Int J Pharma Bio Sci 2019 Jan; 10(1): (B) 18-23

Original Research Article Clinical Biochemistry

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

TISSUE 1, 25-DIHYDROXYCHOLECALCIFROL (DHVD3), VITAMIN D RECEPTOR (VDR) AND MATRIX METALLOPROTEINASE-2 (MMP-2) LEVELS IN WOMEN WITH UTERINE LEIOMYOMA(S)

FATIMAH A. DAWOOD[1], RAYAH S. BABAN[2]* AND ENAS A.A. RASUL[3]

[1]Laboratories of Al-Imamayn Al-Khademeyiyan Medical City/Ministry of Health
[2] Departments of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad-70036.
[3]Department of Gynecology and Obstetrics, College of Medicine, Al-Nahrain University Baghdad-70036

ABSTRACT

Uterine leiomyomas are the most common form of benign uterine tumors. The main purpose of this study is to compare between the levels of 1, 25-Dihydroxyvitamin D_3 (DHVD3), vitamin D receptor (VDR), and Matrix Metalloproteinase-2 (MMP-2) in tissues homogenate of uterine leiomyoma(s) and adjacent normal myometrium. Thirty four women were recruited for this case-control study. For each case, fibroid(s) size was determined. Also, tissue DHVD3, VDR, and MMP-2 levels were measured by ELISA technique. A significant decrease in fibroid tissue homogenate DHVD3 levels was found versus homogenate of normal tissue. Vitamin D receptor and Matrix Metalloproteinase-2 levels revealed insignificance between the fibroid and normal tissues. A reduction of DHVD3 level was obtained in fibroid tissues which represents an important gynecological pathology in women due to its relatively high prevalence and their probable impact on the patient's quality of life.

KEYWORDS: ***Uterine leiomyoma, uterine fibroid, vitamin D3(DHVD3), vitamin D receptor(VDR), Matrix Metalloproteinases-2, and fibroid tissue homogenate***

RAYAH S. BABAN*

Departments of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad-70036

Corresponding Author

Received on: 24-09-2018
Revised and Accepted on: 14-11-2018
DOI: http://dx.doi.org/10.22376/ijpbs.2019.10.1.b18-23

INTRODUCTION

Uterine leiomyomas are the most common pelvic tumor in women of reproductive age. They are the benign monoclonal tumors arising from the smooth muscle cells of the myometrium and may be single or multiple, leiomyomas are unrelated to the common forms of uterine cancer and uterine cervical cancer which typically arise from the endometrium (the uterine lining) or cervical epithelium, respectively [1].The pathogenesis of leiomyoma is not well understood. Genetic predisposition, environmental factors, steroid hormones and growth factors are important in fibrotic processes and angiogenesis, all play a role in the formation and growth of uterine fibroids. Myomas are asymptomatic or with few symptoms in most of the cases but larger myomas can compress surrounding organs, leading to urinary, digestive, or sexual symptoms. It negatively affects fertility, in case of large fibroids or when uterine cavity is distorted. Symptoms of fibroids depend on the growth and location. Smaller are symptomatic if they are within the uterine cavity and larger one is asymptomatic if they are outside of the uterus. The types of fibroid are classified according to their location (Intramural fibroids, Subserosal fibroids, Submucosal fibroids, and Cervical fibroids). Fibroids may be single or multiple. Most of the fibroids start in the muscular wall of the uterus and some lesions may develop towards the outside of the uterus or towards the internal cavity because of further growth. Secondary changes that may develop within fibroids are hemorrhage, necrosis, calcification, and cystic changes [2] . Vitamin D (Vit D) is a prohormone produced in the skin via a sunlight-initiated reaction and metabolism normally starts in the skin, where ultraviolet B (UV-B) radiation from the sun initiates conversion of 7-dihydrocholesterol to cholecalciferol. Cholecalciferol is not biologically active, but has to undergo two enzymatic steps before active 1, 25(OH) 2D3 is formed. Normally, 25-hydroxylation takes place in the liver by CYP2R1, while 1α-hydroxylation is conducted in the kidney following tubular reabsorption by CYP27B1. Converted to the active metabolite 1, 25-dihydroxyvitamin D3 [1, 25(OH) 2D3] [3]. Vitamin D exerts its effects via activation of its cellular receptor VDR, which in turn alters the transcription rates of target genes responsible for various biological responses[4-5.] Local production of 1, 25(OH) 2D3 is dependent on circulating precursor levels, which may explain the association of serum Vit D deficiency with various diseases [5.] Diverse functions for Vit D have been confirmed by the presence of VDR in a wide range of human tissues, including the skin, brain, colon, pancreas, and breast, as well as activated T and B lymphocytes, monocytes, and macrophages [3.] Many authors have demonstrated VDR expression in both the myometrium and the endometrium of the human uterus, throughout the menstrual cycle, in addition to UF tissues [6.] Uterine fibroids grow slowly by the deposition of a wide array of extracellular matrix (ECM) components, and the ECM is found to be abundant and disorganized in uterine fibroids [5.] Uterine fibroids are three to four times more prevalent among African American women when compared with Caucasian women. They also have 10 times higher risk of hypovitaminosis D (a condition in which the vitamin D3 level is lower than normal physiological levels) than Caucasian women (40 versus 4%) [6]. However, the direct relationship between uterine fibroid pathogenesis and vitamin D effect is not yet well established. The growth of uterine fibroids takes place due to an increase in cell proliferation and deposition of the ECM [7] .Uterine fibroids contain abnormal deposition of ECM components that play important role in the pathogenesis. The degradation of ECM is an important characteristic of development, morphogenesis, tissue repair and remodeling. This degradation process is specifically regulated under normal physiological conditions; however, dysregulation of this process is a cause of several diseases such as arthritis, nephritis, cancer, encephalomyelitis, chronic ulcers and fibrosis. Although various types of proteinases are involved in ECM degradation, the major enzymes are considered to be matrix metalloproteinases (MMPs)[8] There are 24 MMPs in humans, although the activities of most MMPs are very low or negligible under normal physiological condition. Their activities are primarily regulated by tissue inhibitors of matrix metalloproteinases (TIMPs), and thus the balance between MMPs and TIMPs are critical for the ultimate ECM remodeling in the tissue. The matrix metalloproteinases (MMPs) are a family of proteases that has been characterized into several subgroups based on their ability to specifically degrade various interstitial matrix and basement membrane components, and those are collagenase, gelatinases, stromelysins, membrane-type (MT)-MMPs and several others. Collagenases (MMP-1, MMP-8 and MMP-13) cleave interstitial collagens I, II and III, but they can also digest other ECM components and soluble proteins. [8] Gelatinases (MMP-2 and MMP-9) digest gelatin via their fibronectin type II repeats that binds to gelatin/collagen, and they can also digest a number of ECM molecules including type IV, V and XI collagens, laminin and others. Matrix metalloproteinase-2 (MMP-2), but not MMP-9 can also digest collagens I, II and III in a similar manner to the collagenases [9.] This study was aimed to evaluate the levels of DHVD3, VDR, and MMP-2 in the homogenate of uterine leiomyoma(s) tissues and normal adjacent cell of myometrium.

MATERIALS AND METHODS

A study was carried out on 34 women from the 1st of July-2015 to the 1st of February-2016. Women included in this study were collected from the Gynecology and the Obstetric Department at Al-Imamayn Al-Khademeyiyan Medical City in Baghdad. The practical part was conducted at Research Laboratory in the Department of Chemistry and Biochemistry, College of Medicine-Al Nahrain University and at the laboratories of Al-Imamayn Al-Khademeyiyan Medical City. This research was approved by the Institutional Higher Scientific and ethical committee in College of medicine at Al Nahrain University before participation, all women involved were given an idea about the study and their written informed consent was taken.

Study Group

Thirty-four women with uterine fibroids were involved in this study. The age range was between (40 – 50) years. They diagnosed with irregular uterine bleeding and/ or with different types of uterine fibroid(s). The presence of uterine leiomyoma(s) was confirmed by ultrasonography, and all uterine leiomyoma(s) were taken for histopathological examination to exclude uterine cancer. Blood samples were collected from women who are already diagnosed with uterine leiomyoma(s) before surgery.

Inclusion criteria

The inclusion criteria for participants were: women aged from 40 to 50 years, without smoking, with uterine fibroids only and exposure to sunlight.

Exclusion criteria

Women with one or more of the following conditions were excluded: prior hysterectomy; {current pregnancy, or pregnancy; currently lactating, or lactating within the 6 prior to this study; and women who had experienced an abortion or miscarriage within the 6 months prior to this study}. Women currently using vitamins, anticonvulsive or any hormonal treatment, including all of the types of hormonal contraceptives (Oral Contraceptive Pill, injectable contraceptive, implantation …etc.) within the 6 months prior to enrollment also will be excluded from the study. In addition, women with history of any fibro adenoma, women with coexisting adnexal pathology, e.g. ovarian cyst, diabetes mellitus, thyroid disease, parathyroid disease, renal disease, liver disease and Body Mass index more than 30 kg/m^2.

Tissue sampling

Tissue samples were collected from women who are already diagnosed with uterine leiomyoma(s) after hysterectomy (fibroid tissue and normal myometrium). The tissues were minced to small pieces and rinse in ice-cold phosphate buffer saline (PBS) (0.01M, pH=7.4) in order to remove excess blood thoroughly. Tissue pieces were weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. The homogenates then were centrifugated for 5 minutes at 5000×g to get the supernatant, divided into aliquots and store frozen at -80 ĉ until analysis. Tissue samples were used to measure 1,25-Dihydroxy cholecalciferol (DHVD3) levels by ELISA Kit Catalog No: E-EL-0016, Elabscience, China; Vitamin D Receptor (VDR) levels by ELISA Kit Catalog No: E-EL-H2043 Elabscience, China, and Matrix Metalloproteinase -2 (MMP-2) levels by ELISA Kit Catalog No: E-EL-H445, Elabscience, China.All were determined by Ezyme linked Immuno assay technique(ELISA).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS (Statistical Package for social Science) version 16, and Microsoft Excel Worksheet 2010. Comparison between groups at baseline was made using paired sample *t*-test. Significant differences in continuous variables among more than two groups were confirmed by the ANOVA test. Significant variation was considered when the p- value ≤ 0.05.

RESULT

There was a significant (p= 0.005) decrease of DHVD3 levels in fibroid tissues in comparison to the normal tissue (normal myometrium). The mean DHVD3values of the fibroid tissues was 120.41±10.22 pg/g protein vis that of the normal tissue 170.21± 20.10 pg/g protein (Table 1).

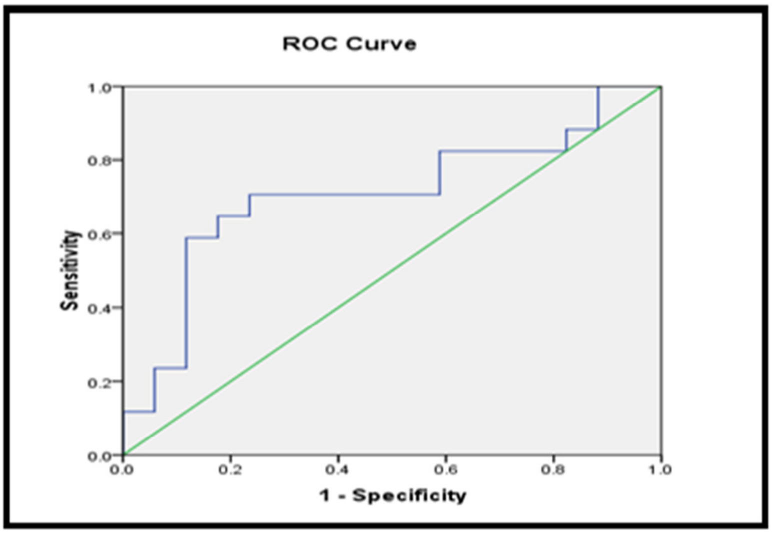
Table-1

Levels of tissue protein, 1,25-dihydroxycholecalciferol, vitamin D receptor, and matrixmetalloproteinase-2 in fibroid and normal tissues

Parameter	Normal tissue Mean ±SE N=34	Fibroids tissue Mean ±SE N=34	P- Value
Tissue protein (mg/g tissue)	1.23 ± 0.07	1.35 ± 0.06	0.50
DHVD3 (pg/g protein)	170.21± 20.10	120.41 ± 10.22	0.005 *
VDR (ng/g protein)	1.10 ± 0.21	0.78 ± 0.11	0.14
MMP-2 (ng/g protein)	60.23 ± 4.10	50.11 ± 3.10	0.23

P value is significant when it is less than 0.01,; SE: standard error; DHVD3: 1,25-Dihydroxy cholecalciferol, VDR: Vitamin D receptor, MMP-2: Matrixmetalloproteinase-2.

The measurement of tissue protein, VDR, and MMP-2 levels revealed insignificant differences between fibroid tissues and normal tissues. The mean tissue protein, VDR, and MMP-2 levels in fibroid tissues were 1.35±0.06 mg/g protein, 0.78±0.11ng/g protein, and 50.11±3.10 ng/g protein respectively. However, in normal tissues, they were 1.23±0.07 mg/g protein, 1.10±0.21ng/g protein, and 60.23±4.10 ng/g protein respectively (Table 1). The Receiver operating characteristic curve (ROC) analysis of tissue DHVD3 demonstrated an ability of this marker to differentiate women suffers from uterine fibroids from normal women (Figure 1). In this figure the cut-off value was obtained to be 130 pg /g protein (p= 0.004), the sensitivity was 70%, the specificity was 70.6% and the area under the curve (AUC) was 73%.



Cut off value	Specificity	Sensitivity	Area under curve	p-value
0.13	76.5%	70.6%	71%	0.004

Figure-1
Receiver operating characteristic curve (ROC) showing sensitivity and specificity for Patients and control by tissue 1, 25-Dihydroxy cholecalciferol levels.

The analysis of variance (ANOVA) revealed insignificant differences in the mean of tissue DHVD3, VDR, and MMP-2 among various types of fibroid (Intramural fibroid, subserosal fibroid, and submucosal fibroid) as shown in (Table 2). The mean DHVD3, VDR, and MMP-2 in intramural fibroid were 110.21±10.13pg/g protein, 0.90±0.21ng/g protein, and 58.11±6.30ng/g protein respectively. The mean DHVD3, VDR, and MMP-2 in subserosal fibroids were 120.13±10.01 pg/g protein, 0.82±0.09 ng/g protein, and 56.21±2.91ng/g protein respectively. While the mean DHVD3, VDR, and MMP-2 in submucosal fibroids were 160.12 ± 40.11 pg/g protein, 1.10 ± 0.16 ng/g protein and 70.11 ± 11.20 ng/g protein respectively (Table 2).

Table-2:
Levels of 1, 25-Dihydroxycholecalciferol, Vitamin D receptor, and Matrixmetalloproteinase-2 in tissues of fibroids.

Parameter	Intramural fibroid (50%) Mean ±SE n=26	Subserosal fibroid (38.5%) Mean ±SE n=20	Submucosal fibroid (11.5%) Mean ±SE n=6	P-Value
DHVD3 (pg/g protein)	110.21 ± 10.13	120.13± 10.01	160.12 ± 40.11	0.14
VDR (ng/g protein)	0.90 ± 0.21	0.82 ± 0.09	1.10 ± 0.16	0.2
MMP-2 (ng/g protein)	58.11 ± 6.30	56.21 ± 2.91	70.11 ± 11.20	0.5

P value < 0.01, SE: standard error; DHVD3: 1, 25-Dihydroxy cholecalciferol, VDR: Vitamin D Receptor, MMP-2: Matrixmetalloproteinase-2.

Note: The overall 52 fibroids were collected from 34 leiomyomas women participated in this study. More than one fibroid type could be taken from the same patient.

DISCUSSION

A significant reduction of DHVD3 mean level was obtained in fibroid tissues when compared with the adjacent normal tissues (normal myometrium). Such decline may indicate to the involvement of DHVD3 in the pathogenesis of uterine fibroid. Al-Hendy A, and Badr M in 2014 have reported that 1, 25-Dihydroxcholecalciferol reduced the expression of the cell proliferation marker, PCNA and MK167, and increased the expression of caspase 3 in Eker rats. Vitamin D3 shrinks UF tumor in the Eker rat by reducing cell proliferation and by activating the intrinsic apoptosis pathway. Such results support the use of 1,25-dihydroxcholecalciferol as an antitumor agent that may be a potential safe, nonsurgical therapeutic option for the treatment of uterine leiomyomas[11]. Thus, DHVD3 supplementation for women with uterine fibroid may be considered as a promising rational to prevent or regress uterine fibroids[7-10-11]. Moreover, the consistency of DHVD3 levels in the various types of uterine fibroids suggesting a compatible role of the vitamin in the development and occurrence of the UF.VDR could not be detected in fibroid and normal tissues isolated from the investigated women. The analysis of VDR was carried out by an ELISA kit with a detection limit of 0.625-40 ng/ml. Metabolically speaking, this is an expected result, since the uterus is well known to be a non-target tissue for vitamin D. However, VDR may be found in uterine tissues in very little concentration, less than the detection limit. These results were in agreement with those reported by using both immunoblotting and in situ immunohistochemical staining with a highly specific and sensitive VDR antibody to determine whether VDR is in mouse, rat, and human muscle (skeletal, cardiac, and smooth) tissues [12]. It is reasonable to consider the indirect impact of vitamin D on the smooth muscle of uterus, it functions to enhance the development and growth of the uterine fibroids, probably through the antioxidant effects on the myometrium tissue [13]. However, the current results are inconsistent with those reported by Halder, et al., 2013, who found VDR to be expressed in reduced amounts in fibroid uterine tissues relative to the adjacent normal tissues [10]. Leiomyomas are characterized by excessive deposition of ECM as well as an increase in cell proliferation.The ECM undergoes degradation in a physiologic process designed to repair and remodel it. Disruption of this degradation process leads to pathology. The major enzymes involved in this degradation process are the matrix metalloproteinases (MMPs), which are in turn regulated by tissue inhibitors of matrix metalloproteinases (TIMPs) [14]. The determination of tissue MMP-2 levels revealed insignificant differences in the fibroid tissues relative to the normal tissues. This result, in contrast to previous studies at which MMP2 levels were demonstrated to be elevated in uterine leiomyomas and is regarded as a biomarker of prognostic values as well as diagnostic for leiomyoma(s) [9-15]. Differences could be due to the small sample size, the type of techniques used and the duration of the disease.

CONCLUSION

A significant reduction in fibroid tissues 1, 25-Dihydroxycholecalciferol level was found compared with adjacent normal myometrium tissues. This finding may considered as a possible risk factor for the occurrence of uterine leiomyoma(s). While the significant increase of serum Matrix Metalloproteinase-2 level in women with uterine fibroids may use as a risk factor for developing uterine fibroids. In other hand, the absence of VDR from these tissues gives the suggestion that the effect of 1, 25-Dihydroxycholecalciferol on muscle function is most likely indirect.

ACKNOWLEDGMENT

This research paper is made possible through the help and support of my supervisors Dr. Rayah S. Baban and Dr. Enas A.A. Rasul for their significant advisors and encouragement, advices on grammar and organization of the manuscript.

AUTHOR CONTRIBUTION STATEMENT

Rayah S. Baban and EnasA.A.Rasul designed the protocol. Fatimah A. Dawood and EnasA.A.Rasul conducted the protocol and performed the experiment. Fatimah A. Dawood and Rayah S. Baban analyzed protocol and wrote the article.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Pejić s, todorović ana, stojiljković v, gavrilović l, popović n, pajović sb. Antioxidant status in women with uterine leiomyoma: relation with sex hormones. An Acad Bras Cienc. 2015;87(3):1771–82. DOI:10.1590/0001-3765201520130416
2. Ciavattini A, Di Giuseppe J, Stortoni P, Montik N, Giannubilo SR, Litta P, et al. Uterine Fibroids: Pathogenesis and Interactions with Endometrium and Endomyometrial Junction. Obstet Gynecol Int. 2013;2013:1–11. DOI:10.1155/2013/173184
3. Norman AW, Bouillon R. Vitamin D nutritional policy needs a vision for the future. Exp Biol Med. 2010;235(9):1034–45. DOI:10.1258/ebm.2010.010014
4. Bikle DD. Vitamin D: an ancient hormone. Exp Dermatol. 2010;20(1):7–13. DOI:10.1111/j.1600-0625.2010.01202.x
5. Halder SK, Goodwin JS, Al-Hendy A. 1,25-Dihydroxyvitamin D3Reduces TGF-β3-Induced Fibrosis-Related Gene Expression in Human Uterine Leiomyoma Cells. J Clin Endocrinol Metab. 2011;96(4):E754–62. DOI:10.1210/jc.2010-2131
6. Baird DD, Hill MC, Schectman JM, Hollis BW. Vitamin D and the Risk of Uterine Fibroids. Epidemiology. 2013;24(3):447–53. DOI:10.1097/ede.0b013e31828acca0
7. Brakta S, Diamond JS, Al-Hendy A, Diamond MP, Halder SK. Role of vitamin D in uterine fibroid biology. Fertil Steril. 2015;104(3):698–706. DOI:10.1016/j.fertnstert.2015.05.031
8. Halder SK, Osteen KG, Al-Hendy A. Vitamin D3 inhibits expression and activities of matrix metalloproteinase-2 and -9 in human uterine fibroid cells. Hum Reprod. 2013;28(9):2407–16.DOI: 10.1093/humrep/det265
9. Korompelis P, Piperi C, Adamopoulos C, Dalagiorgou G, Korkolopoulou P, Sepsa A, Expression of vascular endothelial factor-A, gelatinases (MMP-2, MMP-9) and TIMP-1 in uterine leiomyomas. Clin Chem Lab Med. 2015;53(9):1415-1424. DOI:10.1515/cclm-2014-0798
10. Halder SK, Osteen KG, Al-Hendy A. 1,25-Dihydroxyvitamin D3 Reduces Extracellular Matrix-Associated Protein Expression in Human Uterine Fibroid Cells1. Biol Reprod. 2013;89(6). DOI:10.1095/biolreprod.113.107714
11. Al-Hendy A, Badr M. Can Vitamin D Reduce the Risk of Uterine Fibroids? Women's Heal. 2014;10(4):353–8. DOI:10.2217/whe.14.24
12. Wang Y, DeLuca HF. Is the Vitamin D Receptor Found in Muscle? Endocrinology. 2011;152(2):354–63. DOI:10.1210/en.2010-1109
13. Mokhtari Z, Hekmatdoost A NM. Antioxidant efficacy of vitamin D. J Parathyr Dis. 2017;5(1):12. Available from:

http://www.jparathyroid.com/PDF/JPD-5-11.pdf
14. Leppert PC, Jayes FL, Segars JH. The Extracellular Matrix Contributes to Mechanotransduction in Uterine Fibroids. Obstet Gynecol Int [Internet]. 2014;2014:1–12. Available from: http://dx.doi.org/10.1155/2014/783289
15. Dawood fa, rasulc eaa, baban rs. Serum 1,25-dihydroxycholecalciferol (dhvd3) and matrix metalloproteinase-2 (mmp-2) levels in women with uterine leiomyoma(s). Int J Pharma Bio Sci. 2017;8(2). DOI:10.22376/ijpbs.2017.8.2.b622-627