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Research Article

Evaluation of vitamin D-deficiency effects on the incidence of uterine fibroids

Shafq Al-azzawi*, Dhafir Masheta, Rawnaq Kadhim

[1] College of Pharmacy, University of Babylon, Iraq

ABSTRACT

Uterine fibroids are a very common benign tumor in women of reproductive age due to unknown aetiology. There is growing evidence that vitamin D deficiency has a potential role in developing uterine fibroids. This study aims to investigate the effect of vitamin D levels in patients with uterine fibroids in Hilla city (a sunny city). The study was conducted on 70 women diagnosed with uterine fibroids that visited private clinics and were referred to biochemical laboratories. Vitamin D levels were assessed in patients' sera, and questionnaire data on age, body mass index, symptoms and drugs used were analysed. In addition, information from their ultrasound scans of the fibroids, including the size and the number of fibroids, were also recorded. The results demonstrated that more than half of patients had vitamin D deficiency and 25% of them were with insufficient vitamin D levels. The age group, 41-45 year, showed the lowest values, whereas no significant differences in vitamin D levels were observed in body mass index and severity of symptoms parameters. The results also revealed that a significant decrease in vitamin D levels was associated with large size and multiple fibroids. It can be concluded that vitamin D has a great implication on the incidence of uterine fibroids, even in sunny cities.

Keywords:
Vitamin D, Uterine fibroid, Hypovitaminosis, Small burden, Leiomyoma

1. INTRODUCTION

Uterine fibroid (UF), also known as "small burden", is a localized proliferation of the uterine smooth muscle cells. It is considered a benign tumor that commonly occurred in a woman's genital tract and its aetiology remains unclear. Tumor necrosis factor α (TNF-α) is suggested to be one of the key factors in the transformation of the uterine smooth muscle cells into abnormal and immortal cells, that lead to clonal division and consequently to UF tumor[1]. The mechanisms that control the growth of UF are sophisticated and still not well understood[2]. It is suggested that their growth results from the increased cellular proliferation and abnormal and excessive deposition of "extracellular matrix" (ECM)[3]. The main hormones that can simulate UF development and growth are oestrogen and progesterone[2,4-5]. Cell signaling dysregulation, cytogenetic abnormalities, and miRNA expression have also been implicated in UF etiology. Mutations of the mediator complex gene subunit (MED12) may also result in fibroid formation[6-7]. Fibroids is mainly composed of smooth muscle cells and various amounts of fibrous tissue enclosed by a neurofibrovascular networks[8]. Typically, the symptoms include; heavy menstrual bleeding or menstrual disorders, pelvic pain, anemia and bulky abdomen symptoms. Moreover, fibroids in the uterine could lead to infertility, abortion and several adverse gynecologic and obstetric outcomes with different debilitating morbidities[9-10].

The main applicable treatment of uterine fibroid is their removal by surgical intervention, either myomectomy or hysterectomy and are considered expensive[11]. Ulipristal acetate has recently been introduced as "a selective progesterone receptor modulator" to be a new treatment strategy for uterine fibroids[12]. However, idiosyncratic liver injury was reported after its using as treatment for UF[13]. Obviously, a therapeutic strategy for uterine fibroids should be effective and with low cost and risk.

Thence, it is imperative to search for nonsurgical and efficient alternative strategies for uterine fibroids

***Corresponding author:**
*Shafq Al-azzawi Email: Phar.shafaq.kadhim@uobabylon.edu.iq

management, and in the first place among these strategies is the prevention of its occurrence. It is known that vitamin D3 acts as a regulator of calcium homeostasis in addition to its strong antifibrotic activity[14]. Moreover, studies have reported vitamin D3 as a potent antitumor factor that efficiently inhibits uterine fibroid in cell culture and shrinks fibroid in animal studies. However, there are not enough clinical trials conducted in the human uterine regarding this area of research[1,12,15-16].

Vitamin D deficiency is associated with enormous unfavorable conditions, in particular, osteomalacia and rickets[17]. Low levels of vitamin D have previously been detected in women with osteoporosis and metabolic syndrome, which in turn emphasizes the value of vitamin D in women's general health[18-19].

Vitamin D can be found within the diet, such as cod liver oil, oily fish and dairy products. However, the main vitamin D source is sun exposure, where ultra-violet light transforms 7-dehydrocholesterol in the skin to vitamin D via a nonenzymatic isomerization. After that, vitamin D is subjected to metabolic biotransformation in the liver by 25α-hydroxylase to 25-hydroxyvitamin-D; which is then converted in the kidney to the active form (1,25-dihydroxyvitamin-D) (vitamin D3) by the action of 1α-hydroxylase[20]. Vitamin D3 exerts its biological activities in cells by interacting with its specific receptor, vitamin D receptor (VDR). VDR is "a nuclear transcription factor", it plays an important role in gene expression modulation and transcription. VDR affects the cell signalling such as "growth arrest, differentiation and/or induction of apoptosis", which in turn inhibits cell growth[1,21].

It has been recently suggested that vitamin D deficiency is associated with increasing uterine fibroids risk [1-2,4,22]. Vitamin D acts as a potent antiprogesteronic and antiestrogenic compound and can inhibit leiomyoma cell proliferation. Furthermore, vitamin D was found to inhibit the growth of fibroid cells and also promotes cell apoptosis in *in vitro* culture, whereas in *in vivo* animal models, it reduced the fibroid size.

Several previous epidemiological studies conducted in different populations emphasized the importance of vitamin D deficiency in the development of UFs[24-26]. Nevertheless, no study so far has ever addressed this issue in the Iraqi population (country in the Middle East) especially in Hilla city (located in the middle area of Iraq) where it is considered a hot region with high levels of sunlight exposure. Therefore, this study aims to investigate the relationship of occurrence of fibroids in women diagnosed with uterine fibroid with vitamin D deficiency in Hilla city.

2. SUBJECTS AND METHODS

The cross-section study was conducted in 2021 during the summer season, from June to October, to exclude deficiency of vitamin D associated with the winter season, on a sample of 70 subjects. The participants were recruited among the female patients attended private gynecological clinics and were referred to biochemical analytical laboratories in Hilla city.

A female subject was considered eligible to participate in the study if she presented with uterine fibroid that was detected by ultrasound scan (transvaginal or abdominal) and all types of fibroids (subserosal, submucosal, intramural and pedunculated fibroids) as appeared in the reports were included. Information from their ultrasound scan reports were considered as variables in the study including the size of fibroids; small (<1.9 cm), medium (2-2.9 cm) and large (>3 cm)[27] and number of fibroids (single or multiple).

The medical history and clinical features for each patient were recorded as a questionnaire to include the following parameters: age, "body mass index (BMI)", previous or current intake of vitamin D supplements or not and a history of disease. Fibroids-related symptoms such as menstrual disorders and pelvic pain (which are classified as minor, mild or severe) were also reported. Women with a history of intake of vitamin D supplements and those who followed a special diet regimen due to weight lowering or disease were excluded from the study. In addition, women with chronic disease (such as cardiovascular and central nervous system disorders, diabetes mellitus, autoimmune diseases) were also excluded.

Vitamin D3 levels were obtained from laboratory biochemical analysis reports which were measured in the serum of participants using MINI VIDAS system (France)[28] to study the effect of vitamin D3 levels on the incidence of UF. Vitamin D deficiency was deemed when serum levels were <20 ng/mL and considered insufficient if the levels were 20-30 ng/mL and normal when the levels were >30 ng/mL[29]. Data of the patients with vitamin D3 deficiency and insufficiency were analyzed to evaluate the relationship between vitamin D and the tested variables (age, BMI, severity of symptoms and the size and number of fibroids). To study the relationship of vitamin D concentration with age factor, participants were stratified into four groups (<35, 35-40, 41-45 and >45 years) whereas for BMI factor, they were grouped into four groups (<25, 25-30, 31-40 and >40).

2.1. Statistical analysis

The data in this work were collected and expressed as percentages or means±standard deviation (represented by error bars) using Excel Microsoft. The data were analyzed by ANOVA and Tukeys test using Minitab software, and probability <0.05 is considered for the significant differences.

3. RESULTS AND DISCUSSION

In this study, 70 women diagnosed with fibroids were recruited and their vitamin D3 serum levels were evaluated. The results revealed that 56% of the participants were with deficient vitamin D3 values (<20 ng/mL), while 25% of them were found with insufficient vitamin D3 values and only 19% were normal (>30 ng/mL) (Figure 1).

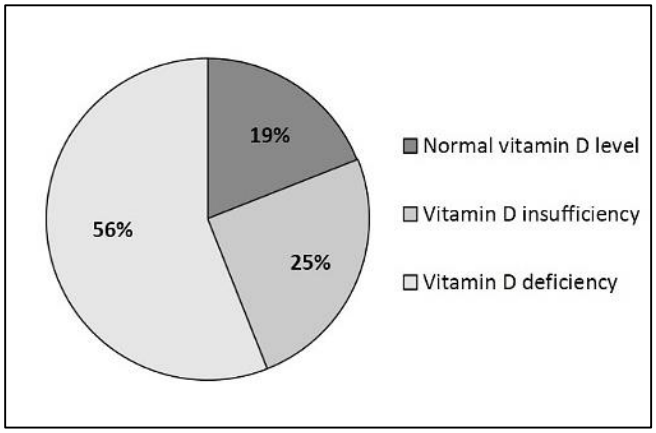

Figure 1. Percentages of women having normal, insufficient and deficient vitamin D levels associated with uterine fibroids.

To evaluate the association of vitamin D deficiency with the age factor, women were stratified into four age groups. It is of note that the youngest women (<35 years) have the highest levels of vitamin D3 while its lowest levels are accompanied with older women. Furthermore, both age groups, 41-45 and >45 showed significant differences (p<0.05) in vitamin D levels from the <35 group. Significant differences (p<0.05) were found in comparison with the group of control women matched per age (Figure 2).

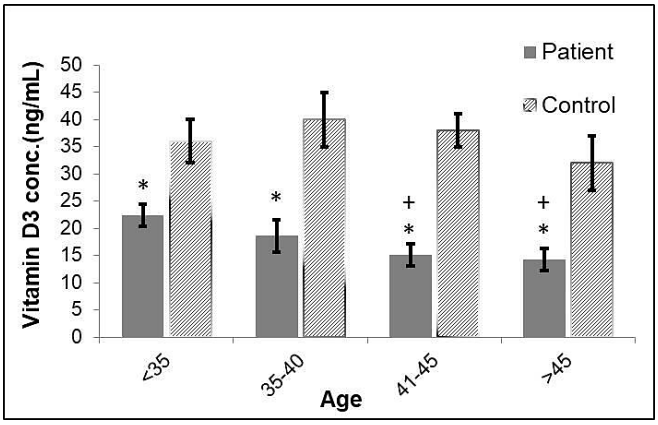

Figure 2. Vitamin D levels within age subgroups for women with uterine fibroids with comparison to control groups. Significant differences (p<0.05) were found between each subgroup with the corresponding age in control group(*), and between >40 subgroups and <40 subgroups(+).

This is consistent with the previous findings that have documented a positive correlation between age and serum vitamin D concentrations[31-32]. It has been reported that low vitamin D levels associated with increasing age is due to several factors including reduction in calcium absorption with increasing "intestinal resistance" to vitamin D[31], low expression of VDR[5], and reduced renal production of vitamin D by the kidneys[31]. Obesity and lack of outdoor activity by the older women may also contribute to the vitamin D deficiency[33]. Therefore, consumption of vitamin D-rich food with increasing sunlight exposure are required for people aged more than 41 years to avoid vitamin D deficiency.

The data regarding BMI showing the lowest vitamin D3 levels were noticed with high BMI (>30) group, whereas the highest levels of the vitamin (19.1±5 ng/mL) were observed with low BMI (<25) group, however there was no significant differences (p>0.05) within the tested subgroups (Figure 3).

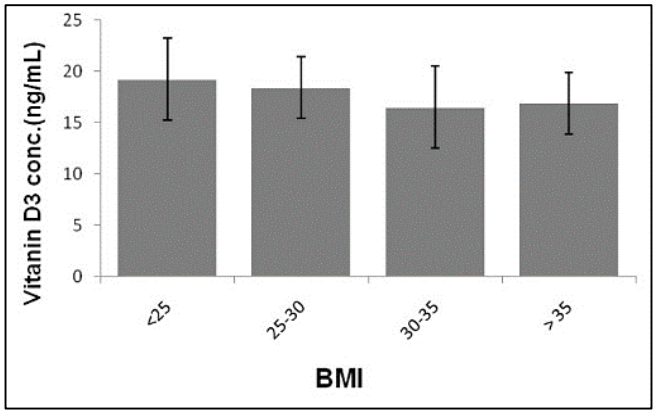

Figure 3. Vitamin D levels within BMI subgroups for women with uterine fibroids.

Considering other studies, it has been shown that obesity is "independently correlated" with vitamin D deficiency[34]. A previous study has demonstrated that vitamin D deficiency was found in more than 50% of obese individuals (BMI ≥40)[35]. The relationship between obesity and vitamin D is not clear and still unknown, and may be explained by the presence of "excess body fat tissue" that retain vitamin D resulting in an increase in the volume of distribution of the vitamin, which in turn causes a decrease in the circulatory vitamin D levels. Alternatively, metabolic syndromes could also be the reason behind the vitamin D deficiency as overweight is considered as one of its main components[34]. It has been found that vitamin D deficiency is associated with metabolic syndrome especially in postmenopausal women[18]. Further explanation may link obesity with vitamin D deficiency is that obese individuals have higher cortical tissue mineral density and cortical thickness than thin ones have or may due to differences between obese and normal subjects in liver enzymes expression that activate vitamin D[33].

The severity of symptoms associated with UF was

also evaluated to detect any suspected effects of vitamin D deficiency. In this regard, women with mild to moderate symptoms were associated with the lowest values of vitamin D (16.3±2 ng/mL) while highest values (20.2± 4 ng/mL) were detected in individuals with minor symptoms (Figure 4). However, there were no significant differences (p>0.05) within groups of severity of symptoms (minor, mild or severe). These controversial results could be attributed to the inaccurate information given by some patients or some of them have tolerated these symptoms or underestimated them (pain-threshold variations from patient to patient).

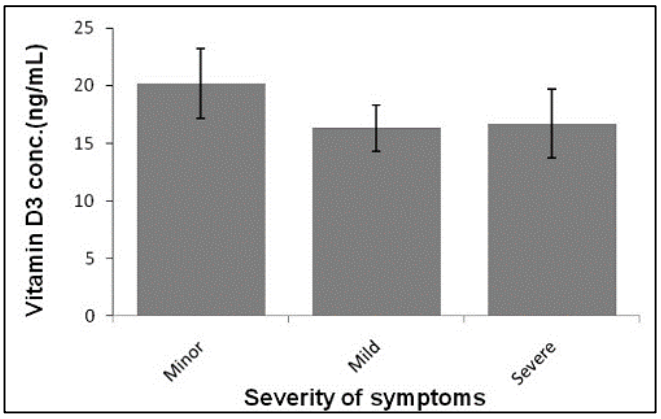

Figure 4. Vitamin D levels according to severity of symptoms associated with uterine fibroids.

To identify any potential correlation between vitamin D deficiency and the incidence of UF, serum levels were investigated according to the size and number of fibroids in the involved subjects. As expected, the concentrations of vitamin D were found to be significantly (p<0.05) lower in patients with large size fibroids (14.8 ±2 ng/mL) than in those have small ones (20.9±3 ng/mL) (Figure 5). Interestingly, the serum levels of vitamin D were found at its lowest concentrations (12.9±4 ng/mL) in the cases with multiple number of fibroids with significant differences (p<0.05) when compared to that of the single fibroid cases (22.6±3 ng/mL) (Figure 5).

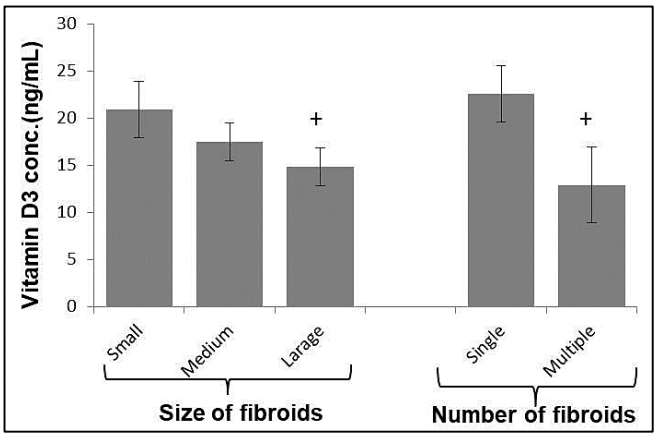

Figure 5. Vitamin D levels according to size and number of fibroids. Significant differences (P<0.05) were found between small and large size subgroups and between single and multiple fibroids subgroups (+).

Thence, current findings demonstrated the crucial role of vitamin D deficiency in developing fibroids and increasing cell growth and proliferation. As previously reported, vitamin D has potent anti-tumor activity by inhibiting "leiomyoma cell proliferation"[4,24], this is also supported by other studies that have proposed its potential role in the non-surgical management of uterine leiomyoma[9,14,16]. It has been previously postulated that vitamin D could exert an antiproliferative action on uterine cells through arresting cell growth and inhibition of "Wnt/β-catenin pathway", suggesting its effective option in stabilizing leiomyoma size and preventing its growth[36]. It has been demonstrated that increasing vitamin D concentration was correlated with inhibition of UF cell growth[15,23]. Further study has shown the inhibitory effect of vitamin D3 on the "transforming growth factor beta" (TGF-β) pathway that is deemed to be the main factor in the developing of "fibrosis-associated diseases" confirming the role of this vitamin in the fibroid pathogenesis. Furthermore, vitamin D may act as a potent antiprogesteronic and antiestrogenic compound, and upon vitamin D supplementation, the progesteronic and estrogenic receptors expression decreased and the VDR up-regulation increased resulting in reducing disease progression[9]. In addition, it is believed that "human uterine leiomyoma" contains lower vitamin D3 concentrations than its adjacent myometrium[30]. Another study has suggested that vitamin D3 reduces UF growth via modulating the up-regulation and activities of "matrix metalloproteinase-2 and -9 and it is considered a promising natural substance with anti-uterine fibroid properties[3]. Other several *in vivo* and *in vitro* studies have shown the effectiveness of vitamin D in reducing the size and the frequency of leiomyomas [25,37]. Recent studies have demonstrated that a combined supplementation of vitamin D plus epigallocatechin gallate could reduce myomas' volume and improve women's quality of life[38].

As a result, vitamin D3 supplementation seems to be beneficial to ameliorate the difficulties associated with, or even preventing, UFs, which in turn may improve women's health. Vitamin D can regulate cells proliferation and differentiation, inhibit angiogenesis, and stimulate cells apoptosis, consequently, this leads to inhibition of tumorous tissue growth and of neoplastic formation associated with fibroids of the uterus[9,36]. Another explanation for the role of vitamin D supplements on reduction of fibroids progression by recovering the damaged DNA, including suppression of the UF's phenotype via orchestrated addressing at multiple molecules of the DNA repairing pathways[39]. Vitamin D3 is found able to shrink the UF growth and reverse several abnormal biological pathways by ameliorating

the developmental exposure-induced DNA damages and inflammatory pathways in primed myometrial stem cells[40].

Vitamin D deficiency has become a common dilemma associated with many health problems and its consequences could not be underestimated. Chronic conditions are usually multifactorial, yet they could easily be overcome; however, vitamin D is considered an effective and potent, safe and low-cost treatment that can also be taken as a prophylactic agent[14,23]. Although sun exposure is the "ideal source" of vitamin D, most individuals, in reality, have hypovitaminosis D and need supplementation. Vitamin D deficiency was found pandemic even in "sun-drenched" countries and associated with tremendous negative health consequences. The skin is capable of production of vitamin D supplying 80-100% of the body's requirements of vitamin D. However, age, time of day, latitude, season and pigmentation can influence the production of vitamin D in the skin[41]. Generally, people avoid the sun because of the overexposure dangers. Moreover, many of "life's obligations" force us to spend most of our time inside under fluorescent lights, keeping us away from natural sunlight. In addition, the cultural differences in clothing and Muslim dress style could have impact on sunlight exposure, as many Iraqi women cover their bodies with hejab or abaya that limits the skin's exposure to sunlight and lowers the ability to synthesize vitamin D[42]. Therefore, health in general and vitamin D levels, in particular, must be checked routinely to unearth any latent disease or even to prevent its manifestation via modifying the risk factors. In Iraq, the temperature in summer may reach over 50°C that prevents most people from getting outside or doing any outdoor activities. Hence, vitamin D supplementation might be considered especially for people at high risk factors and for women at their reproductive age to avoid occurrence or progression of fibroids.

To reach sufficient serum vitamin D3 concentration (75 nmol/L), according to previous studies, different daily vitamin D supplemental intakes were recommended depending on region, status and age of individuals. For instance, the dose of 2250 IU/day for pregnant women and of 2026 IU/day for adults in Middle East area is suggested[37,43]. Adverse effects associated with vitamin D self-administration such as hypercalciuria and hypercalcemia are rare, and usually due to taking it for a long time with extremely high doses[44]. In addition, many nutrients and dietary habits can be associated with myoma development risk, so dietary supplements should be considered for women with UF such as food rich with fibres, and fish oil as well some fruits and vegetables, especially the coloured ones, these can help in improving health and potentiating the action of vitamin D in preventing the progression of diseases[45-46].

4. CONCLUSION

Vitamin D3 plays a potential role in UF pathogenesis and its severity. In spite of the fact that the region where the study was conducted is considered sunny, people may suffer from vitamin D deficiency due to lifestyle and low sun exposure. Natural vitamin D sources and supplements seem to be promising, beneficial, and inexpensive tools in the fight against UFs and in alleviating their progression. A recent study has found the administration of vitamin D supplements could reduce the recurrence rates of UFs and the size of recurrent UFs [47]. Vitamin D with specific anti UF drugs could provide a synergistic effect and is an interesting issue to be further studied.

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Author contributions
SA designed the whole study, collected the data and wrote the manuscript. DM and RK collected the data, analyzed the study data and edited the manuscript.

Conflict of interest
None to declare.

Funding
None to declare.

List of abbreviations
BMI= Body Mass Index
ECM= Extracellular matrix
TNF-α= Tumor necrosis factor α
UF= Uterine fibroid
VDR= Vitamin D receptor

Ethics approval and consent to participate

All patients in this study were asked for their consent to participate and the study protocol was approved by the local ethics committee. Permission was authorized by the Committee of Research Ethical Approval, COP, University of Babylon, Iraq, on 20/April/2021 (Reference no.: COPSEC 2021-O3).

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