Epigallocatechin gallate in combination with Vitamin D3 and Vitamin B6 as a promising alternative in uterine fibroids management

L. Costabile[1], M. Burratti[1], G. Biovi[1], R. Pajalich[1], V. Unfer[1,2]

[1]AGUNCO, Obstetrics and Gynecology Centre, Rome, Italy
[2]Systems Biology Group Lab, Rome, Italy

ABSTRACT — OBJECTIVE: *Recent findings showed the beneficial action of epigallocatechin gallate (EGCG) and vitamin D3 (Vit D3) in the treatment of uterine fibroids (UF). Therefore, we aimed at evaluating the efficacy of the combined supplementation of EGCG with vitamins (Vit) D3 and B6 on the reduction of volume and blood loss related to UF.*

PATIENTS AND METHODS: *19 women (mean age 44.9 ± 7.5), with at least one UF, were treated for 4 months with one tablet of 25 µg of Vit D3, 150 mg of EGCG and 5 mg of Vit B6, via oral route, twice a day. Volume, number of UFs as well as blood loss were evaluated.*

RESULTS: *All the patients experienced a significant decrease in UF volume (16.2 ± 2.7 cm³ at baseline vs. 10.9 ± 2.0 cm³ at the end of treatment; p < 0.0001) with an average reduction of 32.72%. In one patient, a small UF (0.5 cm³) fully disappeared after 4 months of treatment. A significant reduction in blood loss (63% heavy bleeding and 37% normal bleeding at T0 vs. 5% heavy and 95% normal bleeding at T1; p < 0.0001) was reported by patients at the end of the study.*

CONCLUSIONS: *Supplementation with EGCG, Vit D3, and Vit B6 reduced the size of UFs and the abnormal heavy bleeding. Therefore, this novel combination could be an alternative approach to "wait and see" for UF reduction and the correlated symptoms.*

KEYWORDS
Uterine fibroids, Leiomyoma, Vitamin D, Epigallocatechin gallate, EGCG, Menorrhagia.

INTRODUCTION

Uterine fibroid (UF), also known as uterine leiomyoma or myoma, is the most common benign tumor in pre-menopausal women. UF is an estrogens/progesterone dependent monoclonal tumor generated by myometrial smooth muscle cells with an incidence of over 50% in women aged between 35 and 45 years[1]. Even whether 50% of the patients are asymptomatic, UF symptoms can cause high discomfort and, in some cases, require surgical approaches[2]. The broad range of symptoms is acute and chronic pelvic pain, constipation, pollakiuria, infertility, dyspareunia, and iron-deficiency anemia due to abnormal heavy bleeding during menstruation and intra-menstrual cycle[3]. In addition to anemia, abundant blood loss represents the symptom with the highest impact on the quality of life (QoL) in women with UFs[4]. UF

Corresponding Author
L. Costabile, MD; e-mail: loredana.costabile@gmail.com

treatment should take into consideration location, size, symptoms, patient's age, and desire to maintain fertility[3]. The first approach is the medical treatment with hormonal contraceptives, gonadotropins releasing hormone analog (GnRHa), and selective progesterone receptor modulators (SPRM) including ulipristal acetate (UPA)[1]. In particular, UPA is the only drug having a specific indication for the treatment of UF and it is effective in reducing bleeding and tumor size[5,6]. On the other side, UPA showed adverse estrogenic activity and important liver toxicity[7]. In reason of its side effects, UPA was withdrawn from the market in September 2020 due to severe side effects, including liver failure[8,9]. Surgery approaches include hysterectomy, myomectomy, magnetic resonance-guided focused ultrasound surgery, and uterine artery embolization[1,4,10,11]. Nowadays UFs recurrence rate ranges between 3% to 32%, depending on the technique, within 5 years. However, hysterectomy seems to be a definitive remedy, showing no need of re-intervention[12]. Considering that malignant transformations of UFs occur very rarely, the "wait and see" approach is usually recommended to asymptomatic patients, with follow-ups every 6/12 months. However, some studies report UF growth in addition to manifestation of symptoms[13-15].

Recent evidence reports that vitamin D3 (Vit D3) deficiency correlates with UF insurgence[16-18]. Vit D3 is a fat-soluble steroid hormone, 80% of which is synthesized by the skin after sun exposure. This hormone is known for its role in the regulation of intracellular calcium and in bone health[19,20]. Furthermore, Vit D3 deficiency showed a crucial role in the development of different malignant tumors including breast, prostate, lung, and colon cancer[20]. Vit D3 also showed an inhibiting effect on proliferation and the ability to induce apoptosis in leiomyoma cells as well as *in vivo* model[16,18,21,22]. Likewise, epigallocatechin gallate (EGCG) showed a similar effect on UFs, both *in vitro* and *in vivo*[13,23,24]. EGCG is the most abundant green tea flavonol, commonly known as catechins, which include 5 major subtypes: catechin, epicatechin, gallocatechin, epigallocatechin, and EGCG[25].

In recent years, epidemiological and animal studies about EGCG emerged, demonstrating tumor prevention and antitumoral activities exerted by this molecule. These findings suggest a positive correlation between green tea consumption and lower incidence of gastric, ovarian, pancreatic, and colorectal cancer[25,26]. The promotion of apoptosis and the inhibition of cell growth was also reported in UF, both *in vitro* and *in vivo* models[15,23-25].

Therefore, this study aims to evaluate the efficiency of EGCG in combination with Vit D3 and Vit B6 in counteracting UF growth as well as blood loss related to this pathology.

PATIENTS AND METHODS

All patients involved in this observational study were enrolled between June 2019 and April 2020. All women gave their oral informed consent after the explanation of the study purpose. The study was conducted following the Ethical Principles of the Helsinki Declaration and the national laws. Selection of patients was in accordance with the following inclusion criteria: 18 years of age or older, premenopausal stage, at least one UF ≥ 2 cm^3 (intramural, subserosal and/or submucosal) detected by vaginal and abdominal ultrasound, normal to abnormal heavy bleeding, and any medical prescription other than regular observation. Pregnant women or intended to become pregnant during the following four months, on currently breastfeeding, with severe anemia or medical morbidity, eligible to surgery, displaying elevated liver enzymes, taking hormones (estrogen, progestin, oral contraceptives) within the past 3 months, taking corticosteroids, taking food supplements having possible hormonal effects, or using of SPRMs or GnRHa within the past 6 months were excluded. Nineteen women with one or more UFs were enrolled and treated by oral route with one tablet of 25 µg of vitamin D3, 150 mg of EGCG and 5 mg of vitamin B6 (Delphys, Farmares S.r.l., Rome, Italy), twice a day for 4 months.

The primary outcome of this study was the evaluation of the UF size reduction. The secondary outcomes were UF number reduction, and blood loss decrease in concomitance either with the presence or absence of the menstrual cycle. The subjective experience of bleeding was indicated as heavy or normal, through a self-administered bleeding assessment. The entire medical history was collected from each patient at the baseline (T0). The data from ultrasound sonography was collected at the T0 and after 4 months of treatment (T1) and analyzed in compliance with privacy regulation. UF volume was evaluated via ultrasound, recording height and width measurements. Blood loss was self-assessed by the patients at the 2 timepoints T0 and T1.

STATISTICAL ANALYSIS

Statistical analysis was performed using unpaired *t*-test (2018 GraphPad Software, La Jolla, CA, USA), with the results expressed as mean ± standard error of the mean (SEM). The comparison between T0 and T1 was assessed by one-way ANOVA analysis for repeated measures; values are indicated as mean ± SEM. Wilcoxon–Mann–Whitney test was used for blood loss analyses; values are indicated as median, 25th, and 75th percentile. The level of statistical significance was achieved with a p-value ≤ 0.05.

Table I. The baseline characteristics of patients (Treated with Vitamin D + EGCG + Vitamin B6). Abbreviations: Uterine Fibroid (UF); BMI, body mass index.

Parameters	T0	T1	*p*-value
Age (years)	44.9 (+/- 7.5)	45.5 (+/- 7.5)	0.9552
BMI (Kg/m^2)	23.8 (+/- 2.4)	23.6 (+/- 2.3)	0.9524
UFs Number	35	34	
Intramural UFs (%)	20 (57.1%)	19 (55.9%)	
Subserosal UFs (%)	15 (42.9%)	15 (44.1%)	

RESULTS

This study enrolled 19 women with at least one UF, aged between 31 and 58 years and with an average BMI of 23.8 (±2.4) Kg/m^2. All the clinical characteristics of the patients at baseline and at T1 are reported in Table I.

No adverse events or dropouts were reported during the entire study period. The total number of UF reported at the enrollment was 35, being 15 subserosals and 20 intramurals (Tab. I). All the patients experienced a significant reduction of UF volume (16.2 ± 2.7 cm^3 at T0 *vs.* 10.9 ± 2.0 cm^3 at T1; $p < 0.0001$), with an average size reduction of 32.72% (Figure 1). In one patient, a small fibroid (0.5 cm^3) completely disappeared at the end of treatment. No difference in size reduction between the various UF location was found.

Figure 2 reports the quantity of blood loss, expressed in percentage (%), between T0 and T1. These data were collected via patients' self-assessment. At baseline, 37% of the patients showed normal bleeding while 63% of them displayed heavy bleeding. After the treatment, patients reported a significant reduction in blood loss ($p < 0.0001$). Among them, abundant blood loss was reported by 5% of the patients while the remaining 95% showed normal bleeding.

DISCUSSION

The aim of this study was to evaluate the combination of Vit D3, EGCG, and Vit B6 as oral supplementation for the treatment of UFs and related blood loss symptomatology. Our results report a significant reduction in UF volume (32.72%). Besides volume reduction, the patients experienced a significant improvement in blood loss at the end of the treatment. At baseline, patients showed 37% and 63% of normal and heavy bleeding, respectively, while, after 4 months of treatment, 95% of the patients reported normal blood loss showing significant improvements

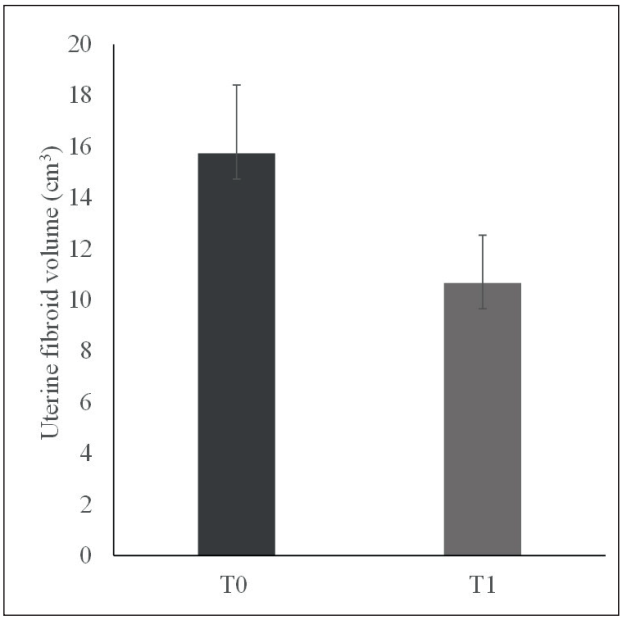

Figure 1. UF volume (cm^3) comparison between baseline and after 4 months of treatment. The volume of UF is indicated (mean ± SEM) as cm^3 at baseline (T0) and the end of the study (T1), The black column represents the volume at baseline (16.2 ± 2.7 cm^3) and the light grey column represents the volume at the end of treatment (10.9 ± 2.0 cm^3); T0 *vs.* T1 *p*-value<0.001; Abbreviations: UF, uterine fibroids; SEM, standard error of the mean.

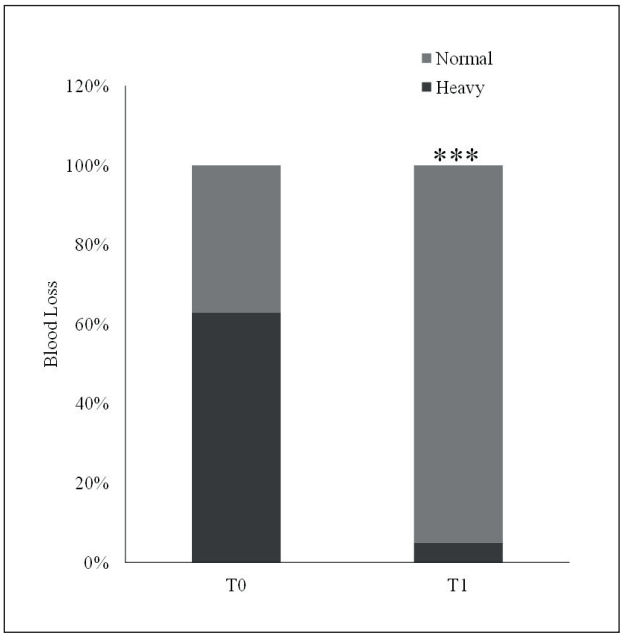

Figure 2. Blood loss quantity defined as percentage (%) at baseline (T0) and at the end of the study (T1); the black part of the column represents the percentage of patients with heavy blood loss while the light grey the percentage of patients with normal bleeding; ***= *p*-value <0.001.

in one of the most problematic symptoms related to UFs. The results from Porcaro et al[13] perfectly match ours. In their study, 30 women with UF were divided into 2 groups, 15 women undergoing our same treatment (EGCG, Vit D, and Vit B6) showing a significant reduction in UF volume after 4 months of treatment with an average of 34.7%. Significant improvements in the general symptomatology as well as in the QoL were also reported in treated patients when compared to the controls[13].

This pathology shows a very high incidence, affecting up to 70% of women in reproductive age. Approximately 50% of patients may display symptoms, that can have an important impact on QoL[27]. Among all the symptoms, heavy bleeding may represent the most distressing because of its repercussions, such as anemia, fatigue, anxiety, productivity reduction, and discomfort. Women displaying UFs often report increased usage of either tampons or pads, with more than 6 changes per day[28]. Most of these cases often require a surgical approach. On the other hand, "wait and see" is the approach commonly taken for asymptomatic UFs, with follow-up every 6-12 months. This alternative is often acceptable since UFs are characterized by a very little chance of converting into a malignant tumor and both surgical and medical treatments are not free from risks and side effects. However, different studies report UF growth after only four months of non-intervention[13, 14], leading to the insurgence of correlated symptoms. In this regard, the combination of EGCG, Vit D3, and Vit B6 may represent a solution to the wait and see approach in asymptomatic women as in those patients suffering from heavy bleeding.

The action of either EGCG or Vit D3 singularly was investigated in patients with UF. Evidence shows significant results in 4 months of treatment with EGCG in women displaying UF, reporting a reduction of 32.6% in (VDR) induction[31]. Other results suggest that vitamin D3 works as an inhibitor of Wnt4/β-catenin and mammalian target of rapamycin (mTOR) signaling pathways, which may play major roles in fibroid pathogenesis[32]. Furthermore, UFs cells secrete abundant extracellular matrix (ECM), consisting mainly of collagen, fibronectin, and proteoglycan[33, 34]. One study demonstrated that the activation of MMP-2 and MMP-9 is associated with ECM deposition as well as fibrosis in the VDR knockout mice model[35]. The group of Halder et al[36] discovered that Vit D3 inhibits expression and activities of matrix metalloproteinase-2 and -9 in human uterine fibroid cells[36]. All these results highlight an important activity of Vit D3 in inhibiting ECM deposition and its remodeling.

Interestingly, Al-Hendy et al[21] report that Estrogens can reduce the expression of VDR in HuLM in a dose-dependent manner. On the contrary, $1,25(OH)_2D_3$ increases VDR expression and reduces estrogen receptor-alpha (ER-α), progesterone receptor A (PRA) and B (PRB) expression in the same cellular model[21]. EGCG showed similar antiproliferative and proapoptotic effects through the downregulation of PCNA, CDK4, and BCL-2[15, 23-25]. The same effects were also observed in nude mouse model after the inoculation of HuLM cell, also reporting a significant tumor size reduction[37].

The current medical approaches in the management of UFs such as GnRHa and UPA are characterized by acting on both size and symptomatology reduction. This efficiency, at the same time, is accompanied by several and severe side effects[1]. For these reasons, we want to point out that in our study, as well as in the study of Porcaro et al[13], no contraindication or side effects were reported in none of the treated patients.

CONCLUSIONS

EGCG in combination with Vit D3 and Vit B6 significantly reduced the volume of UFs as well as heavy bleeding symptoms in women with one or more UFs. Therefore, supplementation with EGCG, Vit D3, and Vit B6 could represent a valuable alternative to the wait and see approach to reduce UF growth and symptomatology rise. Furthermore, this combination may represent an optimal approach in symptomatic patients ineligible for medical and/or physical treatments. Further research with larger cohorts of patients and randomized controlled group are strongly suggested.

Authors' contribution:
C.L., B.M., G.B., R.P. and V.U. contributed to the design and implementation of the research, to the analysis of the results, to the writing and reviewing of the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest:
Vittorio Unfer is employed at Lo.Li. Pharma srl, Rome, Italy. Other authors declare that they have no conflict of interest.

Informed consent:
All women gave their oral informed consent after the explanation of the study purpose. The study was conducted following the Ethical Principles of the Helsinki Declaration and the national laws.

Funding:
No funding is declared for this article.

References

1. Donnez J, Dolmans MM. Uterine fibroid management: from the present to the future. Hum Reprod Update 2016; 22: 665-686.

2. Sparic R, Mirkovic L, Malvasi A, Tinelli A. Epidemiology of uterine myomas: a review. Int J Fertil Steril 2016; 9: 424-435.
3. De La Cruz MS, Buchanan EM. Uterine fibroids: diagnosis and treatment. Am Fam Physician 2017; 95: 100-107.
4. Stewart EA. Uterine fibroids. Lancet (London) 2001; 357: 293-298.
5. Giarrè G, Franchini M, Castellacci E, Malune ME, Di Spiezio Sardo A, Saccone G, Angioni S. Ulipristal acetate in symptomatic uterine fibroids. A real-world experience in a multicentric Italian study. Gynecol Endocrinol 2020; 36: 171-174.
6. Ghonim M, Magdy R, Sabbour M, Ghonim M, Nabhan A. A systematic review and meta-analysis of ulipristal acetate for symptomatic uterine fibroids. Int J Gynaecol Obstet 2019; 146: 141-148.
7. Bouchard P, Chabbert-Buffet N, Fauser BC. Selective progesterone receptor modulators in reproductive medicine: pharmacology, clinical efficacy and safety. Fertil Steril 2011; 96: 1175-1189.
8. Rabe T, Saenger N, Ebert AD, Roemer T, Tinneberg HR, De Wilde RL, Wallwiener M. Selective progesterone receptor modulators for the medical treatment of uterine fibroids with a focus on ulipristal acetate. Biomed Res Int 2018; 2018: 1374821.
9. Meunier L, Meszaros M, Pageaux GP, Delay JM, Herrero A, Pinzani V, Dominique HB. Acute liver failure requiring transplantation caused by ulipristal acetate. Clin Res Hepatol Gastroenterol 2020; 44: e45-e49.
10. Nieboer TE, Johnson N, Lethaby A, Tavender E, Curr E, Garry R, van Voorst S, Mol BW, Kluivers KB. Surgical approach to hysterectomy for benign gynaecological disease. Cochrane Database Syst Rev 2009; 3: CD003677.
11. Bhave Chittawar P, Franik S, Pouwer AW, Farquhar C. Minimally invasive surgical techniques versus open myomectomy for uterine fibroids. Cochrane Database Syst Rev 2014: Cd004638.
12. Yoo EH, Lee PI, Huh CY, Kim DH, Lee BS, Lee JK, Kim D. Predictors of leiomyoma recurrence after laparoscopic myomectomy. J Minim Invasive Gynecol 2007; 14: 690-697.
13. Porcaro G, Santamaria A, Giordano D, Angelozzi P. Vitamin D plus epigallocatechin gallate: a novel promising approach for uterine myomas. Eur Rev Med Pharmacol Sci 2020; 24: 3344-3351.
14. Ciavattini A, Delli Carpini G, Serri M, Vignini A, Sabbatinelli J, Tozzi A, Aggiusti A, Clemente N. Hypovitaminosis D and "small burden" uterine fibroids: opportunity for a vitamin D supplementation. Medicine (Baltimore) 2016; 95: e5698.
15. Roshdy E, Rajaratnam V, Maitra S, Sabry M, Allah AS, Al-Hendy A. Treatment of symptomatic uterine fibroids with green tea extract: a pilot randomized controlled clinical study. Int J Womens Health 2013; 5: 477-486.
16. Baird DD, Hill MC, Schectman JM, Hollis BW. Vitamin d and the risk of uterine fibroids. Epidemiology (Cambridge, MA) 2013; 24: 447-453.
17. Paffoni A, Somigliana E, Vigano P, Benaglia L, Cardellicchio L, Pagliardini L, Papaleo E, Candiani M, Fedele L. Vitamin D status in women with uterine leiomyomas. J Clin Endocrinol Metab 2013; 98: E1374-E1378.
18. Brakta S, Diamond JS, Al-Hendy A, Diamond MP, Halder SK. Role of vitamin D in uterine fibroid biology. Fertil Steril 2015; 104: 698-706.
19. Adams JS, Kantorovich V, Wu C, Javanbakht M, Hollis BW. Resolution of vitamin D insufficiency in osteopenic patients results in rapid recovery of bone mineral density. J Clin Endocrinol Metab 1999; 84: 2729-2730.
20. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004; 80: 1689s-1696s.
21. Al-Hendy A, Diamond MP, El-Sohemy A, Halder SK. 1,25-dihydroxyvitamin D3 regulates expression of sex steroid receptors in human uterine fibroid cells. J Clin Endocrinol Metab 2015; 100: E572-E582.
22. Bläuer M, Rovio PH, Ylikomi T, Heinonen PK. Vitamin D inhibits myometrial and leiomyoma cell proliferation in vitro. Fertil Steril 2009; 91: 1919-1925.
23. Ahmed RS, Liu G, Renzetti A, Farshi P, Yang H, Soave C, Saed G, El-Ghoneimy AA, El-Banna HA, Foldes R, Chan TH, Dou QP. Biological and mechanistic characterization of novel prodrugs of green tea polyphenol epigallocatechin gallate analogs in human leiomyoma cell lines. J Cell Biochem 2016; 117: 2357-2369.
24. Zhang D, Al-Hendy M, Richard-Davis G, Montgomery-Rice V, Rajaratnam V, Al-Hendy A. Antiproliferative and proapoptotic effects of epigallocatechin gallate on human leiomyoma cells. Fertil Steril 2010; 94: 1887-1893.
25. Singh BN, Shankar S, Srivastava RK. Green tea catechin, epigallocatechin-3-gallate (EGCG): mechanisms, perspectives and clinical applications. Biochem Pharmacol 2011; 82: 1807-1821.
26. Negri A, Naponelli V, Rizzi F, Bettuzzi S. Molecular Targets of Epigallocatechin-Gallate (EGCG): a special focus on signal transduction and cancer. Nutrients 2018; 10.
27. Parker WH. Etiology, symptomatology, and diagnosis of uterine myomas. Fertil Steril 2007; 87: 725-736.
28. Wegienka G, Baird DD, Hertz-Picciotto I, Harlow SD, Steege JF, Hill MC, Schectman JM, Hartmann KE. Self-reported heavy bleeding associated with uterine leiomyomata. Obstet Gynecol 2003; 101: 431-437.
29. 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: 125-131.
30. Sharan C, Halder SK, Thota C, Jaleel T, Nair S, Al-Hendy A. Vitamin D inhibits proliferation of human uterine leiomyoma cells via catechol-O-methyltransferase. Fertil Steril 2011; 95: 247-253.
31. Ali M, Shahin SM, Sabri NA, Al-Hendy A, Yang Q. Hypovitaminosis D exacerbates the DNA damage load in human uterine fibroids, which is ameliorated by vitamin D3 treatment. Acta Pharmacol Sin 2019; 40: 957-970.
32. Al-Hendy A, Diamond MP, Boyer TG, Halder SK. Vitamin D3 inhibits Wnt/β-Catenin and mTOR signaling pathways in human uterine fibroid cells. J Clin Endocrinol Metab 2016; 101: 1542-1551.
33. Stewart EA, Friedman AJ, Peck K, Nowak RA. Relative overexpression of collagen type I and collagen type III messenger ribonucleic acids by uterine leiomyomas during the proliferative phase of the menstrual cycle. J Clin Endocrinol Metab 1994; 79: 900-906.
34. Berto AG, Sampaio LO, Franco CR, Cesar RM, Jr., Michelacci YM. A comparative analysis of structure and spatial distribution of decorin in human leiomyoma and normal myometrium. Biochim Biophys Acta 2003; 1619: 98-112.
35. Rahman A, Hershey S, Ahmed S, Nibbelink K, Simpson RU. Heart extracellular matrix gene expression profile in the vitamin D receptor knockout mice. J Steroid Biochem Mol Biol 2007; 103: 416-419.
36. 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: 2407-2416.
37. Zhang D, Al-Hendy M, Richard-Davis G, Montgomery-Rice V, Sharan C, Rajaratnam V, Khurana A, Al-Hendy A. Green tea extract inhibits proliferation of uterine leiomyoma cells in vitro and in nude mice. Am J Obstet Gynecol 2010; 202: 289.e1-289.e9.