

Uterine fibroid treatment with Vitamin D combined with Epigallocatechin gallate and Vitamin B6: a controlled pilot study

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ABSTRACT — OBJECTIVE: Uterine fibroids are the most common benign tumor in women and a specific treatment is not available. The combination of epigallocatechin gallate (EGCG), vitamin D and B6 in treating uterine fibroids (UFs) recently showed a promising efficacy. Here we tried to evaluate the efficiency of the combination to improve gynaecological and cardiological parameters.

PATIENTS AND METHODS: 43 women with a diagnosis of UF were enrolled and divided into two groups: a) study group, treated twice a day with 150 mg EGCG, 25 µg vitamin D and 5 mg vitamin B6, for 4 months; b) control group, with no intervention. Volume, number of UFs, menstrual bleeding, pelvic discomfort, anemia and hypertension were monitored and analyzed.

RESULTS: One UF developed in the control group, but none in the treated group. UF size significantly decreased from $10.73 \pm 5.52 \text{ cm}^3$ at baseline to $7.98 \pm 4.00 \text{ cm}^3$ after 4 months of treatment ($p < 0.0001$), while it remained unchanged in the control group ($10.21 \pm 5.83 \text{ cm}^3$ at T0 to $10.62 \pm 6.28 \text{ cm}^3$ at T1). Menstrual bleeding and anemia ameliorated only in the treated women.

CONCLUSIONS: Supplementation with EGCG, vitamin D and B6 is a safe and novel approach for the management of UFs, reducing volume and improving menstrual bleeding and anemia of women presenting such symptoms.

KEYWORDS

Uterine fibroids, Vitamin D, Epigallocatechin gallate, EGCG, Anemia.

INTRODUCTION

The effectiveness of vitamin D combined with epigallocatechin gallate (EGCG) in the management of uterine fibroids (UFs) is now consolidated^{1,2}. Indeed, our previous study proved that the combined use of these two molecules for 4 months reduced the volume of fibroids and improved patients' quality of life². Earlier evidence demonstrated the individual use of vitamin D or EGCG in treating UFs, but more recent data indicate that the combination of the two molecules leads to better outcomes. Roshdy et al³ investigated the role of the sole EGCG in the treatment of symptomatic UFs, showing a reduction of their volume and improvement of women's health. Cia-

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vattini et al⁴ demonstrated that the supplementation of vitamin D increased its serum levels in women with hypovitaminosis D and concomitant UFs, while decreasing the rate of surgical or medical therapy. Both vitamin and EGCG act on different pro-apoptotic, proliferative and extra-cellular matrix (ECM) remodeling pathways crucial for UF onset and growth⁵. All these pathways are depicted in Figure 1.

A recent meta-analysis indicated that serum vitamin D levels were significantly lower in patients with UFs than those without⁶. Similarly, previous reports revealed that low vitamin D levels correlated with UFs in the white but not black population, possibly due to differences in sun exposure, racial and individual factors. The combination of EGCG and vitamin D seems to exert a synergistic action on UFs, leading to a greater decrease in UF size and fibroid-specific symptom severity, as well as a better quality of life, when compared to the single substances². UFs are classified into subserous, intramural, and submucous types according to their location in the uterus. The submucous type lies beneath the endometrium and seems to be the most threatening as it may present many clinical complications such as menorrhagia, metrorrhagia, dysmenorrhea, infertility, and abortion³. Indeed, among all types of fibroids, submucosal or those with an intracavitary component are associated with decreased reproductive outcomes, and surgery may be required⁴. UF-associated symptoms consist of heavy menstrual bleeding and pelvic pain or discomfort. Excessive or

prolonged menstrual bleeding may lead to anemia⁷, which causes fatigue and has negative effects on reproductive health and well-being in premenopausal women^{8,9}. Indeed, fatigue is significantly more severe in young women with heavy menstrual bleeding as compared to healthy controls¹⁰. Elevated diastolic blood pressure (DBP) may represent a risk factor for fibroid onset, through injury of uterine smooth muscle. Indeed, UFs are more frequent among hypertensive (42%) than normotensive (37%) women. The increase in DBP is positively correlated with the risk of UF formation, by 8% (5-11% nonusers of antihypertensive medications) or 10% (7-13% users of antihypertensive medications) for every 10 mmHg¹¹. Since anemia and hypertension were not considered in our previous study², we investigated the role of EGCG and vitamin D on these two parameters, as well as on volume and other symptoms correlated to UFs.

PATIENTS AND METHODS

In this pilot study women with UFs, referred to our Outpatient Unit between September 2019 and May 2020, were enrolled. Following the explanation of the study aim, all women gave their oral consent to participate in this clinical trial. The study was conducted according to the Ethical principles of the Helsinki Declaration and the national laws. Inclusion criteria were: 18 years of age, or older; at least one UF ≥ 2 cm³

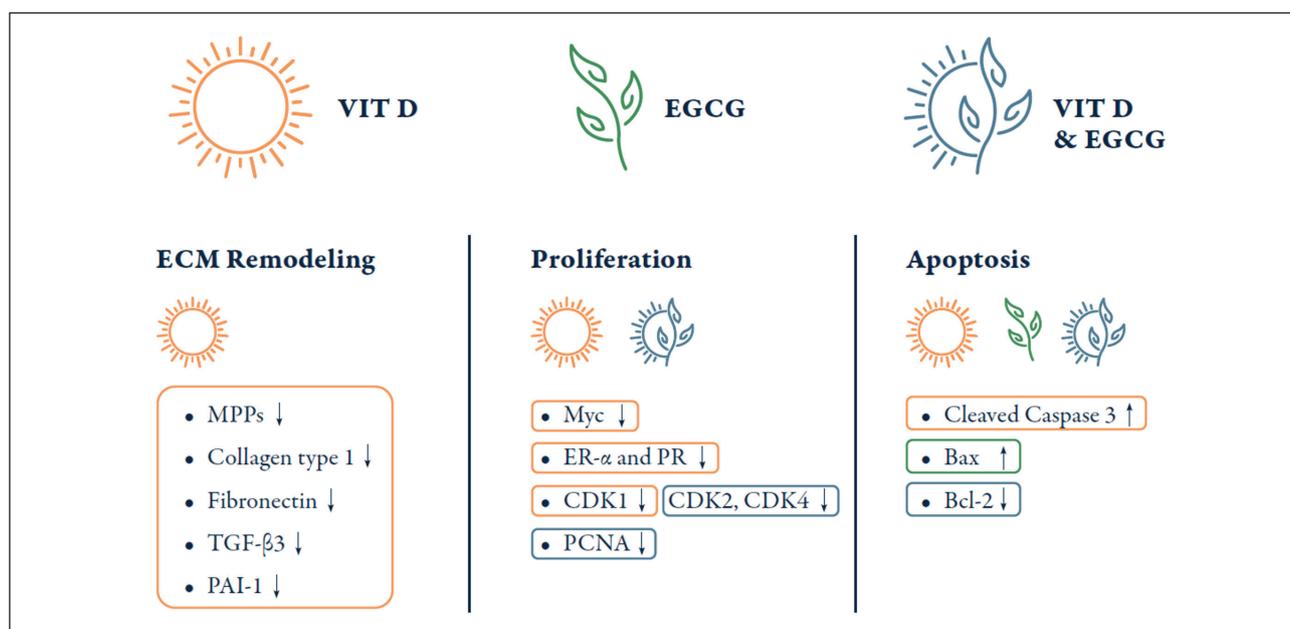


Figure 1. The figure summarizes all the molecular mechanisms in which Vitamin D (yellow boxes), EGCG (green box) or both (blue boxes) act for counteracting uterine fibroids growth. *Abbreviations:* Vit D: vitamin D; EGCG: epigallocatechin gallate; ECM: extracellular matrix; MPPs: Matrix metalloproteinases; TGF-β3: transforming growth factor-β3; PAI-1: Plasminogen activator inhibitor-1; ER-α: estrogen receptor-α; PR: progesterone receptor; CDK: Cyclin dependent kinase; PCNA: Proliferating cell nuclear antigen; Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2.

(intramural, subserosal and/or submucosal). Pregnant women or seeking pregnancy within the following four months, currently breastfeeding, with elevated liver enzymes, medical co-morbidities, undergoing treatment with hormones (estrogen, progestin, oral contraceptives), gonadotropin-releasing hormone analogs, corticosteroids, or food supplements with possible hormonal effects were excluded from the study. A total of 43 women were enrolled and divided into two groups: one study group (22 patients) treated with a daily dosage of two tablets of EGCG, vitamin D and vitamin B6 (Delphys®, Farmares S.r.l., Rome, Italy), for 4 months; one control group (21 patients) received no treatment. Patients with anemia (hemoglobin <11.0 g/dL) started or continued the standard oral iron supplementation. Also, women with high blood pressure maintained their anti-hypertensive medication. The primary outcome was the reduction of size and number of UFs, analyzed by transvaginal and/or transabdominal ultrasonography with Voluson™ E8 ultrasound (GE Healthcare). The secondary outcomes were the reduction of blood loss indicated as heavy, medium, and normal, through a self-administered bleeding assessment. Furthermore, fatigue and the feeling of pressure in the pelvic area were evaluated. Anemia and hypertension (systolic blood pressure, SBP, and diastolic blood pressure, DBP) were monitored throughout the study. A complete medical history was gathered from all women and a careful physical examination and ultrasound instrumental evaluation were performed at baseline (T0) and after 4 months (T1). All data were treated and analyzed anonymously.

STATISTICAL ANALYSIS

Unpaired Student's *t*-test was used to compare and evaluate the statistical significance of treatment effect between groups (2018 GraphPad Software, La Jolla, CA, USA). Data are expressed as mean ± standard deviation (SD). Comparisons for repeated measures were assessed for intragroup analysis by one-

way ANOVA: values are indicated as mean ± SD. Pearson's chi-square test was used for the statistical analysis of the distribution (%). A *p*-value ≤ 0.05 is considered statistically significant.

RESULTS

In this clinical trial, we enrolled 43 women with UFs attending our Outpatient Unit between September 2019 and July 2020. Mean age of all patients was 37.60 ± 5.77 years old. The clinical characteristics of patients by group at baseline are illustrated in Table 1.

At baseline, the two groups were comparable for all parameters and no dropouts were recorded throughout the study. In the treated group, 34 UFs (14 intramural, 6 subserosal and 14 submucosal) were reported, while 27 in the control group (12 intramural, 3 subserosal and 12 submucosal). The number of UFs did not change in treated women, whereas 1 UF developed in the control group after 4 months of observation. In the treated group a significant reduction of UF size was observed (from 10.73 ± 5.52 cm³ at baseline to 7.98 ± 4.00 cm³ (-35%) after 4 months of treatment; *p* < 0.0001), while the size of UFs remained unchanged in the control group (from 10.21 ± 5.83 cm³ at baseline to 10.62 ± 6.28 cm³ (+4%) at the end of the study; *p*= 0.8076) (Figure 2).

The self-administered bleeding assessment revealed that treatment with EGCG, plus vitamin D and vitamin B6 reduced the bleeding in all the patients that reported heavy blood loss. Normal bleeding was restored in 82% of patients after 4 months of treatment (Figure 3).

On the other hand, bleeding remained unchanged in the control group at the end of the study, with respect to baseline (19% normal bleeding, 43% medium bleeding and 38% heavy bleeding) (Figure 2). At T1 in the treated group, fatigue was reported in only 13% of women, while 52% of patients in the control group reported fatigue either at the beginning and at the end of the study (Figure 4).

Table 1. Clinical characteristics of patients by group at baseline.

	Control Mean ± SD	Treated Mean ± SD	<i>p</i> -value
Number patients	21	22	
Age	37.33 ± 6.71	37.86 ± 4.86	0.7674
Height (cm)	166.81 ± 4.21	164.45 ± 6.57	0.1717
Weight (kg)	63.14 ± 5.61	61.50 ± 7.68	0.4294
BMI (kg/m ²)	22.68 ± 1.70	22.74 ± 2.62	0.9271
N° UFs	27	34	
Mean n° UFs	1.29 ± 0.64	1.55 ± 0.74	0.2268
Volume UFs (cm ³)	10.21 ± 5.83	10.73 ± 5.52	0.7212

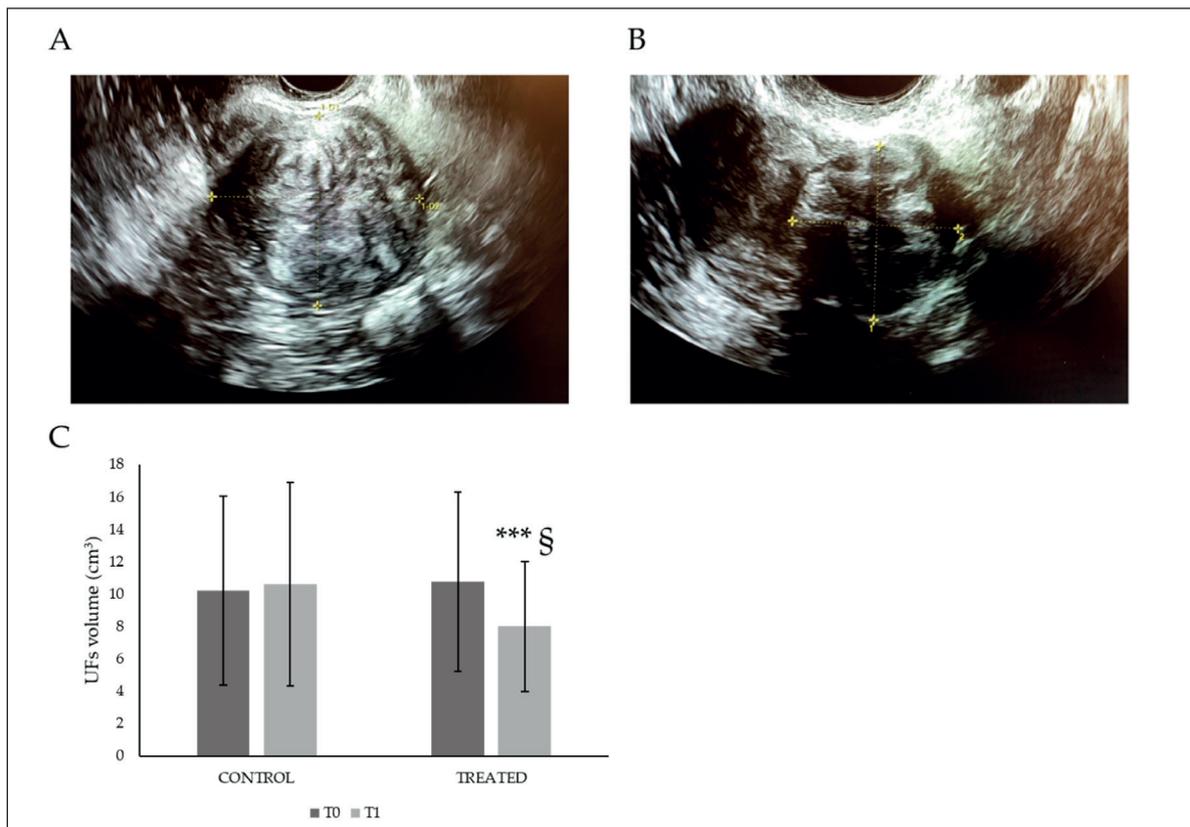


Figure 2. Transvaginal ultrasonography performed with Voluson™ E8 ultrasound (GE Healthcare) of a single patient before (A) and after the treatment (B). Figure 1A reports the intramural uterine fibroid of a patient at the baseline (T0), with the following dimension: diameter 1 (D1): 5.7 cm; diameter 2 (D2): 6.2 cm. Figure 1B reports the intramural uterine fibroid of the same patients at the end of the treatment (T1), with the following dimension: D1: 3.6 cm; D2: 2.89 cm. (C) UFs volume (cm³) at baseline (T0) and after the 4-month study period (T1). Treated group (vitamin D + EGCG + vitamin B6 for 4 months). Control group (no treatment for 4 months). Data are expressed as mean ± standard deviation (SD). Comparisons for repeated measures were assessed for intragroup analysis by one-way ANOVA: values are indicated as mean ± SD. Pearson’s chi-square test was used for the statistical analysis of the distribution (%). A *p*-value ≤ 0.05 is considered statistically significant. *Significance T0 vs. T1. §Significance between control vs. treated group at T1.

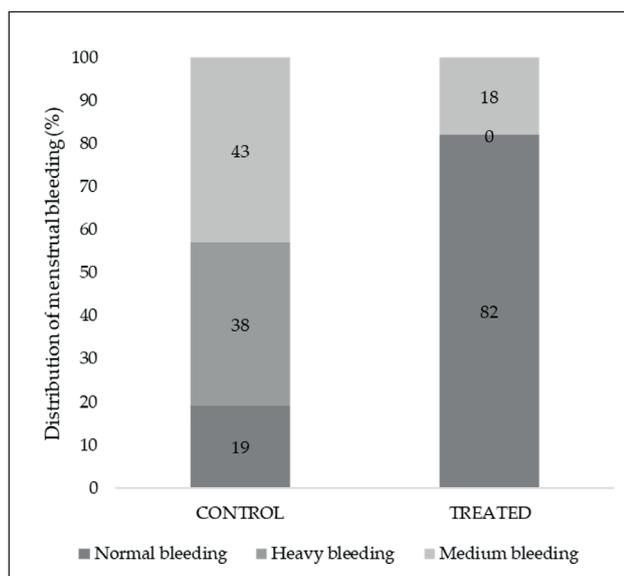


Figure 3. Distribution of bleeding assessment reported by the patients. The distribution (%) of the normal, medium and heavy bleeding was statistical different between Treated and Control group. Statistical analysis was performed using the Pearson’s chi-square test. Significance was *p*<0.001.

At study entry, 21 women (11 in the treated group and 10 in the control group) reported pelvic pain. After four months, all the patients in the control group affected by pelvic pain at the baseline continued to report it, while in the treated group only one patient experienced the symptom (Figure 4). Anemia was present in almost 50% of patients at baseline and ameliorated only in the treated women (4.5% had anemia at the end of the study). The mean hemoglobin (Hb) levels significantly improved after treatment with EGCG, vitamin D and vitamin B6, increasing from 11.06 ± 0.70 g/dL at baseline to 11.43 ± 0.48 g/dL at T1 (*p* < 0.05); in the control group Hb levels decreased from 11.06 ± 0.91 g/dL at T0 to 11.00 ± 0.83 g/dL at T1 (*p* < 0.0001) (Table 2). The values of blood pressure (SBP and DBP) of patients in both groups at T0 and T1 are illustrated in Table 2, but no significant changes were observed.

DISCUSSION

In this study, the combination of EGCG with vitamin D and vitamin B6 proved to be effective in

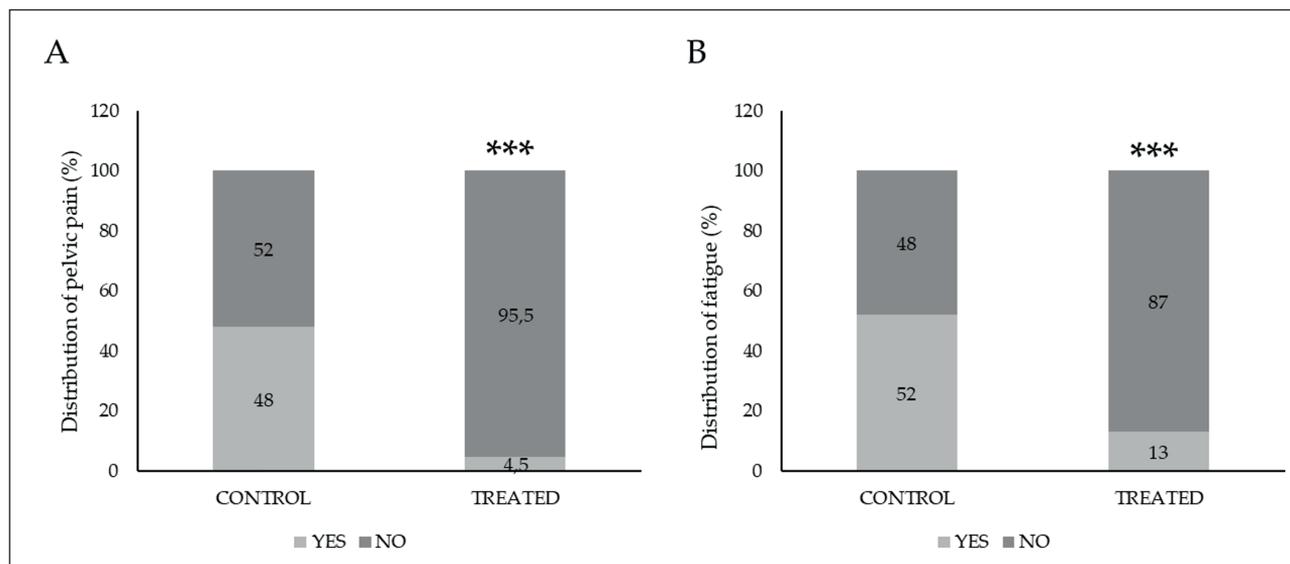


Figure 4. Distribution of pelvic pain (A) or fatigue (B) perceived by the patients. The distribution (%) of pelvic pain (A) and fatigue (B) was statistically different between Treated and Control. Statistical analysis was performed using the Pearson’s chi-square test. Significance was $p < 0.001$.

reducing the volume of UFs, and the associated pelvic pain and fatigue. Moreover, the treatment improved menstrual bleeding and anemia status. Notably, we confirmed our previous results, with a reduction of 35% in the UF volume size after treatment with EGCG, vitamin D and vitamin B6 for four months. Instead, the control group received no treatment (following the “wait and see” approach) and experienced a 4% increase in the UF volume size. Furthermore, the menstrual bleeding was completely reduced or normalized, and no women reported heavy blood loss following the treatment. Anemia was present in almost 50% of patients at baseline and ameliorated only in the treated women (present only in the 4.5% of women at the end of treatment). This result may be due to the lower blood loss after the use of EGCG combined with vitamin D and vitamin B6, thus decreasing metrorrhagia and consequently anemia. Even under iron treatment, the anemia status of women in the

control group failed to improve due to the heavy menstrual bleeding. As a result, women with anemia in the control group continued the iron therapy throughout the study, while women in the treated group were able to suspend iron supplementation, thanks to the effect of EGCG, vitamin D and vitamin B6. This is an outstanding result as iron supplementation can cause bothersome side effects that may lead to diminished compliance. These include headache, muscle pain, chills, dizziness, fainting, fast heartbeat, fever with increased sweating, flushing, metallic taste, nausea or vomiting, etc. For this reason, a safe and effective medical treatment is essential to maximize adherence when choosing various therapeutic options. Many women with UFs present with heavy menstrual bleeding, with a significant physical, social and emotional impact on the quality of their life¹². However, the mechanisms linking heavy menstrual bleeding and UFs remain only partially understood.

Table II. Incidence of number, type and volume of UFs and correlated symptoms, at baseline and after the 4-month study period. Parameters analysis pre and post-treatment in Control and Treated groups. Treated group (vitamin D + EGCG + vitamin B6 for 4 months); Control group (no treatment for 4 months). A p -value < 0.05 was considered statistically significant using one-way ANOVA. *Significance T1 vs. T0 in treated group.

	Control		Treated		p-value
	T0	T1	T0	T1	
n° UFs intramural	12	12	14	14	-
n° UFs subserosal	3	3	6	6	-
n° UFs submucosal	12	13	14	14	-
Mean n° UFs	1.29 ± 0.64	1.33 ± 0.80	1.55 ± 0.74	1.55 ± 0.74	NS
SBP (mm Hg)	126.67 ± 11.22	126.43 ± 10.86	130.68 ± 11.98	130.00 ± 11.44	NS
DBP (mm Hg)	82.86 ± 6.81	82.38 ± 6.82	80.91 ± 7.18	80.45 ± 7.06	NS
Anemia – Hb (g/dL)	11.06 ± 0.91	11.00 ± 0.83	11.06 ± 0.70	11.43 ± 0.48*	*0.0404

Concerning hypertension, no changes in blood pressure were observed in either group and the hypertensive therapy was unaffected by treatment with EGCG, vitamin D and vitamin B6. Hypertension is the main risk factor for cardiovascular diseases¹³, and it is now well established that hypertension is related to the onset and growth of UFs¹⁴⁻¹⁶. Therefore, blood pressure monitoring should be part of routine care, especially for patients with UFs¹⁴. All risk factors for the onset of UFs must be carefully considered to prevent health complications. It is also interesting to note that UFs are associated with other inflammatory diseases, such as obesity, insulin resistance, and polycystic ovary syndrome (PCOS)¹⁷. In particular, UFs are very frequent in PCOS. The reason may be ascribable to the high luteinizing hormone levels and/or of unopposed estrogens, as well as to deregulation of the insulin-growth factor and growth hormone axis (commonly present in women with PCOS) that may influence the development of UFs¹⁷.

Natural active compounds may be beneficial for patients with UFs, especially those who want to preserve their future fertility. EGCG, vitamin D and vitamin B6 have been used as natural alternatives to UF treatments or to the “wait and see” approach^{1,2}. We have previously demonstrated that 4-month supplementation with EGCG, vitamin D and vitamin B6 reduces UF size and severity of symptoms, improving the quality of life of women with UFs². In this study, we confirmed our previous results and extended the investigation to three common and critical signs and symptoms that can exacerbate women’s health status, such as heavy blood loss, anemia and hypertension. We observed that the oral supplementation of EGCG, vitamin D and vitamin B6 has a positive impact on the general health status of women presenting with UFs, preventing a possible increase of their size.

CONCLUSIONS

The oral supplementation with EGCG, vitamin D and vitamin B6 may represent a suitable alternative to the “wait and see” approach, and also an adjuvant treatment that could be administered along with other pharmacological therapies, even before surgery to reduce the occurrence of possible complications. In conclusion, the supplementation with EGCG, vitamin D and vitamin B6 proved effective in managing UFs and the related symptoms, thus opening new therapeutic scenarios and medical options for women with UFs. We suggest validating these preliminary results with further randomized controlled trials, with a larger cohort of patients and different ethnic groups.

AUTHOR CONTRIBUTIONS:

Conceptualization, supervision and project administration, G.P.; data curation, writing—review and editing and investigation, P.A. All authors have read and agreed to the published version of the manuscript.

INFORMED CONSENT:

All women gave their oral consent to participate in this clinical trial. The study was conducted according to the Ethical principles of the Helsinki Declaration and the national laws.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest

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