihyroxyvitamin D analogue with lower hypercalcemic effects, may offer a safer alternative (3).

Although we are not yet sure whether vitamin D or other related therapies will constitute an effective and safe treatment for uterine fibroids, studies demonstrating the striking prevalence of vitamin D deficiency across the world suggest that we could all use some more sunshine in our lives (2). For those of us who spend most of our life indoors, moderate-dose vitamin D supplementation would seem to be safe and possibly beneficial to our general health, even if this dose is insufficient for fibroid treatment. Our advice, as we watch the story of vitamin D and uterine fibroids unfold, is to open up the windows and (with apologies The 5th Dimension's 1969 hit song) let the sunshine in!

Vaishnavi Purusothaman, M.D., M.A.
Steven L. Young, M.D., Ph.D.
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

https://doi.org/10.1016/j.fertnstert.2020.09.151

REFERENCES

1. Corachán A, Trejo MG, Carbajo-Garcia MC, Monleón J, Escrig J, Faus A, et al. Vitamin D as an effective treatment in human uterine leiomyomas independent of mediator complex subunit 12 (MED12) mutation. Fertil Steril 2020; 114:XXX-XX.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Enocrinol Metab 2011;96:1911–30.
3. Brakta S, Diamond JS, Al-Hendy A, Diamond MD, Halder SK. The role of vitamin D in uterine fibroid biology. Fertil Steril 2015;104:698–706.
4. Hajhashemi M, Ansari M, Haghollahi F, Eslami B. The effect of vitamin D supplementation on the size of uterine leiomyoma in women with vitamin D deficiency. Caspian J Intern Med 2019;10:123–31.
5. Kampjarvi K, Park MJ, Mehine M, Kim NH, Clark AD, Butzow R, et al. Mutations in exon1 highlight the role of MED12 in uterine leiomyomas. Hum Mutat 2014;35:1136–41.