



Association between serum TNF and uterine fibroid and evaluate its level in symptomatic patients

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Abstract

Background: -The most common benign lesion of the uterus are leiomyoma (fibroids) in the genital tract of the female. Uterine fibroids incidence has been assessed 30-70%. Tumor necrosis factor - α is a cell-signaling protein released in systemic inflammation. It is one of the cytokines present in acute inflammatory state in the body.

The aim of the study: - was to estimated relationship between TNF- α in female with and without uterine fibroids then evaluate the role of TNF - α and its level in clinically symptomatic women this uterine lesions (leiomyoma or the other name fibroids).

Material and methods: - overall of 97 women were included: 59 patients with uterine fibroid and 38 women without fibroid, serum TNF- α level between the two samples were matched. Mean of TNF- α in control group 5.26 ± 3.2 pg/mL while in patients group about 17.64 ± 5.6 pg/mL. The result was statistically significant. We found that serum TNF- α level of 7 pg/mL is used as a positive indicator for uterine fibroids in particular women. In this study, we established elevated serum TNF- α level in women which had symptoms such as pain and bleeding.

Conclusion: - In reproductive age group female, fibroids are the commonest benign gynecological lesion of uterus (30-70%). Many biological factors are reported to have an associated with them, TNF-a , one of the important factor, which is cytokine that interfere with presence and behavior of uterine fibroid, which has major role in symptoms and complaints of patients with fibroid mostly due to inflammatory like state

Keywords: TNF- α , Cytokines, Inflammation, Fibroids, Symptoms

الارتباط بين عامل نخر الورم في الدم والورم الليفي الرحمي وتقييم مستواه لدى المرضى الذين يعانون من أعراض

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المستخلص

خلفية: - الأفة الحميدة الأكثر شيوعا في الرحم هي الورم العضلي الأملس (الأورام الليفية) في الجهاز التناسلي للأنثى هي الورم العضلي الأملس (الأورام الليفية) في الجهاز التناسلي للأنثى. تم تقييم حدوث الأورام الليفية الرحمية بنسبة 30-70% عامل نخر الورم - α هو بروتين يشير إلى الخلايا يتم إطلاقه في التهاب الجهاز. وهو واحد من السيتوكينات الموجودة في الحالة الالتهابية الحادة في الجسم

هدفت الدراسة: - تم تقدير العلاقة بين عامل نخر الورم α في الإناث المصابات وبدون الأورام الليفية الرحمية ثم تقييم دور عامل نخر الورم α ومستواه لدى النساء المصابات بأعراض سريرية هذه الآفات الرحمية (الورم العضلي الأملس أو الاسم الآخر الأورام الليفية)

المواد والطرق: - تم تضمين 97 امرأة بشكل عام: 59 مريضة مصابة بالورم الليفي الرحمي و 38 امرأة بدون ورم ليفي، تمت مطابقة مستوى α مصل TNF بين العنيتين. متوسط عامل نخر الورم α في المجموعة الضابطة 5.26 ± 3.2 بيكوغرام / مل بينما في مجموعة المرضى حوالي 17.64 ± 5.6 بيكوغرام / مل. وكانت النتيجة ذات دلالة إحصائية. وجدنا أن مستوى TNF- α المصل البالغ 7 بيكوغرام / مل يستخدم كمؤشر إيجابي للأورام الليفية الرحمية يستخدم كمؤشر إيجابي للأورام الليفية الرحمية وخاصة النساء. في هذه الدراسة، أنشأنا لدى النساء اللواتي عانين من أعراض مثل الألم والنزيف

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معلومات البحث

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الخلاصة: - في الفئة العمرية الإنجابية للإناث ، الأورام الليفية هي الآفة النسائية الحميدة الأكثر شيوعا في الرحم (30-70%).

الكلمات المفتاحية : $TNF-\alpha$ ، السيتوكينات، الالتهاب، الأورام الليفية، الأعراض.

Introduction

Uterine fibroid originate from uterine smooth muscle cells which is benign tumor of reproductive tract , were developed . High percent (30% - 70%) of female may had uterine fibroids, those are related to several risk factors, mostly age, obesity and ethnic factor [1,2] . According to the site, number, size and dimensions, uterine fibroids cause a lot of clinical symptoms, such as menorrhagia , anemia, painful menstruation, chronic pelvic pain and pressure symptoms such as constipation, urinary symptoms, dyspareunia in addition to element of subfertility [3,4]

Numerous risk factors may be responsible for uterine fibroids, including hormones, genetic predispositions, vitamin D deficiency , extracellular matrix , and other growth factors, like tumor necrosis factor , which cause uterine fibroids development[5,6,7]. There were a rising in the number of researches concentrating on the correlation between TNF and fibroid, our study will scientifically evaluate the relationship between them. [8]

Fibroid are perceived in women (rising to 70% by age of fifty years) and, in 30% of women with fibroid , cause discomforting symptoms due to pressure symptoms in pelvis, chronic pelvic pain and abnormal uterine bleeding. African-American females have a higher chance of affection by these lesions. These benign lesions happen at any age between menarche and menopause however are greatest in female (30-50) years of age. Uterine

fibroids naturally resolve spontaneously, after menopause.[9]

Effects of fibroids on pregnancy:

1. Sub-fertility: - fertility was affected mostly by sub mucous and intramural fibroids. Interference with implantation of conceptus and obstructing the ascending of spermatozoa by distorting the uterus and tubes and related disorder of ovulation could be the most common causes of subfertility. Majority of cases, occurrence of fibroids does not affect pregnancy.
2. Minority Of cases, it cause complication in pregnancy, there is high risk for miscarriages and preterm labor. [10]

Tumor necrosis factor – alpha is a cell signaling protein elaborated in any systemic inflammatory processes. This study estimate existing facts about difference in serum $TNF-\alpha$ in control and cases, the serum level of $TNF-\alpha$ in the symptomatic patients also its level according to fibroids' size, site and number in uterus. Its serum level has been established to be raised in symptomatic women with uterine fibroids. The existing facts propose occurrence of "inflammation-like" picture in female with uterine fibroids in which tumor necrosis factor – α level is high which represent a marker for inflammatory process. Causes of several complaints described by female with uterine fibroid are drawn back to tumor necrosis factor - α effect. However, the information on this topic still inadequate and tumor necrosis factor - α

related pathway in pathophysiology of uterine fibroid must be studied more. [11]

Uterine fibroid growing was affected by degree of cell differentiation, proliferation, angiogenesis, apoptosis and extracellular matrix production [11, 12].

Uterine fibroids are deliberated as a form of a fibrotic disturbance with extreme extracellular matrix deposition [8, 10]. Enrolment of the inflammatory cells and fibroblasts activation are the major pathways to cause fibrosis [10]. Immune system cells form and release cytokines, which are low-molecular-weight proteins [12]. Large number of cytokines are being recognized to have an important role in the biological behavior of uterine fibroid tissue [13, 14, 15].

While various biological and inflammatory molecules are elaborate in uterine fibroids occurrence, it seems that tumor necrosis factor- α may be one of the greatest important myometrium-related cytokines. Tumor necrosis factor- α has a double biological behavior as it may cause a lot of troubled symptoms for the patients. Tumor necrosis factor- α is a pleiotropic cytokine which is being recognized as the main director of the process of inflammation [11]. Furthermore, it plays a main role in the cell cycle, being the director of cell differentiation, apoptosis and growth [11,12] .

Increased tumor necrosis factor- α appearance has been institute in fibroid in comparison to the nearby normal uterine muscle. Uterine fibroid proliferation enhances by tumor necrosis factor- α secreted by adipocytes. Various human diseases, including cancers has been demonstrated by abnormal regulation of tumor necrosis factor - α

production, deposition and distribution. [11] .

It was stated that serum tumor necrosis factor- α concentration in symptomatic females with uterine fibroid were significantly elevated than in the normal females. Tumor necrosis factor- α rises expression of activin-A, the extracellular matrix promoter, in uterine fibroids, signifying that tumor necrosis factor- α rises the deposition of extracellular matrix, leading to uterine fibroids pathology [16,17].

Though the actions of tumor necrosis factor- α in uterine fibroids look to be tumorigenic, the study has described a role for tumor necrosis factor- α in vascular dysfunction elsewhere. For example, Tumor necrosis factor- α was revealed to down regulate the manufacture of nitric oxide (NO) via inhibition of endothelial nitric oxide synthase (eNOS), thus weakening smooth muscle relaxation and encouraging abnormal function of endothelium . This was more reinforced by discovery that tumor necrosis factor- α stimuli abnormal function of endothelium in animal models with diabetes and metabolic syndrome by oxygen species manufacture. Therefore, assumed the current suggestion that fibroids of the uterus possibly will be related to cardio metabolic risk factors and atherosclerosis, inflammation may cause abnormal function of vessels in uterine fibroids in a comparable approach to that seen in risk factors of cardiac metabolism [18 , 19, 20] .

Material and methods:

Cross-sectional study was done from 1st of January, 2022 to 30th of April, 2023. The case studied sample comprised women with the diagnosis of uterine fibroids, those consult gynecological outpatient unit in Babil teaching hospital for maternity and children, AL-Qathia primary health care unit and outpatient clinics in

AL- Hilla city. The control sample involved female without recognized uterine fibroids who were enrolled from general population, both groups were diagnosed by abdominal or vaginal ultrasound. Informed consent was achieved from women. Overall of 97 participants were enrolled. Exclusion criteria were include the following:-

- 1- Suspected adenomyosis.
- 2- Polycystic ovarian females.
- 3- Any women with autoimmune diseases such as systemic lupus erythematosus rheumatoid arthritis, and inflammatory bowel diseases
- 4- Women with acute inflammation (symptoms of infection like sneeze, fever, cough).
- 5- Active inflammation or allergy.
- 6- Postmenopausal women.
- 7- Malignancy.
- 8- Pregnancy.

Transvaginal or transabdominal ultrasound scanning was done for all participant women by specialist sonography a heterogenous hypoechogenic site in uterus with distinct margins(subserous, intramural and submucous) was determined as uterine fibroids, its site, size and number was determined.

Data about women age, BMI and parity were included. Any history of infertility and previous pregnancy complications such as history of recurrent miscarriages and preterm deliveries were collected, then history of symptoms such as pain, bleeding, constipation and other pressure symptoms also were collected.

Blood samples were aspirated from all participant women for bio chemical analysis. The plasma was separated from venous blood samples which frozen at -80°C until wanted for tested. The homogenates are then centrifuged for 5-10 min. to get the supernatant to carry out the assay, Tumor necrosis factor- α basal plasma levels were measured, using ELISA kit (USA) which is a high-sensitivity enzyme linked immunosorbent assay. Assay sensitivity was 4.69pg/ml. Specificity:-the kit recognizes human tumor necrosis factor in sample and no cross- reactivity or interference between it and correspondents was detected. The intra assay coefficient of variation was $< 10\%$.

Results

No significant correlation between fibroid and women age, BMI, parity in sample study, P value > 0.05 as shown in Table (1), Figure (1)

Table 1: demographic variables of the study groups (n=97)

Variable		Cases (n=59)	Controls (n=38)	P value
Age(years)	Mean \pm SD	34.19 \pm 6.9	32.03 \pm 7.64	0.457
	Range	20-45	20-45	
	20-25	6	10	
	26-30	15	5	
	31-36	12	10	
	>36	26	13	
BMI(kg/m ²)	Mean \pm SD	28.68 \pm 3.78	29.21 \pm 3.51	0.786
	Normal	14	5	
	Overweight	27	22	

	Obese	18	11	
Parity	Mean±SD	2.68 ± 1.4	3.28 ± 1.5	0.352
	Range	0_6	1_6	
	0	3	0	
	1	10	6	
	2_3	30	14	
	>3	16	18	

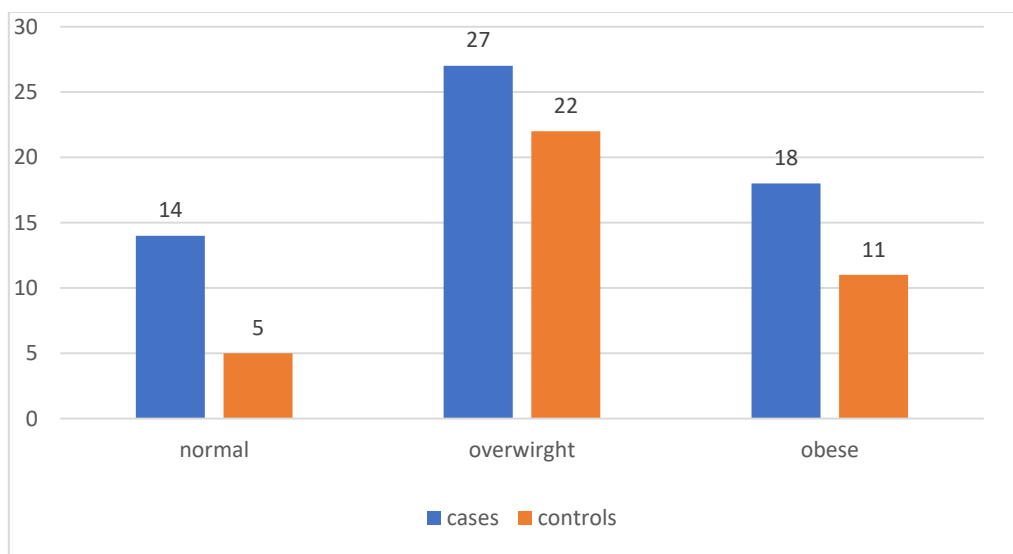


Figure 1: BMI distribution of the study sample (n=97).

Show distribution of fibroid in women according to their BMI .which revealed increase incidence of fibroid in overweight group.

Characters of fibroid in study sample, Intramural fibroid is more common than other site, most of

presented women with single fibroid and average size was 39.88 mm and about 27 women present with symptom Of complications. as shown in Table (2), Figure (2)

Table 2: fibroid characteristics of the cases (n=59)

Size(mm)	Mean ± SD	39.88 ± 10.72	
	Range	22-67	
		Frequency	%
Site	Intramural	36	37.1
	Subserosal	15	15.5
	Submucosal	8	8.2
Number	Single	41	42.3
	Multiple	18	18.6

Complications during pregnancy	Absent	32	33
	Present	27	27.8
Symptoms	Absent	32	33
	Present	27	27.8

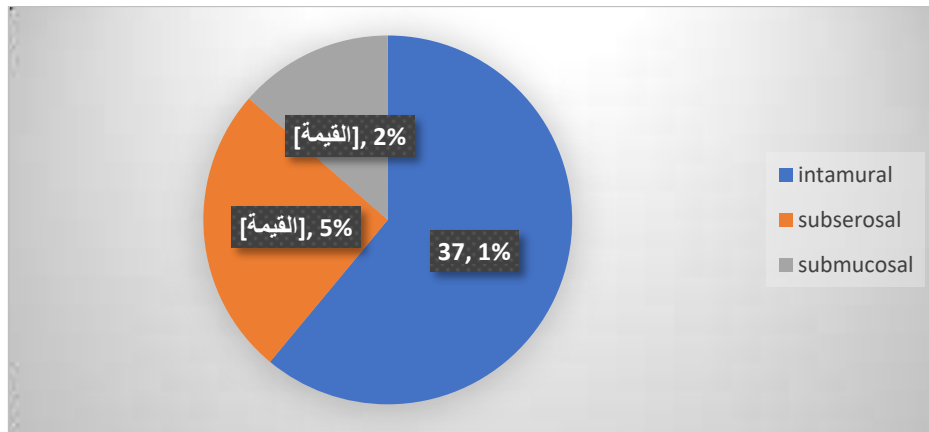


Figure 2: fibroid site distribution

Percentage of uterine fibroid site illustrated, the intramural one more frequent than other types in whole study sample.

There are significant correlation between serum TNF level women with uterine fibroids and those without, P value < 0.05. as shown in Table (3), Figure (3).

Table 3: TNF values in cases vs controls

TNF(pg/ml)	Cases	Controls	P value
Mean ± SD	17.64 ± 5.6	5.26 ± 3.2	<0.0001
Range	10_30	2_16	

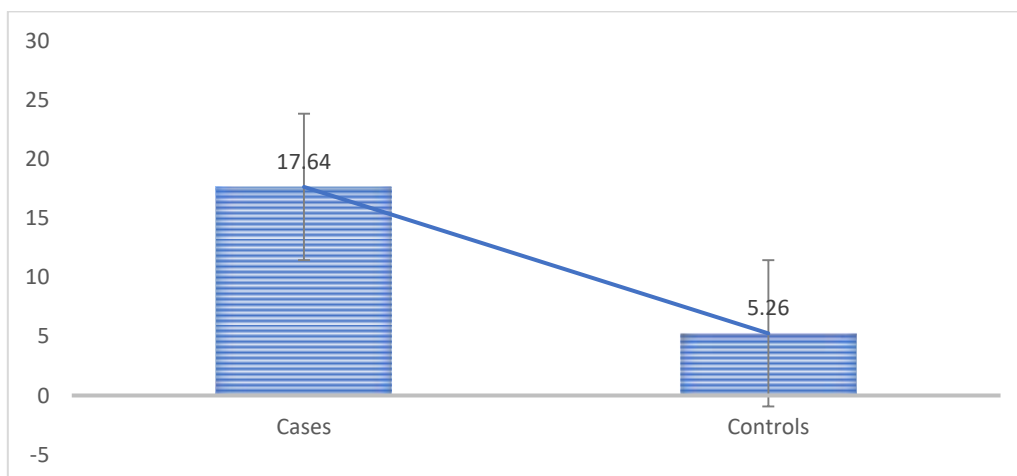


Figure 3: mean TNF between cases and control

Highly significant correlation between serum TNF level and women with uterine fibroid than those without fibroids.

No significant correlation between serum TNF level with age and BMI in women with fibroid P value > 0.05 , except that we found inverse

significance with parity which mean TNF level decrease with increase parity, P value - 0.007. (This finding support that majority of women with uterine fibroid especially smallest one has no effect on pregnancy outcome). as shown in Table (4).

Table 4: statistical correlation between TNF and Age, parity, and BMI.

	TNF(pg/ml)	Mean	P value	Comment
Age(years)	20-25	9.25	0.169	Not sig.
	26-30	15.5		
	31-36	12		
	>36	13.3		
BMI(kg/m²)	Normal	13.95	0.967	Not sig.
	Overweight	12		
	Obese	13.38		
Parity	0	20	0.007	Inverse sig.
	1	14.3		
	2_3	13.66		
	>3	10.32		

There are significant correlation between serum TNF level in women with uterine fibroid in number and size, P value < 0.05. That mean TNF

level increase with increase in number and size of fibroid, while there is no relation with site of fibroid in the uterus. as shown in Table (5).

Table5: statistical correlation between TNF and size site and number of fibroids.

	TNF(pg/ml)	Mean	P value	Comment
Site	Intramural	19.56	0.066	Not sig.
	Subserosal	12.27		
	Submucosal	19.13		
Number	Single	16.02	<0.0001	Positive sig.
	Multiple	21.3		
Size(mm)	20-30	13.31	<0.0001	Positive sig.
	31-40	15.72		
	>40	20.89		

There are significant correlation between serum TNF level in women with uterine fibroids regarding clinical symptoms and history of pregnancy complications, P value < 0.05, which mean woman with clinical symptoms such as

bleeding or pain and those with past history of pregnancy complications had higher level of TNF than those woman who had fibroid but without symptoms or previous pregnancy complications. as shown in Table (6).

Table 6: statistical correlation between TNF and complications during pregnancy and fibroid symptoms.

TNF(pg/ml)		Mean	P value
complications during pregnancy	absent	13.81	<0.0001
	present	22.19	
Symptoms	absent	13.81	<0.0001
	present	22.19	

Discussion:

About uterine fibroids, those pathogenesis has not yet assumed at all, a obvious inflammatory method important to an overstated tissue repair may clarify the copious ECM, a typical feature of the uterine fibroids that, just because of this specific behavior , is considered a representative fibrotic tissue. In particular, a key role in this complex system can be played by the macrophages when a deregulation in their action occurs.[23]

The fibroids number, size, and site had no substantial role with manifestation of fibroids symptoms, sings and complications during pregnancy. This according to the study of (Poovathi and Ramalingam 2016) [24] which agree with our study about site of fibroid but disagree with our study about size and number of uterine fibroids.

TNF-α are elaborated in vital ways in the pathophysiology of uterine fibroids, in additions to

other cytokines which have an consequence on the, neoangiogenesis, inflammation and the regulation of tissue alteration. These cytokines may also be responsible for symptoms caused by fibroids, such as , abdominal pain, subinfertility, and pregnancy complications. These data agree with the results of our study that stated about the role of elevated TNF-α in patient who have symptoms in the presence of uterine fibroid.

TNF-α is released by significant numbers of macrophages whose can be established in fibroids tissue. High TNF-α presence has been institute in fibroid tissue with compares to the nearby normal myometrium According to Nair et al., TNF-α released by adipocytes enhances the proliferation of uterine fibroids [25] , this data correlate with our results in this study and agreed our finding about elevated level of tumor necrosis factor -α in fibroid tissue which is unlike to nearby normal tissues [11]

A lot of researches informed that TNF- α is connected with uterine fibroids. Uterine fibroids are very mutual benign gynecological lesions of reproductive age females. Many issues are stated to have connotation with uterine fibroids, such as TNF- α .

Uterine fibroids interrupt the usual uterus by changing the vascular strategy, fading the usual contractility, and altering the manufacture of angiogenic factors, cytokines (TNF- α), chemokine, growth factors, prostaglandins (PGF2 α), and factors elaborate in coagulation and fibrinolysis. It is of dominant importance to examine the mechanisms causal heavy menstrual bleeding and subfertility secondary to reduced accessibility and implantation in females with uterine fibroids [26]

Immune cells have TNF- α , a reaction to tissue damage or due to initiation immune response to TNF- α in smooth muscle cells. A wide variety of intracellular signal events can encourage TNF- α (e.g. cell growth, apoptosis, and inflammatory processes), (Ciebiera et al., 2018) study ensure that, high serum TNF- α level is one of the causes of complaints and symptoms of the patient [11].

In this study, we discover that female with established uterine fibroids had virtually mean serum TNF- α levels three times higher (17.64 ± 5.6 pg/ml) when matched to controls (5.26 ± 3.26 pg/ml). (Statistically significant at P value <0.0001) that agree with (Plewka A. et al., 2013) study [20].

In our study, With increasing fibroid size, we found increasing serum TNF concentration (direct proportion) so TNF serum levels can be regarded as indicators for clinical confirmation and used to evaluate the severity of the symptoms caused by

uterine fibroid similar finding were recognized in other several studies [21].

All the results in our study, propose the existence of an “inflammation-like” picture in female with uterine fibroids.

Because of the pathophysiological complexity of the evolution in which TNF- α shares, we consider that TNF is not be a definite indicator to uterine fibroids. Probably, presence of additional biochemical factors might rise sensitivity. [27]

The data of this study, revealed those clinically symptomatic patients with uterine fibroids had raised serum level of TNF- α . It is probable that TNF- α may demonstrate beneficial as further indicator for the diagnosis, treatment and prognosis of fibroids, so we must do numerous studies about this subject including larger number of participants to support the results.

Finally, uterine fibroids are widely common as far as it is a benign uterine lesion in females at reproductive age. Numerous biological factors are described to have association with them, like TNF- α , which has major role in symptoms and complaints of patients with fibroid mostly due to inflammatory like state. [28,29,30].

Conclusions

We suppose that the outcomes of this study may offer helpful signal for clinical training and future studies. TNF- α is an very important cytokine related with the biology of uterine fibroids. Its concentration has been established to be raised in females with fibroid symptoms. The existing facts suggest the presence of an “inflammation-like” state in females with uterine fibroids where TNF- α is a strong inflammation markers. The source of many symptoms described by females can be

drawn back to the TNF- α . However, our information on this topic still incomplete. It appears vigorous to study the pathophysiological ways reliant on TNF- α , specifically its relations with progesterone and activin A. Positively, the consequences of that study will be the conclusive factor in choosing the suitable, separately designer uterine fibroid treatment approaches. It is probable that TNF- α will prove valuable as an extra biomarker for the diagnosis and treatment of uterine fibroids. The significance of anti-TNF- α drugs in the handling of uterine fibroids and medications with established anti-fibroid accomplishment disturb the TNF- α reliant on symptoms should be examined.

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