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Systematic Review

A Systematic Review of Clinical Studies Investigates the Role of Serum Prolactin Levels in Women with Uterine Fibroids

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Abstract

Background: Uterine fibroids are high-prevalence benign tumors originating from the smooth muscles of the uterus, noticed in approximately 70% of women of reproductive age. Prolactin is a hormone secreted from the anterior pituitary gland involved in various physiological functions, including lactogenesis and the regulation of reproductive functions. High serum prolactin levels may cause infertility and other reproductive disorders in women. Increasing evidence has focused on the critical pathogenetic role of prolactin in uterine fibroid diseases, as well as premalignant and malignant conditions. Subsequently, the objective of this current systematic review is to determine the status of serum prolactin in women of uterine fibroids.

Methods: In this systematic review, we comprehensively searched several databases, including PubMed, Google Scholar, and the Cochrane Library. A critical assessment has been conducted on articles related to the epidemiological and biological correlation of prolactin with uterine fibroids. Both observational and clinical trial studies were included from inception to March 15, 2024. The quality of the eligible studies was independently evaluated, retrieved, and assessed.

Results: Out of 275 studies, a total of 5 studies (1 Clinical Trial, 3 Case-Control, and 1 Cross-sectional) were included in this review. The 3 case-control studies concluded that serum prolactin levels are significantly higher in leiomyoma patients compared to healthy controls. Serum total protein and prolactin can be used as adjuvant biomarkers to confirm the diagnosis of uterine fibroids. Similarly, one cross-sectional study found a highly significant difference between prolactin levels in leiomyomas and in patients' myometrium. Additionally, a clinical trial revealed that patients' serum prolactin levels prior to surgery were significantly higher than post-surgery levels and higher than those in healthy, normal women. Furthermore, this clinical trial demonstrated a highly significant correlation between serum prolactin levels and their ratio with serum total protein. These results ensure and support the relationship between patients' serum prolactin and serum total protein levels with their fibroid numbers.

Conclusion: The evidence from available clinical studies suggests that uterine fibroids synthesize prolactin, and the ectopic production of prolactin from fibroid tissue results in a rise in serum prolactin levels, leading to infertility and other reproductive disorders that affect the quality of life. Hence, serum prolactin analysis should be taken into consideration in patients with uterine fibroids and infertility disorders. Furthermore diagnostic studies are required to validate the increased serum level of prolactin in women with uterine fibroids.

Keywords: Prolactin; Uterine Fibroid; Leiomyomas; Myomas; Infertility; Biomarkers

Abbreviations

ECM: Extracellular Matrix; UF: Uterine Fibroids; PRL: Prolactin; MAP: Mitogen-activated Protein; TNF: Tumor Necrosis Factor

Introduction

Uterine fibroids, or leiomyomas, are the most common benign pelvic tumors in females of reproductive age [1]. The incidence of uterine fibroids ranges from 5.4% to 77%, and they typically grow in the uterine smooth muscle of premenopausal women [2]. Uterine fibroids are very rare before menarche but tend to regress after menopause [3]. It is one of the most common pathologies of the female genital tract [4]. UF is characterized by an excess production of extracellular matrix (ECM) [5], which contains collagen, fibronectin, and proteoglycan [6]. Several clinical and epidemiological opinions suggest that genetic and chromosomal alterations play a significant role in the growth of uterine fibroids [7]. Growth factors are relatively small and stable, secreted or membrane-bound polypeptide ligands, and play a vital role in proliferation, differentiation, angiogenesis, survival, inflammation, and tissue repair or fibrosis [8]. Uterine fibroids are the foremost indication for hysterectomy in the United States [5].

The majority of leiomyomas are asymptomatic [9]. Symptomatic women typically suffer from menstrual disorders, heavy menstrual bleeding, anemia, pelvic pain [10], menorrhagia, dysmenorrhea/dyspareunia, pressure-associated symptoms, miscarriage, and subfertility [11]. Infertility can also be based on the position, size, number, and wide variety of leiomyomas [12]. Ultrasonography is the ideal preliminary imaging modality for fibroids; transvaginal ultrasonography is about 90% to 99% sensitive for identifying uterine fibroids, but it may miss subserosal or small fibroids. Surgery, including hysterectomy, myomectomy, uterine artery embolization, and magnetic resonance-guided focused ultrasound surgery [13], is the main treatment approach for leiomyomas, which imposes a significant burden on the economy as well as morbidity and mortality consequences [14]. In India, the incidence is high, and it inflicts a heavy burden on women's health and the healthcare system [15]. Therefore, the advancement and development of alternatives to surgery and preventative approaches come into prominence.

Prolactin (PRL) is a polypeptide hormone produced and secreted from specialized cells of the anterior pituitary gland [16]. It was first identified as a lactation hormone [17] and is known for supporting reproduction and lactation in mammalian females [18]. Prolactin is a protein hormone involved in various mammalian physiological functions, such as lactogenesis [19]. The human uterine fibroid is an extra-pituitary source of prolactin [20]. Prolactin has a dual function, acting as both a circulating hormone and a cytokine. This interpretation is based on the production of prolactin and its distinct regulation in extra-pituitary sites. Prolactin exhibits binding capacity with receptors on the cell membrane of the cytokine receptor superfamily, encouraging signaling pathways that promote cell progression and survival [21]. Hyperprolactinemia has several physiological and pathological causes. Regarding pathological causes, several neoplasms of differing origins can give rise to hyperprolactinemia through various mechanisms [17]. There is rising evidence that prolactin plays a role in different types of tumors in both reproductive and non-reproductive tissues through ectopic production or accumulation [21].

Rationale for the study

Studies have shown that fibroids can synthesize prolactin, leading to an increase in serum prolactin levels due to ectopic production of prolactin from uterine fibroids. The results of the study revealed that infertility might be caused by the presence of

prolactin-secreting fibroid tissues [22]. Additionally, another study demonstrated that the effects of prolactin on the proliferation of cultured human uterine leiomyoma-derived smooth muscle cells suggested that prolactin stimulates the proliferation of human leiomyoma cells via the mitogen-activated protein (MAP) kinase cascade [23]. Other studies have concluded that prolactin plays an important role in ovarian and endometrial tumorigenesis and may represent a risk factor for ovarian and endometrial cancers [24]. According to the review of existing literature, it is evident that no systematic review or meta-analysis has been conducted so far to investigate the status of serum prolactin levels in women of uterine fibroids. Hence, the current systematic review was designed to establish the status of serum prolactin levels in in women of uterine fibroids.

Methodology

A systematic review was carried out to identify studies evaluating the effects of vitamin D on uterine fibroids. The reporting procedures were performed according to the guidelines presented the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses).

Search strategy

Many database searches had been performed for this systematic review using keywords and Boolean expressions for keywords such as prolactin, uterine fibroids, uterine leiomyomas, hyperprolactinemia, and infertility. The search strings included “prolactin and uterine fibroids,” “prolactin and uterine leiomyomas,” “uterine leiomyomas or uterine fibroids and prolactin,” “hyperprolactinemia and uterine fibroids,” and “hyperprolactinemia and uterine leiomyomas.” These searches were conducted across databases including Google Scholar, PubMed, and the Cochrane Library.

The earliest data search was conducted on September 10, 2022. Subsequently, additional research was conducted on March 15, 2024, to gather more recent studies. The search encompassed literature from the earliest available records to March 15, 2024, with a specific focus on English-language publications. Additionally, manual searches were conducted to identify relevant and appropriate literature from the references of eligible articles.

The search plan follows the PICO tool standards as outlined below

- **Population-** Adult females.
- **Intervention-** Not applicable.
- **Comparison**- Individuals with uterine fibroids (cases) compared to those without fibroids (controls).
- **Outcome-** Hyperprolactinemia resulting from ectopic production by uterine fibroids.
- **Study design-** Clinical studies (Randomized controlled clinical trials and observational studies).
- We have excluded animal studies, duplicate studies, research involving adjuvant therapies that interfere with prolactin secretion, abstracts, posters, and literature not in English.

Study selection and assessment of quality

The study was independently reviewed by the impartial authors (AA, MK) according to the eligibility criteria for inclusion. The studies were initially assessed based on title and abstract, followed by a review of the full article for eligible studies. Any discrepancies were resolved through mutual discussion and agreement among the authors, as illustrated in Figure 1.

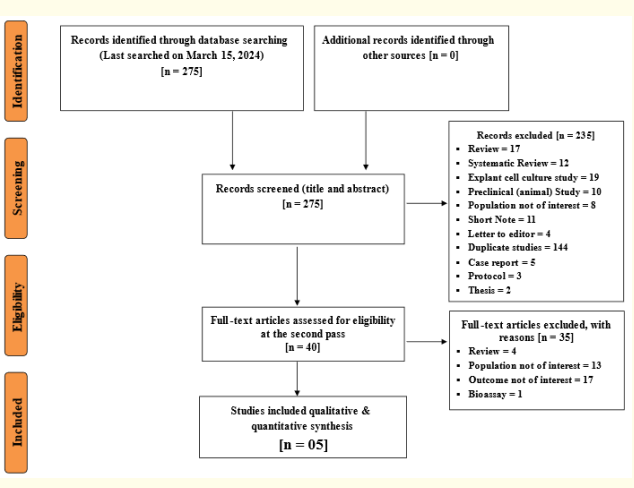

Figure 1: Flowchart for study selection.

Data extraction

The data were extracted and further summarized in tabular form for the eligible studies. The following information was extracted: authors’ details, years of the study, study design, type of study, number of samples, population, number of exposed cases, number of exposed controls, age of populations in years, study country, diagnosis of fibroids, and measurement of serum prolactin.

S. No.	Authors	Study Design	Sample Size	No. of Exposed in Cases	No. of Exposed in Controls	Age (Years)	Study Populations	Study Country	Diagnosis of Fibroids	Measurement of Serum Prolactin	Remark
1	ElSokary., *et al*. (2020)	Case-control study	100	50	50	29-51	Outpatient Clinic, Dept of Obs and Gynae at King Abdulaziz University Hospital.	Saudi (Jeddah)	Ultrasonography	Enzyme-Linked Immunosorbent Assay (ELISA) kit, (Cat No. 422619, Cortez, Diagnostics, California)	Higher levels of serum prolactin are a common clinical outcome in several gynecological disorders, including uterine leiomyomas. Increased levels of serum prolactin among patients with uterine leiomyomas are not linked to somatic mutations in the prolactin gene. However, genetic polymorphisms in prolactin may indirectly influence the etiology of the ailment.
2	Ahmed., *et al*. (2019)	Randomi-zed controlle-d clinical trial study	100	48	48	20-50	Outpatient Clinic, Dept of Obs and Gynae at AL-Hussien University Hospital	Iraq (Baghdad)	Transvaginal Ultrasound	Not specified	An important correlation was noted between serum prolactin and its ratio with serum total protein. This outcome ensures and supports the correlation between the patient's serum prolactin, serum total protein, and their fibroid number. This encourages us to suggest these variables as simple biochemical markers to assist in other clinical decisions and findings.
3	Baban, Rayab S. (2009)	Case-control study	62	32	30	28-53	Dept of Obs and Gynae at Al- Kharch Hospital	Iraq (Baghdad)	Ultrasonography	mini-VIDAS (ELFA technique (Enzyme-Linked Fluorescent Assay)	Serum prolactin and serum total protein can be treated as adjunct biomarkers to validate and confirm the diagnosis of uterine fibroids.
4	Abdulla., *et al*. (2008)	Cross-sectional study	53	53	-	Reproductive age	Dept of Obs and Gynae Dept. at Al-Khadimiy, Al-Noor, Al-Kharck, and Al-Saadoon Hospital.	Iraq (Baghdad)	Ultrasound	Prolactin Kit (Biomérieux)	A highly significant difference was found between the prolactin levels in leiomyomas and in the myometrium of the same patients.
5	Baban., *et al*. (2008)	Case-control study	93	53	40	30-49	College of Medicine Al- Nahraine University, Dept of Obs and Gynae.	Iraq (Baghdad)	Ultrasonography	Immunohistochemical staining method with monoclonal antibody (Biocare Detection Kit)	The increase in prolactin receptors in leiomyoma is expected, given that the underlying host myometrium is abnormal.

Table 1: Summary of data extraction included studies.

Quality of the studies

The quality of the studies was assessed using the Newcastle-Ottawa Scale for observational studies and the Jadad scale for clinical trials. The Newcastle-Ottawa Scale primarily evaluates studies based on three main criteria: selection, comparability, and exposure in case-control studies or cross-sectional studies; then selection, comparability, and outcomes are assessed. Meanwhile, the Jadad scale primarily evaluates clinical trials based on three basic elements in the studies: randomization, blinding, and accountability of the subjects.

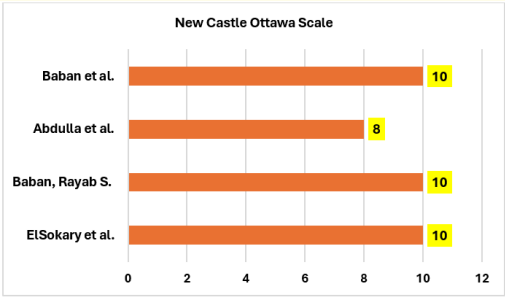

Figure 2: Quality Assessment Scores for Observational Studies (Newcastle-Ottawa Scale).

S. No.	Authors	Study design	Selection (Max 5 stars)	Comparability (Max 2 stars)	Outcome (Max 3 stars)	Total study quality score
1	ElSokary., *et al.*	Case-Control	5	2	3	10
2	Baban, Rayab S.	Case-Control	5	2	3	10
3	Abdulla., *et al.*	Cross-Sectional	5	0	3	8
4	Baban., *et al.*	Case-Control	5	2	3	10

Table 2: Quality Assessment Scores for Observational Studies (Newcastle-Ottawa Scale).

S. No.	Authors	Study design	Randomization (2 points)	Blinding (2 points)	An account of all patients (1 point)	Total study quality score
1	Ahmed., *et al.*	Randomized Controlled Clinical Trial	2	2	1	5

Table 3: Quality assessment score for clinical trials using Jadad score.

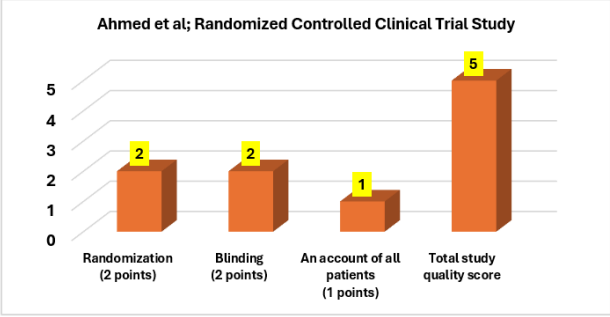

Figure 3: Jadad Score of the Clinical Trial Included.

Results

Search results

A total of 275 potentially relevant articles were searched. After screening and reviewing the titles and abstracts, considering the inclusion and exclusion criteria, 235 articles were excluded. The full texts of the remaining 40 articles were reviewed, of which 35 were excluded because they were review, preclinical and explant cell culture studies or did not match the population and outcomes of interest. Studies with sufficient data for tables of sensitivity and specificity were chosen. Finally, 5 studies were included in this systematic review. All 5 studies were clinical studies (1 Clinical Trial, 3 Case-Control, and 1 Cross-sectional). A flowchart illustrating the study selection process was drawn as suggested by

the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA statement) in Figure-1.

Evidence from the randomized controlled clinical trial study

Only 1 randomized controlled clinical trial was included among the 5 studies in this systematic review. A recent randomized controlled clinical trial provided evidence that the complete serum protein and albumin levels in patients with higher serum TNF levels were significantly lower ($p < 0.05$) than the subsequent values in patients with untraceable serum TNF levels. The research established that prolactin levels decrease after surgical treatment in patients with uterine fibroids. Additionally, a slight increase in serum total protein was found after surgery in patients with uterine fibroids [9].

Other evidence from the randomized controlled clinical trial conducted at the Obstetrics and Gynecology department in Al-Hussein University and Hospital, Baghdad, Iraq in 2019 showed that in a sample size of 100 subjects, the patients' serum prolactin levels prior to surgical procedure (n = 48) were 169.64 ± 133.11 ng/ml, which were greater than post-surgical procedure levels (19.69 ± 9.54 ng/ml) and greater than those in healthy normal women (18.6 ± 2.3 ng/ml) (n = 48). A reduction was noticed in patients' total serum protein (5.56 ± 9.66) prior to surgery compared to total serum protein in the controls (18.93 ± 5.16 ng/ml) and post-surgery (19.69 ± 9.54 ng/ml). The mean ± SE size of fibroids collected from various uterine fibroid patients was 28.52 ± 27.64 cm^3. Only 6 out of 48 (12.5%) uterine fibroid patients had normal serum prolactin levels, despite their fibroid sizes varying from 1-100 cm^3. The remaining 42 patients (87.5%) all had elevated levels of serum prolactin and were therefore considered nonpituitary hyperprolactinemic. The serum prolactin levels decreased after surgical therapy in patients with uterine fibroids compared to patients' serum prolactin levels before surgery [9]. It was concluded that fibroid tissue may secrete prolactin due to ectopic production.

Evidence from the observational studies

Out of the 5 observational studies, 3 were case-control studies that met our eligibility criteria, among which a recent case-control study indicated that uterine leiomyoma patients showed a significant elevation of prolactin levels in serum samples compared to controls ($p \leq 0.01$) [25]. Out of 50 uterine fibroid patients, 44 (88%) were indicated to have increased prolactin levels, with a mean value of 172.1 ± 139.2 (Pmol/l). The prolactin levels of 50 control subjects showed a mean value of 15.18 ± 7.52 (Pmol/l). Statistical analysis revealed an extremely significant difference ($P < 0.01$; OR (95% CI) = 156.92 [117.79–196.04]) between the groups, suggesting a significant elevation of serum prolactin levels in uterine fibroid patients [25].

Other case-control studies provided evidence that the patient's serum prolactin level prior to surgery (n = 32) was 169.64 ± 133.11 ng/ml, which was higher than post-surgery levels (19.69 ± 9.54 ng/ml), and greater than in healthy normal subjects (18.6 ± 2.3 ng/ml) (n = 30). There was a decline in the patient's total serum protein (5.56 ± 9.66) prior to surgery compared to total serum protein in the controls (18.93 ± 5.16 ng/ml) and post-surgery (19.69 ± 9.54 ng/ml). Prolactin receptors were positively identified in all cases, including patient and control tissues, and prolactin receptors increased in uterine leiomyoma patients compared to control patients [26].

Out of 5 observational studies, there was one cross-sectional study that showed the mean serum prolactin levels for patients were 143.1 ± 106.9 ng/ml, for leiomyomas were 18.2 ± 14.0 ng/ml, and for patients' myometrium were 8.0 ± 3.3 ng/ml respectively. A significant difference was observed between the serum prolactin levels of leiomyoma patients and myometrium patients [27].

One more study, a bioassay, showed that out of 103 participants, 45 (43.69%) were noticed to have primary infertility, while the remaining 58 (56.31%) had secondary infertility. The serum prolactin level was observed to be elevated approximately 9-fold in participants with primary infertility and 8-fold in those with secondary infertility prior to surgery compared to their levels post-surgery. Prolactin was found to be highly significant with a P-value < 0.001 in both groups [22].

Discussion

Uterine fibroids (UFs, Leiomyoma) are the most common benign tumors that arises in the myometrium of women of reproductive age. They constitute one of the most common pathologies of the female genital tract and occur in 5–70% of all women [28].

Increasing evidence has focused on prolactin and its significant pathogenetic role in premalignant, malignant, and benign diseases of the uterus [29].

There are 3 case-control studies suggesting that patients with uterine leiomyoma show a significant elevation of serum prolactin levels ($P \leq 0.01$), and no somatic coding province mutations in the prolactin gene were detected. Nevertheless, four germline variants (c.570G>A, c.205-102T>A, c.312+177T>C, and c.269C>T) were identified. This study is the first to confirm that serum prolactin level elevation among uterine leiomyoma patients is not connected to somatic mutations in the prolactin gene. However, prolactin genetic polymorphisms may indirectly contribute to the disease etiology [25].

Another study provided evidence of ectopic production of prolactin by colorectal adenocarcinoma. They assessed circulating prolactin and carcinoembryonic antigen using immunoradiometric assay and radioimmunoassay kits, respectively, in tumor-draining venous and preoperative blood samples of colorectal carcinoma patients. The study concluded that these multiple approaches confirmed that prolactin is produced by colorectal carcinoma cells. Examining its prognostic value and correlation with disease activity may offer new insights into treatment for patients with colorectal carcinoma [26].

Other cross-sectional studies have reported that the mean serum prolactin among leiomyoma patients is (143.1 ± 106.9) ng/ml, compared with the normal serum prolactin level range in women of reproductive age, which is typically (5-35 ng/ml). The average serum prolactin level of leiomyoma was higher than that of myometrium in the same group (18.2 ± 14.0 ng/ml and 8.0 ± 3.3 ng/ml respectively), and it was observed that the mean leiomyoma prolactin was significantly higher than the mean myometrium prolactin ($P < 0.0001$). Prolactin secretion from leiomyoma is significantly higher than prolactin secretion from myometrium in the same participants, and they observed that leiomyoma prolactin secretion enhanced over time, whereas myometrial prolactin secretion did not increase with time. The conclusion of this study aligns with the mean outcomes of this analysis, in which the mean prolactin level in leiomyoma was significantly higher than in myometrial tissue [27].

Another bioassay evidenced that the concentration of serum prolactin, a trophic hormone secreted by the pituitary gland, has been indicated to be increased in specific groups of patients with cancer. It was recognized in 0-20% of colon tumors by Immunohistochemistry and in the plasma in 6-53% of the patients. The research proved that the removal of cervical carcinoma by surgical procedure resulted in the regulation and normalization of serum prolactin concentration. This study confirms the elevated level of serum prolactin due to ectopic production and it can be reduced to its normal level after surgery.

Prolactin has dual functions, serving as both a circulating hormone and a cytokine. The justification of this function is based on the production and regulation of prolactin in extra-pituitary sites, as well as its binding capability to membrane receptors of the cytokine receptor superfamily. This binding leads to the activation of signaling pathways that promote cell progression and survival. Increasing evidence regarding prolactin indicates that it plays a significant role in various types of cancer in both reproductive and non-reproductive tissues through ectopic production and accumulation. Prolactin, considered an effective contributor to tumorigenesis, should encourage and promote the advancement and development of novel treatments targeted at reducing tumor growth by controlling the production of prolactin or by preventing its receptors [30]. In this study, hyperprolactinemia is attributed to the ectopic production and secretion of prolactin from the patient's fibroid tissues, and this prolactin exhibits distinct profiles and bioactivity. Hence, the intervention theory causing infertility [22].

The strength of this study is its inclusion of all types of recent literature with various study designs such as randomized controlled clinical trials, cross-sectional studies, and case-control studies conducted in populations from Iraq and Saudi Arabia. A total of 5 clinical studies have been included in our systematic review. However, a limitation in the majority of the studies is related to population and sample size. For instance, in randomized clinical trials, the sample size should be increased, and clinical trials with longer follow-up periods need to be considered for better precision and clarity.

Conclusion

Prolactin plays a significant role in various physiological functions, including lactogenesis, hormone circulation, and

cytokine regulation. Evidence from available literature suggests that higher levels of serum prolactin are commonly understood in various gynecological disorders and pathologies, including uterine fibroids. Prolactin genetic polymorphisms may indirectly contribute to the etiology of fibroid diseases. Serum total protein and prolactin can be used as adjuvant biochemical indicators to support the diagnosis of uterine fibroids. Furthermore, evidence suggests that a rise in serum prolactin levels is expected due to ectopic production from fibroid tissues. Additionally, the presence of fibroids that secrete prolactin, leading to hyperprolactemia, may be the cause of infertility. Hence, serum prolactin analysis should be taken into consideration in patients with uterine fibroids and infertility disorders.

Conflict of Interest

The authors declare no conflict of interest.

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Bibliography

1. Ahmad Azaz., *et al*. "Low Serum Vitamin D3 Levels among Premenopausal Women with Uterine Fibroids: A Cross-Sectional Study". *International Journal of Nutrition, Pharmacology, Neurological Diseases* 14.1 (2024): 103-110.

2. Hajhashemi Maryam., *et al*. "The effect of vitamin D supplementation on the size of uterine leiomyoma in women with vitamin D deficiency". *Caspian Journal of Internal Medicine* 10.2 (2019): 125.

3. Ahmad Azaz., *et al*. "Diagnosis and management of uterine fibroids: current trends and future strategies". *Journal of Basic and Clinical Physiology and Pharmacology* 34.3 (2023): 291-310.

4. Ciebiera Michał., *et al*. "Vitamin D and uterine fibroids—review of the literature and novel concepts". *International Journal of Molecular Sciences* 19.7 (2018): 2051.

5. Baird Donna Day., *et al*. "Vitamin D and the risk of uterine fibroids". *Epidemiology* 24.3 (2013): 447-453.

6. Khan Aamir T., *et al*. "Uterine fibroids: current perspectives". *International Journal of Women's Health* (2014): 95-114.

7. Jukic Anne Marie Z., *et al*. "Lower plasma 25-hydroxyvitamin D is associated with irregular menstrual cycles in a cross-sectional study". *Reproductive Biology and Endocrinology* 13 (2015): 1-6.

8. Islam Md Soriful., *et al*. "Growth factors and pathogenesis". *Best Practice and Research Clinical Obstetrics and Gynaecology* 34 (2016): 25-36.

9. Ahmed Abd Al Azim Mohamed., *et al*. "Serum protein and prolactin in evaluation of uterine fibroids". *The Egyptian Journal of Hospital Medicine* 76.3 (2019): 3653-3658.

10. Ciavattini Andrea., *et al*. "Hypovitaminosis D and "small burden" uterine fibroids: Opportunity for a vitamin D supplementation". *Medicine* 95.52 (2016): e5698.

11. Sabry Mohamed., *et al*. "Serum vitamin D3 level inversely correlates with uterine fibroid volume in different ethnic groups: a cross-sectional observational study". *International journal of women's health* (2013): 93-100.

12. Protic Olga., *et al*. "Possible involvement of inflammatory/reparative processes in the development of uterine fibroids". *Cell and Tissue Research* 364.2 (2016): 415-427.

13. De La Cruz Maria Syl D and Edward M Buchanan. "Uterine fibroids: diagnosis and treatment". *American Family Physician* 95.2 (2017): 100-107.

14. Oskovi Kaplan Zeynep Aslı., *et al*. "25-Hydroxy vitamin D levels in premenopausal Turkish women with uterine leiomyoma". *Gynecological Endocrinology* 34.3 (2018): 261-264.

15. Singh Vinita., *et al*. "Vitamin D 3 level in women with uterine fibroid: an observational study in eastern Indian population". *The Journal of Obstetrics and Gynecology of India* 69 (2019): 161-165.

16. Freeman Marc E., *et al*. "Prolactin: structure, function, and regulation of secretion". *Physiological Reviews* (2000).

17. Sendur Suleyman Nahit., *et al*. "Hyperprolactinaemia associated with giant uterine myoma, description of a case and review of literature". *Journal of Obstetrics and Gynaecology* 39.7 (2019): 1034-1036.

18. Upreti Rita., *et al*. "Uterine fibroid causing hyperprolactinemia and paradoxical prolactin rise with dopamine agonist: case report and systematic review". *SN Comprehensive Clinical Medicine* 2.4 (2020): 464-467.

19. Levy Gary., *et al*. "Biomarkers in uterine leiomyoma". *Fertility and Sterility* 99.4 (2013): 1146-1152.

20. Chapitis Jane., *et al*. "Physicochemical characterization and functional activity of fibroid prolactin produced in cell culture". *American Journal of Obstetrics and Gynecology* 158.4 (1988): 846-853.

21. Ben-Jonathan Nira., *et al*. "Prolactin as an autocrine/paracrine growth factor in human cancer". *Trends in Endocrinology and Metabolism* 13.6 (2002): 245-250.

22. Abdulla Maysaloun M., *et al*. "Role of Ectopic Prolactin on Thyroid Hormones Level in a Sample of Iraqi Infertile Women with Uterine Fibroids". *Iraqi Journal of Medical Sciences* 10.4 (2012): 334-338.

23. Nohara Ataru., *et al*. "Prolactin stimulates mitogen-activated protein kinase in human leiomyoma cells". *Biochemical and Biophysical Research Communications* 238.2 (1997): 473-477.

24. Levina Vera V., *et al*. "Biological significance of prolactin in gynecologic cancers". *Cancer Research* 69.12 (2009): 5226-5233.

25. ElSokary Hanan Abdelhalim., *et al*. "Assessing the role of serum prolactin levels and coding region somatic mutations of the prolactin gene in Saudi uterine leiomyoma patients". *Archives of Medical Sciences* 10 (2020).

26. Baban Rayah S., *et al*. "Prolactin receptors in uterine leiomyomas". *Saudi Medical Journal* 29.11 (2008): 1593-1596.

27. Abdulla Maysaloun Muhammad. "A study of prolactin hormone and other factors in relation to uterine leiomyomas among a group of Iraqi Women". *Iraqi Journal of Community Medicine* 21.2 (2008): 104-108.

28. Ali Mohamed., *et al*. "Vitamin D and uterine fibroids: preclinical evidence is in; time for an overdue clinical study!". *Fertility and Sterility* 113.1 (2020): 89-90.

29. Auriemma Renata S.., *et al*. "The interplay between prolactin and reproductive system: focus on uterine pathophysiology". *Frontiers in Endocrinology* 11 (2020): 594370.

30. Baban Rayah S. "Serum protein and prolactin as diagnostic markers". *Saudi Medical Journal* 30.11 (2009): 1411-1415.