



## SERUM 1,25-DIHYDROXYCHOLECALCIFEROL (DHVD3) AND MATRIX METALLOPROTEINASE-2 (MMP-2) LEVELS IN WOMEN WITH UTERINE LEIOMYOMA(S)

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### ABSTRACT

Uterine fibroids (leiomyomas), the most common benign tumors in the female reproductive system, arise from smooth muscle cells of the uterus and contain extracellular matrix. This study was aimed to identify whether low serum 1,25-Dihydroxycholecalciferol (DHVD3) levels correlate with increased risk of uterine leiomyoma(s). So, a total of 64 women were recruited for this case-control study. For each case, fibroid size was determined according to the prolate ellipse formula. Serum 1,25-Dihydroxycholecalciferol (DHVD3), and Matrix Metalloproteinase-2 (MMP-2) levels were measured by ELISA kit. Low serum 1,25-Dihydroxycholecalciferol levels in leiomyoma patients were significantly correlated inversely with total fibroid's size. Also, a significant difference ( $p < 0.05$ ) in mean level of MMP-2 was found between patients and control group. In conclusion, lower serum 1,25-Dihydroxycholecalciferol levels were inversely correlated with uterine fibroid size and the 1,25-Dihydroxycholecalciferol levels deficiency is a possible risk factor for the occurrence of uterine fibroids. Insignificant correlation was found between patient's serum Matrix Metalloproteinase-2 and their fibroid's size even when the level of Matrix Metalloproteinase-2 was high.

**KEYWORDS:** Uterine leiomyoma, uterine fibroid, vitamin D3, Matrix Metalloproteinases, MMP-2



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## INTRODUCTION

Uterine fibroids (leiomyoma), the most common benign tumors in the female reproductive system, arise from smooth muscle cells of the uterus and contain extracellular matrix<sup>1</sup>. Many fibroids are asymptomatic, but their presence may be associated with symptoms including excessive vaginal bleeding, pelvic pain, recurrent spontaneous abortion, preterm labor and are the major indication for hysterectomy<sup>2</sup>. Vitamin D can be obtained from the diet or generated through photolysis of 7-dehydrocholesterol in the skin by UV light<sup>3-4</sup>. It is then activated by two sequential hydroxylation reactions.<sup>3</sup> The active metabolite 1,25(OH)<sub>2</sub>D<sub>3</sub>, is a calcium/phosphorus-regulating hormone, indispensable for calcium and phosphate homeostasis. This function provides for normal skeletal mineralization and neuromuscular activity. 1,25(OH)<sub>2</sub>D<sub>3</sub> acts by binding to the vitamin D receptor (VDR), which is a transcriptional factor that regulates gene expression in a ligand-dependent manner.<sup>3</sup> The growth of uterine fibroids takes place due to an increase in cell proliferation and deposition of the extracellular matrix (ECM) components.<sup>5</sup> Uterine fibroids contain abnormal deposition of ECM components that play important role in the pathogenesis.<sup>6</sup> 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.<sup>7</sup> Although various types of proteinases are involved in ECM degradation, the major enzymes are considered to be matrix metalloproteinases (MMPs).<sup>8</sup> The matrix metalloproteinases 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.<sup>7</sup> 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.<sup>8</sup> 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.<sup>9</sup> This study was aimed to evaluate levels of serum 1,25-Dihydroxycholecalciferol (DHVD3) and Matrix Metalloproteinase-2 (MMP-2) in women with and without uterine leiomyoma(s), and also the study the correlation of DHVD3 and MMP-2 levels on the size of the uterine fibroid(s).

## MATERIALS AND METHODS

A case control study was carried out on 64 women from the 1<sup>st</sup> of July to the 1<sup>th</sup> of December-2015 in Baghdad. Women included in this study were collected from the Gynecology and the Obstetric Department at Al-Imamayn Al-Khademeyyan Medical City. 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-Khademeyyan Medical City. This research was approved by the Institutional Higher Scientific and ethical committee in College of medicine at Al Nahrain University in the 20<sup>th</sup> of May 2015. Before participation, we have to explain clearly about the study to the participants and a written informed consent was taken from all of them.

### Study groups

Sixty-four women involved in this study were matched in age and parity. Age range was between (40 – 50) years. They were divided into two groups:

- A- Patients group: Includes 34 women 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.
- B- Control group: Includes 30 apparently healthy women were examined by senior gynecologist during their attendance to the gynecology and obstetrics out patient at Al-Imamayn Al-Khademeyyan Medical City. All of them were free from fibroids. The exclusion criteria for control women were the same as for patients.

### Inclusion criteria

The inclusion criteria for participates 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 months 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, diabetic mellitus, thyroid disease,

parathyroid disease, renal disease, liver disease and Body Mass index more than 30 kg/m<sup>2</sup>.

### Sampling and method

About 5-6 milliliter of venous blood was aspirated from all patients prior to surgery, and the same amount was aspirated from control women. Blood was put in a plain tube, left to clot at room temperature before centrifugation for 15 minutes at 1000 x g. Sera were removed, divided into aliquots and stored, frozen at -80 °c until the time of analysis. All samples were avoided to be repeated freeze-thaw cycles. Blood samples were used to measure 1,25-Dihydroxycholecalciferol (DHVD3) levels by ELISA Kit Catalog No: E-EL-0016, Elabscience, China and Matrix Metalloproteinase -2 (MMP-2) levels by ELISA Kit Catalog No: E-EL-H445, Elabscience, China.

### STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS (Statistical Package for Social Science) version 16, and Microsoft Excel Worksheet 2010. Unpaired *t*-test was used to assess significant difference between means (SE, standard error). The Pearson Correlations test was performed to examine the correlation between serum

DHVD3 and MMP-2 levels with fibroids size. Significant variation was considered when *p*-value < 0.05.

### RESULTS

In the present study, results of serum of 1,25-Dihydroxycholecalciferol (DHVD3), and Matrix Metalloproteinase-2 (MMP-2) levels in both uterine leiomyoma(s) patients and control groups were summarized in Table 1. The Mean ± SEM of serum DHVD3 level was significantly lower in patients with uterine leiomyoma(s) compared with control groups (223.21 ± 15.23 pg/ml *vis* 333.85 ± 23.09 pg/ml, respectively, *P* = 0.0001). The number of women with DHVD3 deficiency in cases and controls were 24 (70.5%) and 9 (30%), respectively. The adjusted odds ratio for the presence of leiomyoma(s) in women with serum levels of DHVD<sub>3</sub> deficiency was 5.6. Also there was a significant differences (*P* = 0.047) between MMP-2 mean levels of patients with uterine leiomyoma(s) groups compared with MMP-2 levels of control groups, (23.00 ± 1.15 ng/ml *vis* 19.54 ± 1.23 ng/ml respectively). The number of women with MMP-2 increment in cases and controls were 21 (67.8%) and 12 (40%), respectively. The adjusted odds ratio for the presence of leiomyoma(s) in women with serum levels of MMP-2 increment was 2.4.

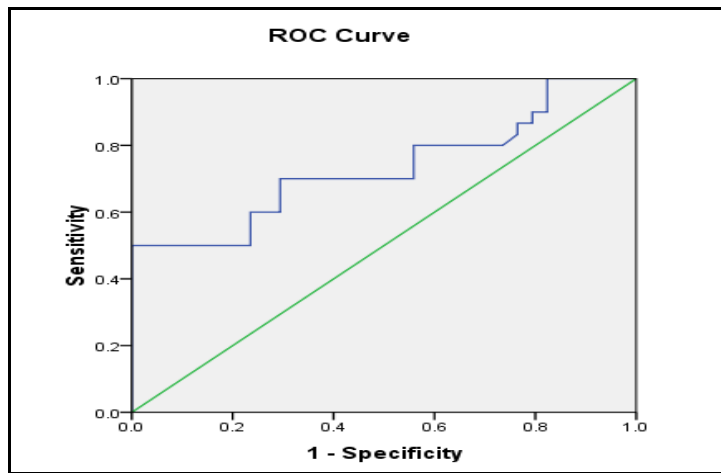
**Table 1**  
The Mean ± SEM levels of 1,25-Dihydroxycholecalciferol (DHVD3) and Matrix metalloproteinase-2 (MMP-2) in sera of patient and control groups

Parameters	Controls N=30	Patients N=34	P- Value
<i>DHVD3 (pg/mL)</i>	333.85 ± 23.09	223.21 ± 15.23	0.0001*
<i>MMP-2 (ng/mL)</i>	19.54 ± 1.23	23.00 ± 1.15	0.047*

\* Significant at *p* < 0.05, *DHVD3*: 1,25-Dihydroxycholecalciferol, *MMP-2*: Matrix metalloproteinase-2, and *SEM*: standard error of mean.

Figure-1 shows the Receiver Operating Characteristic curve (ROC) analyses of DHVD3. In this figure the cut-off value = 244.125 pg/ml, the sensitivity was 70%, specificity was 70.6% and the area under the curve (AUC) was 73%. While Figure-2 shows the Receiver Operating Characteristic curve (ROC) analyses of MMP-2. In this figure the cut-off value = 20.95 ng/ml, the

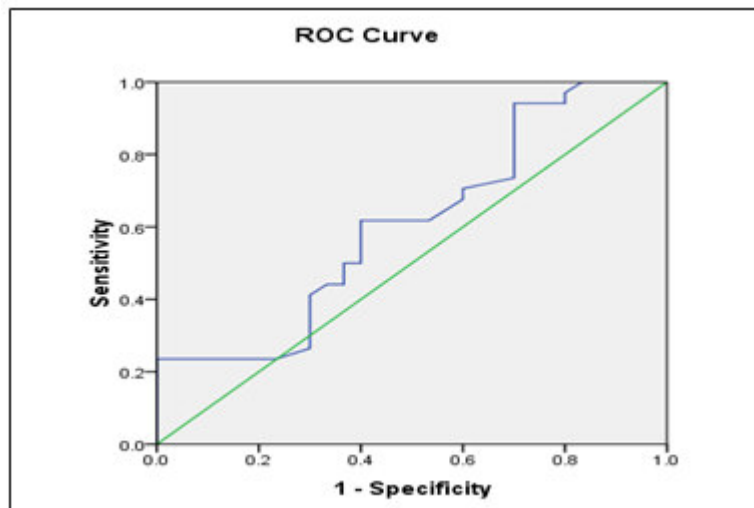
sensitivity was 58.8% specificity was 60% and the area under the curve (AUC) was 61%. Pearson's correlation analysis showed a negative significant correlation between fibroids size and serum DHVD3 levels (Figure-3), while no significant correlation was found with serum MMP-2 levels as presented in Table-2.



Cut off value	Specificity	Sensitivity	Area under curve	p-value
244.125pg/ml	70.6%	70%	73%	0.001

Figure 1

Receiver Operating Characteristic curve (ROC) showing Sensitivity and specificity for patients and control serum 1,25-Dihydroxycholecalciferol (DHVD3) levels.



Cut off value	Specificity	Sensitivity	Area under curve	p-value
20.95 (ng/ml)	60%	58.8%	61%	0.14

Figure 2

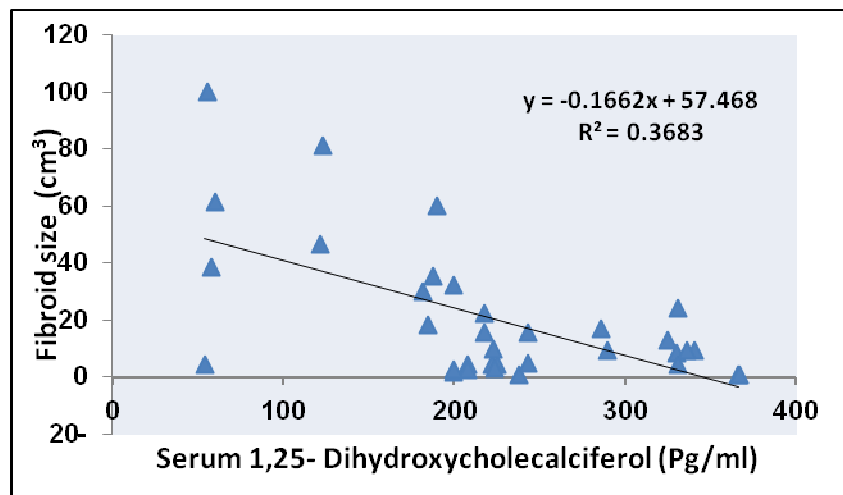
Receiver Operating Characteristic curve (ROC) showing Sensitivity and specificity for patients and control serum Matrix metalloproteinase-2 (MMP-2) levels.

Table 2

Correlation between serum 1,25Dihydroxycholecalciferol (DHVD3) and Matrix metalloproteinase-2 (MMP-2) levels with fibroids size in uterine leiomyoma(s) patients.

Parameters	Fibroids size (cm <sup>3</sup> )	
	R	P-value
Serum DHVD3 (pg/ml)	-0.61	0.0001*
Serum MMP-2 (ng/ml)	+0.08	N.S

\*Significant at  $p < 0.05$ ; N.S: Non significant; DHVD3:1,25-Dihydroxycholecalciferol, MMP-2: Matrix metalloproteinase-2.



**Figure 3**  
**Correlation of Serum 1,25- Dihydroxycholecalciferol (DHVD3)**  
**with fibroids size in uterine fibroids patients.**

## DISCUSSION

In present study, a highly significant difference ( $p=0.0001$ ) in serum vitamin 1,25- Dihydroxycholecalciferol (DHVD3) levels was found in women with uterine leiomyoma(s) group (70.6% of cases) compared with sera levels of control group (30%). This result was in agreement with Sabry *et al.*, 2013; Baird *et al.*, 2013; and Paffoni *et al.*, 2013 when they have found that women with leiomyomas had lower value of vitamin D3 and was statistically significantly when compared with healthy control women.<sup>10-11-12</sup> Moreover, 1,25-Dihydroxycholecalciferol (DHVD3) functions as a strong anti-fibrotic factor. Where other studies have demonstrated that vitamin D3 is a potent antitumor agent that effectively human inhibits uterine fibroid cells *in-vitro* and shrinks fibroid lesions in preclinical animal studies. They detected that 1,25-Dihydroxycholecalciferol reduced the expression levels of the cell proliferation marker PCNA and MK167 and increased expression of caspase 3 in 1,25-Dihydroxycholecalciferol treated Eker rats when compared with vehicle-treated controls Eker rats; they suggest that vitamin D3 shrinks uterine fibroid tumor size in the Eker rat preclinical model by reducing cell proliferation and by activating the intrinsic apoptosis pathway. These results support that 1,25-Dihydroxycholecalciferol is an antitumor agent that may be a potential safe, nonsurgical therapeutic option for the treatment of uterine leiomyomas.<sup>13-14</sup> Furthermore, adjusted odds ratio was 5.6. This indicated to women with vitamin D deficiency were more likely to have leiomyoma(s). By accident with Baird *et al.*, 2013 found that only 10% of African Americans and 50% of Caucasians had sufficient vitamin D levels.<sup>11</sup> In addition, women with sufficient levels of vitamin D were less likely to have uterine fibroids with an adjusted odds ratio of 0.68. Similarly, Paffoni *et al.*, 2013 found that women

with vitamin D deficiency were more likely to have leiomyomas with an adjusted odds ratio of 2.4.<sup>12</sup> Also, there was a significantly association between lower serum 1,25-Dihydroxycholecalciferol levels and the occurrence of UFs. A statistically significant inverse correlation was noticed between serum DHVD3 levels and total UF sizes ( $r = -0.61$ ,  $P = 0.0001$ ) (Figure-3). Patients with larger fibroid sizes had lower serum DHVD3 levels and vice versa. These results were confirmed by other study who found that serum Vitamin D3 levels inversely correlates with UFs sizes.<sup>10</sup> In the present study it was found that ROC study of DHVD3 in serum demonstrated the ability of this marker to differentiate patients suffers from uterine fibroids from normal women (Figure-1). In this figure the cut-off value = 244.125 pg/ml, the sensitivity was 70%, specificity was 70.6% and the area under the curve (AUC) was 0.73. The specificity and sensitivity of ROC study will reveal the accuracy of this test for evaluation patients with uterine fibroid. The mean of serum Matrix Metalloproteinase-2 (MMP-2) levels of patient with uterine leiomyoma(s) groups were higher than control groups ( $P=0.047$ ). This due to the effects of proteins of the Matrix Metalloproteinases (MMPs) family which involved in the breakdown of extracellular matrix (ECM) by degrading type IV collagen, the most abundant component of the basement membrane. The basement membrane is important for maintaining tissue organization, providing structural support for cells, and influencing cell signaling. This results were agreed with other report who found circulating levels of MMP-2 were significantly elevated in leiomyomas patients compared to controls ( $P=0.004$ ).<sup>15</sup> Furthermore, DHVD3 level was with invers correlation with MMP-2 level among patients with uterine leiomyoma(s) group. This was agreed with other researchers who found that there were a remarkable reduction of the mRNA levels of MMP-1, MMP-3, MMP-13 and MMP-14 in human fibroid cells

after treatment with vitamin D<sub>3</sub>. The reduction of MMP-2 and MMP-9 protein was in a vitamin D<sub>3</sub> concentration-dependent manner in uterine fibroid cells ( $p > 0.05 - p > 0.001$ ).<sup>14-16</sup>

## CONCLUSION

Lower serum 1,25-Dihydroxycholecalciferol levels are inversely correlated with fibroid(s) size and 1,25-Dihydroxycholecalciferol levels deficiency is a possible risk factor for the occurrence of uterine leiomyoma(s). High levels of serum Matrix Metalloproteinase-2 was found in patients with uterine leiomyoma(s) compared with control group, but not reached to significant correlation with fibroids sizes.

### Recommendations

Further investigations are needed to know the safety

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dose of Vitamin D3 as a therapeutic/preventive agent in the animal model of uterine fibroids (with no side effects and no negative effects on liver function tests) and this in turn may have a major positive impact on women's health in future.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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