Assessment IL-6, TGF-1, CRP, and Vit-D in Women With Uterine Fibroid

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KEYWORDS

Uterine Fibroids, Age, IL-6, TGF-1, CRP, and Vit-D

ABSTRACT

Background: Uterine fibroids, also known as UFs, are the most common type of tumor that occurs in the female reproductive system. They affect a significant majority, around 70-80%, of women during their lifetime. Various factors that increase the incidence of Uterine Fibroids (UFs) have been found, including as vitamin D deficiency, inflammation, DNA repair deficit, and exposure to endocrine-disrupting chemicals (EDCs) in the environment. **Objective:** The purpose of this study to evaluated the level of IL-6, TGF-1, CRP, and Vit-D in women with uterine fibroid and study the relation these parameter with different ages. **Materials and methods:** 90 blood samples were collected and divided into (50) blood samples from women with uterine fibroids who received a diagnosis from a qualified medical specialist, and (40) blood samples from healthy women, whose ages ranged from (20-50). Assessment level of **IL-6, TGF-1, CRP, and Vit-D** have been done by using ELISA technique. **Result:** The results showed that there was no significant difference at the probability level ($P>0.05$) in the level of IL-6 in women with uterine fibroids, as it reached (44.9±4.4 pg/ml) when compared with healthy women (42.5±4.1 pg/ml). The results of this study showed a significant increase in the levels of both β-TGF and CRP in women with uterine fibroids at the probability level ($P<0.05$), reaching (2.870±0.760ng/ml, 62.9±6.4 pg/ml), respectively, when compared with Healthy women: As for Vit.D3, it showed a significant decrease in women with uterine fibroids at the probability level ($P\leq0.05$), as it reached (5.92±1.87 ng/ml). **Conclusion:** This result concluded no differences in the level of IL-6 in women with uterine fibroids, while increase in the levels of both β-TGF and CRP . Furthermore, decrease level of Vit.D3.

1. Introduction

Uterine fibroids, also known as leiomyoma, are the most common tumors found in the pelvic region of women who are of reproductive age. Fibroids have a prevalence of 70-80% among women during their lifetime and often necessitate hysterectomies in premenopausal women[1]. The range from 1 to 3, inclusive, with a step size of 1. While these lesions are often considered harmless, they are frequently linked to substantial morbidity. Approximately 30% of affected patients have symptoms, which commonly include: Abnormal uterine bleeding, pelvic discomfort, gastrointestinal troubles, voiding problems, bulk symptoms, obstetric complications, and infertility are all potential symptoms and complications that can arise in relation to the uterus[2]. Several environmental and genetic risk factors exist for the development of uterine leiomyomas, including local injury or infection, stress, obesity, hypertension, race, and parity— the latter having a protective effect[3,4]. Inflammation plays a role in the development of uterine leiomyomas. Briefly, factors such as localized injury, infection, stress, and obesity cause the body to be in a chronic, low-grade inflammatory state. If the uterus suffers an insult or multiple insults (i.e., menses), the immune response will become pro-inflammatory, leading to smooth muscle cell proliferation and fibrous tissue formation[4]. Macrophages and neutrophils are initially attracted and release a variety of growth factors and cytokines that have chemotactic, proinflammatory, and angiogenic qualities. These include TNF-α, IL-1, IL-6, IL-8, and vascular endothelial growth factor (VEGF)[5]. The proliferative effects of estrogens and progesterone are believed to be demonstrated by proinflammatory mediators such as growth factors including transforming growth factor b (TGF-b) and basic fibroblast growth factor (FGF basic), as well as tumor necrotizing factor a (NFa) [6].

2. Methodology

Sample collection

90 blood samples were collected and divided into (50) blood samples from women with uterine fibroids who received a diagnosis from a qualified medical specialist, and (40) blood samples from healthy

women, whose ages ranged from (20-50).

Samples were collected from _____ Hospital after the diagnosis of the disease, and for the period from October 2023 until March 2024. A personal interview was conducted for each person, through which a questionnaire was filled out that included sequence, age, marital status, family history, number of children, number of miscarriages, and gender. the blood.

Samples for the current study were also collected by drawing 5 ml of venous blood from both patients and healthy people. The blood samples were placed in gel tubes and left at room temperature for approximately half an hour until coagulation occurred. Then a centrifugation process was performed at a speed of 3000 revolutions/minute for 10 minutes to obtain the serum. (Serum), and then the separated serum was stored in Appendroff tubes. After that, the separated serum was kept under freezing until it was used in serological tests.

Measurement of IL-6, TGF-1, CRP, Vit-D

The level of IL-6, TGF-1, CRP, Vit-D were estimated by sandwich ELISA technique. The Microelisa plate for each was coated with the appropriate antibodies to IL-6, TGF-1, CRP, Vit-D, after which the standard solution or samples were added to the holes of the plate. Microelisa stripplate is appropriate and combined with the specific antibodies, then HRP-Conjugate reagent for IL-6, TGF-1, CRP, Vit-D is added to each hole of the microelisa plate and incubated well, then it is washed well and then chromogen solutions A and B are added, the color of the liquid will turn blue. After adding the solution Stop Solution: The color turns yellow, and then the absorbance is measured at a wavelength of 450 nanometers, as the absorbance intensity value is proportional to the concentrations of IL-6, TGF-1, CRP, Vit-D in the blood serum.

Statistical analysis

The results were analyzed statistically using the Minitab-17 statistical program, applying the analysis of variance (ANOVA) test, and the arithmetic means were compared with the Duncan-test with a probability level of 0.05

3. Result and Discussion

As shown in Table (1). The findings indicated that there was no statistically significant disparity, with a probability level ($P>0.05$), in the concentration of IL-6 among women diagnosed with uterine fibroids, as it reached (44.9±4.4 pg/ml) when compared with healthy women (42.5±4.1 pg/ml). The results of this study showed a significant increase in the levels of both β-TGF and CRP in women with uterine fibroids at the probability level ($P<0.05$), reaching (2.870±0.760ng/ml, 62.9±6.4 pg/ml), respectively, when compared with Healthy women: As for Vit.D3, it showed a significant decrease in women with uterine fibroids at the probability level ($P\leq0.05$), as it reached (5.92±1.87 ng/ml).

Table(1): Comparison of IL-6, CRP, V i t level. D3, T G F in women with uterine fibroids

Parameters	**Women with uterine fibroid**	**Control**	**P-Value**
	Mean ± SD		
Vit.D3 ng/ml	5.92 ±1.87	7.56 ±1.61	0.016
CRP ng/ml	2.870±0.760	1.879±0.749	0.001
IL-6 pg/ml	44.9±4.4	42.5±4.1	0.431
T G F pg/ml	62.9 ± 6.4	43.8 ± 8.0	0.057

As The results showed that there was no significant difference in the levels of IL-6, among women with uterine fibroids in the age groups, as they reached (45.79±7.48)pg/ml in the G1, (42.01±5.92) pg/ml, in the G2, while in the G3 was (45.64±4.05) pg/ml

As for CRP, increased in the G3 group (3.089±1.057 ng/ml), and decreased in the G1 and G2 groups, which reached (2.675±0.511, 2.482±0.744 ng/ml), respectively. Vit-D3 also increased in the G1 group,

while it decreased with age, which was recorded (5.291±1.368 ng/ml) in the G2 age group, and recorded a further decrease in the G3 age group, which reached (3.089±1.057 ng/ml). As for TGF, it decreased. It increased in age group G3 (61.20±7.90 pg/ml), decreased in G2 (54.96±7.93 pg/ml), and reached a further decrease in G1 (49.56±6.01 pg/ml).

Table(2): Comparison of IL-6, CRP, V i t level. D3, T G F, Leptin, TNF-a according to age

Parameters	**Women with uterine fibroid**		
	G1=20-30 n=5	**G2=31-40 n=19**	**G3=41-50 n=26**
IL-6 pg/ml	45.79±7.48 a	42.01± 5.92a	45.64± 4.05a
CRP ng/ml	2.482±0.744b	2.675±0.511b	3.089±1.057a
Vit-D3 ng/ml	6.826 ±1.365 a	5.291 ±1.368 b	3.582 ±1.110 c
TGF pg/ml	49.56 ±6.01 b	53.96 ±7.93 b	61.20±7.90 a

4. Conclusion and future scope

The findings of the present study indicate that there is no statistically significant disparity in the IL-6 levels between women diagnosed with uterine fibroids and healthy women, as determined by a probability level of p = 0.431. Our results agreed with the study conducted by [7], indicated that there are no differences in the level of IL-6 between healthy people and women who have uterine fibroids, and the reason for this was attributed to differences in the mucous membranes of fibroid tumors in women. The study conducted by [8] indicated that There were no significant differences in the levels of TNF, IL-6, and CRP between women with uterine fibroids and healthy controls. When comparing the titer levels with women after surgical removal of uterine fibroids, a significant increase was observed. The reason for this was attributed to the occurrence of severe post-operative infections, which could effect on ovarian hormones due to the trauma to the patient's uterus. Our study did not agree with the study [9], which observed an increase in the level of IL-6 in women with myometrial fibrosis.

The results agreed with the study [10], which noted an increase in CRP in women with uterine fibroids, which is considered an inflammatory indicator and a Th1-dependent immune response. CRP is considered one of the most widely used acute phase indicators and is of great value for monitoring disease activity. Other studies have indicated that an increase in its concentration is associated with the severity of the disease, and this explains the high level of CRP in the serum of people with uterine fibroids [11]. Hemilä et al., 1987 has shown that acute-phase CRP is synthesized by cells. The liver responds to pro-inflammatory cytokines and has been shown to be of great value as an inflammatory indicator in uterine fibroids. On the other hand, it has anti-inflammatory properties such as reducing the adhesion of neutrophils to the endothelium[12]. Results were not consistent with [13] who observed a decrease in CRP in women with uterine fibroids.

The study findings demonstrated a notable reduction in the concentration of Vitamin D in women diagnosed with uterine fibroids, with statistical significance at a probability level of p=0.016, as our results agreed with the study [14], which observed a decrease in the level of Vit D in women with fibroids. In the uterus, he also indicated a decrease in the incidence of uterine fibrosis under the influence of vitamin D. The reason for the effect was attributed to the fact that vitamin D prevents cell growth and stimulates programmed cell death in myoma cells. Fibroids are affected by vitamin D, which changes the gene expression of the BCL-2 and COMT genes in uterine fibroid (UF) cells [15]. Vitamin D deficiency is considered a risk factor for developing uterine fibroids, as American women

of African origin are more Caucasian women are susceptible to developing uterine fibroids [16,17]. Polymorphisms in genes are also associated with vitamin D metabolic processes, including the gene responsible for vitamin D transport (GC) and the DHCR7 gene, which is responsible for the production of Cholesterol and the CYP2R1 and CYP24A1 genes, which are important genes in the vitamin D synthesis pathway [18]. Vitamin D reduces the occurrence of tumors by regulating cell differentiation, blood vessel formation, and stimulating programmed cell death [19,20]. The development of uterine fibroids is associated with vitamin D, as patients with uterine fibroids have low levels when compared to control, as vitamin D is considered a protective factor to protect against uterine fibroids [21]. Uterine fibroids exhibit reduced amounts of vitamin D receptors in comparison to normal myometrium [22]. A negative correlation has been found between the levels of vitamin D receptors and the increased expression of estrogen and progesterone receptors in uterine fibroids (UFs). This indicates that vitamin D serves as an antagonist to sex hormone receptors in the tumor tissue of uterine fibroids [23]. Vitamin D plays a role in controlling the production of sex hormones in fibroids by interacting with its nuclear receptor.TGF is considered one of the growth factors that is important in the pathology of fibroid tumors, especially uterine fibroids. Many studies have shown that the level of TGF increases from three to five times in Uterine fibroid compared to the normal uterus, which works to increase the production of Extracellular matrix (ECM) and increase Expression of Type I collagen, fibronectin, proteoglycans[24]. The results of the study agreed with the researcher [16], who noticed an increase in the level of TGF in women with uterine fibroids, and who indicated a decrease in the levels of TGF when treated with vitamin D, which has an influential role in reducing the size of the fibroid. Through the controversy, we note that there is no Differences in IL-6 levels between different age groups. Our results agreed with [25], which showed that there were no significant differences in the level of IL-6 among age groups. Holub et al., 2003 also recorded that there was no significant difference in the level of IL-6 among age groups. Between (27-42)[26].

The results showed a higher level of CRP in the G3 age group compared to the G1 and G2 age groups. The results agreed with the study conducted by [27], which showed an increase in CRP in women aged 40-50 years and who noted positive correlations between CRP and uterine fibroids. The researcher [28] also showed that there were no significant differences in the level of CRP in women with uterine fibroids and in the age groups less than 35 years and more than 35 years.

The results showed an increase in the level of V i t - D3 in the G1 age group compared to G2, G3, as V i t - D3 decreases in the age group after 40 years. Another study revealed that vitamin D decreases with age, which decreased by double in the older age group when compared to the younger age group. The reason for this was attributed to the ability of human skin to synthesize vitamin D, which is affected by the age factor [29]. Our results did not agree with the study conducted by [30]. He noted that there were no significant differences in the level of V i t - D3 in the age groups of women with uterine fibroids.

Through the study, we notice that the level of TGF-B increases with age, as it was highest in the age group (41-50), followed by the age group (31-40), and then the age group (20-30). The study conducted by [31] indicated a high level of TGF-B in women of childbearing age, which indicated its importance as a diagnostic tool and early monitoring of uterine fibroids to control them. The researcher [32] indicated that TGF-B is affected by the age factor. And weight, as it increased in women who had uterine fibroids compared to the normal uterus in the age group (41.36±7.08).

TGF-B is considered one of the serum markers that has a role in the growth and metastasis of fibroids

in the uterus [33]. Fibroids secrete larger amounts of TGF-B compared to a normal uterus, which works to inhibit the secretion of bone morphogenetic protein 2 (BMP). -2) Which in turn causes infertility in women who have fibroids in the uterus [34], and also affects the embryos in terms of implantation or embryo survival [35,36]. It is believed that the tumors Breast fibroids among younger women are due to their higher estrogenic activity (Nawar et al., 2023). A translocation between chromosomes 12 and 14 also occurs in adolescent girls, which is a confirmed risk factor for uterine fibroids [37,38].

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