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EVALUATION OF TUMOR NECROSIS FACTOR ALPHA (TNF-A) IN SYMPTOMATIC UTERINE LEIOMYOMA

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ABSTRACT

Background: Most benign female genital tract tumours are uterine leiomyomas. One-third of women with uterine fibroids have severe clinical symptoms that hinder everyday life. TNF-alpha is one of the most significant myometrium-associated cytokines in uterine fibroid biology, however several interleukins are implicated. **Aim of the study**: Evaluation of tumor necrosis factor alpha in symptomatic uterine leiomyoma. **Method**: one hundred women was included in this study,50 of them with uterine fibroid (38 symptomatic and 12 asymptomatic) and the other 50 control group women without ultrasonographically identified uterine fibroid. Blood samples were collected from all patients for biochemical analysis. Tumor necrosis factor alpha was measured in the sera by an immune enzymatic assay using Tumor necrosis factor alpha ELISA kit. **Results:** Serum tumor necrosis factor alpha level was compared between the two study groups using Student's t-test. There was a highly significant difference between cases (3.08±0.83) pg./mL and controls (2.04±0.84) pg./mL with a mean difference of (1.04) pg./mL, P-value < 0.001. Also there's no significance difference with age, BMI and parity with P value of (0.713, 0.922, 0.051) respectively. Serum tumor necrosis factor was highly significant in patient with fibroid who present with heavy menstrual bleeding and dysmenorrhea with a P value of (0.0267, 0.038) respectively. Also it shows no significant deference in patient who present with irregular menstrual cycle (P value is 0.267), urinary symptoms (P value is 0.115) and even in asymptomatic patient. **Conclusion:** Uterine fibroids and elevated blood TNF-a levels were substantially correlated. TNF-a did not correlate with age or BMI. TNF-a did not correlate with fibroid location, size, MC regularity, or infertility.

KEYWORDS: Evaluation, Tumor necrosis factor alpha, symptomatic, uterine leiomyoma.

INTRODUCTION

Uterine fibroids or leiomyoma are the most frequent benign tumours of the female genital tract, resulting from myometrium smooth muscle cell neoplasia.[1] "They have much of extracellular matrix (ECM) with collagen, fibronectins, and proteoglycans. They are the most frequent benign tumour in reproductive-age women, affecting 20-40% by menopause and 70–80% by menopause.[2] Abnormal uterine haemorrhage, pelvic pressure, and tenesmus cause morbidity in 30% of cases. It causes excessive menstrual bleeding, pelvic discomfort, pelvic mass, infertility, and obstetric complications.[3,4] Fibroids might be invisible seedlings or enormous masses that deform and expand the uterus. Several fibroids may stretch the uterus to the rib cage and add weight.[5] One third of premenopausal women and nearly half of postmenopausal women with abnormal uterine bleeding had benign localised intracavitary lesions like fibroids. Focal intracavitary lesions increase in premenopausal women.[6] Histologically, uterine leiomyomas are benign monoclonal tumours from smooth muscle cells and fibroblasts. They have a narrow pseudocapsule of areolar tissue with squeezed muscle fibres and a lot of extracellular matrix. Nonetheless, "leiomyoma variations are classed as benign or malignant depending upon" histologic findings and clinical behavior.[7] FIGO classifies eight kinds of fibroids. This categorization provides a more accurate map of myoma distributions that may be utilised to develop novel treatment methods[8]. UFs occur in 20–40% of populations and 70% of uteri excised by hysterectomy.[10] Race was a major factor in fibroids "African-Caribbean women had a two-to-nine-fold higher risk. Compared to Caucasian women, it presents earlier. Fibroid risk decreases with parity and oral

contraceptives "oral contraceptives (OC) and fibroids is unclear. Combined OC users and non-users had different fibroids rates.[11] Fibroid risk was also affected "environment. Smoking reduces fibroid risk. Why Black American women get fibroids is unknown. Several research implies that black fat women with high blood pressure are more likely to develop fibroids. Others propose "that the propensity of African American women to eat food with less than" the daily vitamin D requirements.[12] Many studies have linked vitamin D deficiency to uterine fibroids. Vitamin D3 reduces uterine leiomyomas in animal models and suppresses leiomyoma cell growth in vitro. Uterine fibroids show lower amounts of vitamin D receptor (VDR) than surrounding myometrium, suggesting that vitamin D functions, such as serum vitamin D3 and VDR expression, may have a role in the formation of uterine fibroids.[13] Tumor necrosis factor (TNF, cachexin, or cachectin; frequently termed TNF-α) is an apotent pro-inflammatory cytokine, a tiny protein employed by the immune system for cell communication and homeostasis.[14] Immune and smooth muscle cells express TNF-α "tissues or immunological responses. TNF-α activates several intracellular signal pathways (e.g. apoptosis, cell survival, and inflammation). Clinical signs and patient complaints often result from high serum TNF-α. **Aim of the study:** Evaluation of tumor necrosis factor alpha (TNF- a) in symptomatic uterine liomyoma.

METHOD

The Babylon Maternity and Pediatric Teaching Hospital Department of Obstetrics and Gynecology performed a case-control research from to (1st of March 2020 – to 1st of December 2020). following Iraqi board of medical specialities permission and patient and control woman verbal consent. One hundred non-pregnant women, aged 24–45, were studied. Fifty had ultrasound evidence of fibroid. 38 (76%) of them were hospitalised with fibroid symptoms such heavy menstrual flow, dysmenorrhea, abdominal distention, and others, while 12 (24%) were symptom-free. Abdominal and endovaginal ultrasounds assessed fibroids by number, size, and location. The control group (50 women) was abnormally healthy and leiomyoma-free, as proven by U/S. A risk factor, symptom, and sign questionnaire guided history and physical examination for both groups. Intermenstrual bleeding, heavy menstruation, abdominal discomfort, abdominal mass, urinary symptoms, dyspareunia, and dysmenorrhea were studied.

Exclusion criteria had been set during patient selection included women with the following:-

1. Pregnancy
2. Gynecological conditions such as (endometriosis, adenomyosis, PCOS, ovarian cancer and other malignancy)
3. Medications for fibroid as hormonal or non-hormonal therapy.
4. Other causes of increase TNF –a like (Rheumatoid arthritis, inflammatory bowel disease).

Patients selection started by taking detailed history regarding: Age, marital status, parity, occupation and residence. Patients complaint which was either menstrual disturbance, pelvic pain and infertility. Gynecological history includes: age of menarche, menstrual cycle details (frequency, duration, amount and dysmenorrhea), date of marriage and use of contraception, history of genital infection. Past medical history was taken to exclude other cause of heavy menstrual bleeding such as thyroid disease and coagulated disorder. Drug history ex. Contraception. Substance abuse such as smoking. **Examination included:** general examination and vital sign. Height: standing height is measured from the top of the women's head to her heels. Weight: weight was measured in (kg) by using a portable digital scale. Body mass index was calculated.[15] Abdominal examination: as any abdominal mass or abdominal distention. Pelvic examination: may reveal enlarged uterus or mass. **Ultrasound examination:** In supine position abdominal ultrasound and trans vaginal ultrasound to assess number, size, site of the fibroid in each woman in cases group and control. Study group venous blood was aspirated. Five millilitres (ml) of blood were drawn from each patient by venepuncture, placed in a gel tube, and allowed to clot at room temperature for 30 minutes before being centrifuged for 3 minutes at 4000 rpm and kept at -20 C until analysis. The manufacturer (Elabscience) recommended measuring serum tumour necrosis factor alpha (TNF-a) using ELISA. SPSS 26 calculated every statistical analyses. Continuous data were mean±SD. Absolute numbers and percentages represented categorical variables. Kolmogorov-Smirnov tests data normality. For properly distributed data, Independent Samples T or Oneway ANOVA was used to compare continuous variables. For non-normal data, Mann-Whitney U or Kruskal Wallis was used. Spearman's test measures correlation if the continuous variable is not regularly distributed. Pearson's chi-squared test assessed categorical variables. 0.05 or less was significant. TNF-a patients and controls matter. TNF-a increases in uterine fibroid patients with HMB and dysmenorrhea. TNF-a does not affect uterine fibroid location or size.

RESULTS

Mean age of cases was (34.38±7.03) and range of (24-45) years. The mean of control group was (33.88±6.519). There was no significance deference in age was observed between cases and controls, P value = 0.713. Regarding BMI, the mean of cases was (26.434 ± 2.255) while the mean of control was (26.484 ± 2.841), there was no significant difference in BMI between cases and controls, P value = 0.922 as shown in table (1).

Table 1: Association of clinical data in control and cases (uterine fibroid).

Parameters	case group (n = 50) mean ± SD	Control group (n = 50) mean ± SD	*P value
Age/years	34.38±7.03	33.88±6.519	0.713
BMI (kg/m2)	26.434±2.255	26.484±2.841	0.922

Also according to Independent Samples T-Test there is high significance between cases and controls regarding level of TNF-a in which mean of cases was (3.08±0.83) and mean of controls was (2.04±0.838) with P value = 0.001 as shown in table (2)

Table (2): Association of biochemical data in control and cases (uterine fibroid).

Parameter	case group (n = 50) mean ± SD	Control group (n = 50) mean ± SD	*P value
TNF-a	3.08±0.83	2.04±0.838	< 0.001

*Independent Samples T-Test

Two-way ANOVA Test was used in order to assess the correlation between serum TNF-a level and each of age and BMI. These comparisons were carried out for each of the two study groups. Within the cases group, there was no significant difference between age and serum TNF-a level. Similarly, no significant correlation was found between BMI and serum TNF-a level. In a similar manner, the correlation between age and serum TNF-a level within the control group was also found to be non-significant. In addition, BMI correlation with serum TNF-a level was also non-significant. Table (3) summarized the correlations between serum TNF-a level and each of age and BMI within each of the two study groups.

Table (3): Comparison demographic characteristics between control and cases in association to TNF-α.

Parameters		Case group (n = 50)	Control group (n = 50)	*P value
		TNF-α (pg/ml) mean±SD		
20-30	Age/years	3.15±0.8327	1.87±0.6967	0.514
31-40		2.995±0.739	2.032±0.7572	
41-50		3.158±1.0282	2.373±0.1.1429	
Normal (18.5-24.9)	BMI (kg/m2)	2.78±1.0064	2.172±0.9718	0.131
Overwieght (25-29.9)		3.16±0.7768	1.969±0.7592	

*Two-way ANOVA Test

In figure (1) show the correlation between uterine fibroid site and frequency of HMB and dysmenorrhea.

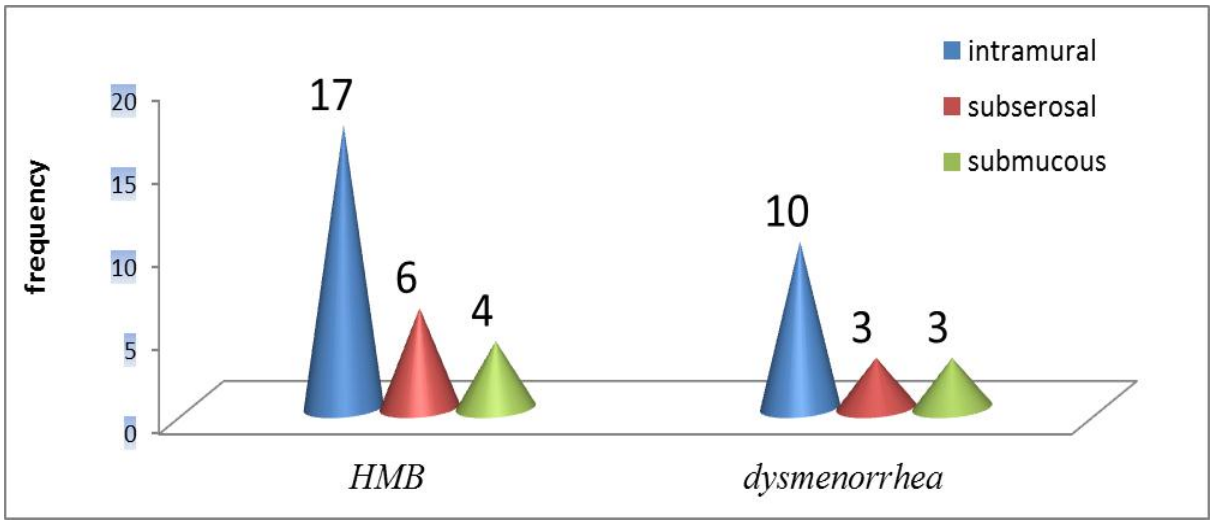

Figure 1: correlation between uterine fibroid site and frequency of HMB and dysmenorrhea.

In figure (2) show the correlation between uterine fibroid site and regularity of MC.

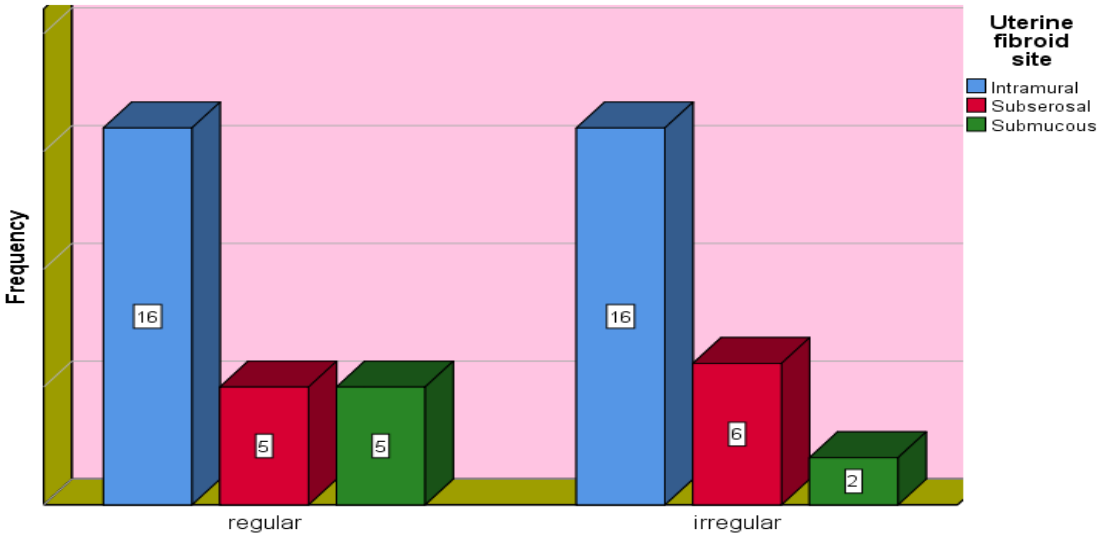

Figure 2: uterine fibroid sites and their effect on menstrual cycle.

In figure (3) show the correlation between uterine fibroid according to its site and their effect in fertile state.

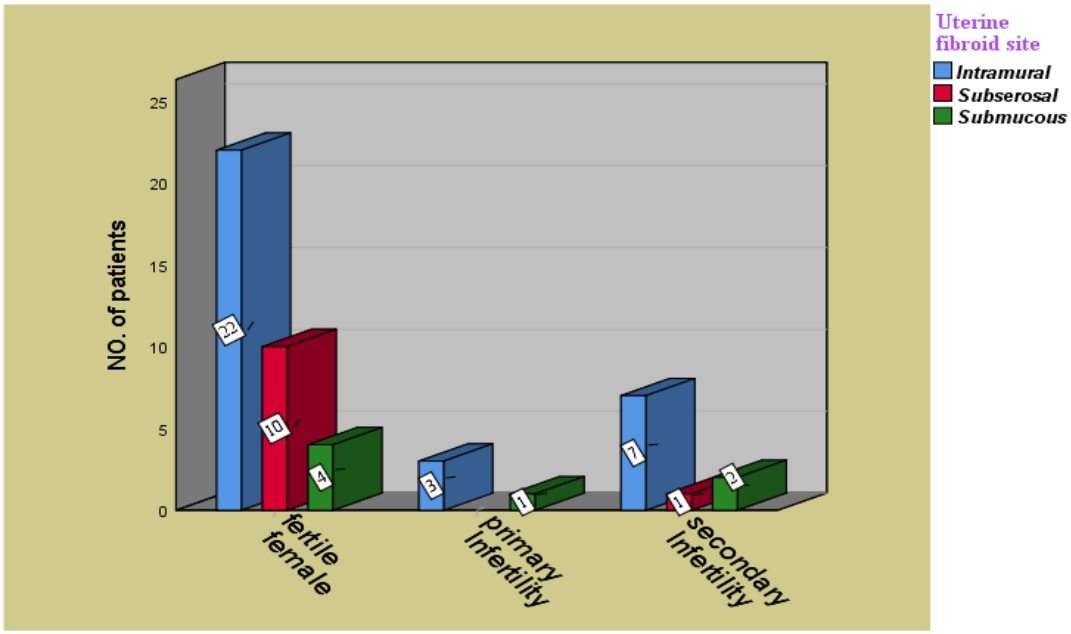

Figure 3: Frequency of patients with uterine fibroid according to its sites and their effect in fertile state.

By using Spearman's correlation, we found that there's no correlation between serum TNF-α with the site of uterine fibroid. As shown in table (4).

Table 4: The correlation between serum TNF-α with the site of uterine fibroid.

serum TNF α and uterine fibroid site	Uterine fibroid patient	
	R	*P value**
TNF α -All sites	-0.033	0.820
TNF α -Intramural	-0.033	0.859
TNF α -Subserosal	-0.082	0.810
TNF α -Submucous	-0.393	0.383

* Spearman's correlation

By using Pearson's correlation, we found that there's no correlation between serum TNF-α with uterine fibroid size of uterine fibroid. As shown in table (5). Fig (4)

Table (5): The correlation between serum TNF-α with uterine fibroid size in patients with uterine fibroid.

serum TNF α and uterine fibroid size	Uterine fibroid patient	
	*P value**	R
TNF α -fibroid size	0.066	0.262
*Pearson's correlation		

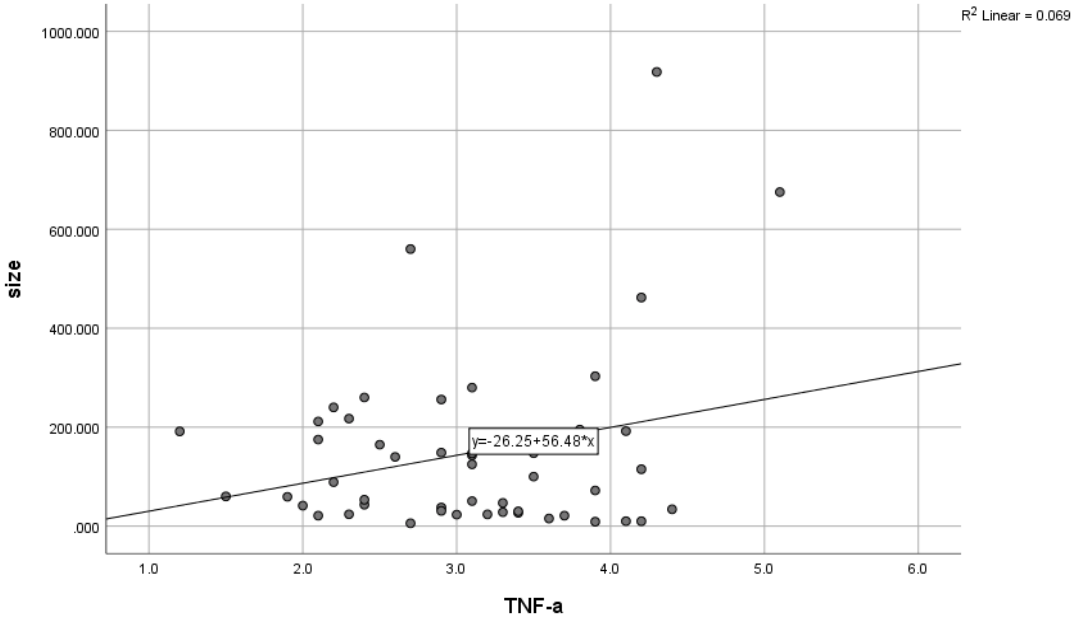

Figure 4: The correlation between serum TNF-α and fibroid size in patients with uterine fibroid.

DISCUSSION

The most prevalent kind of benign uterine tumours that may have a negative influence on fertility is uterine leiomyoma. Tumor necrosis factor alpha serum levels are higher in Caucasian women with uterine fibroids, according to a research by Ciebiera M, et al. (2018).[16] This is consistent with our data, but there is one important distinction: the study by Ciebiera M, et al. (2018) only included Caucasian women. These results are significant because they show that TNF- is a second factor that is more prevalent in blood samples from the group of women who have UF. TNF- may even be used to calculate the likelihood that UFs may develop or to gauge the success of a certain therapy. Similar conclusions were made by Plewka et al. in 2013[17], who discovered that fibroids have "much greater TNF- immunoreactivity as compared to normal uterine smooth muscle cells. Our findings demonstrated that women with clinically symptomatic UFs had higher serum TNF- levels. TNF- upregulates the mRNA levels of MMP-2 in UF cell cultures, according to Wang et al.[18] The same findings were reached regarding protein levels, however in the normal myometrium, this influence was negligible. Their discovery could significantly contribute to the release of soluble versions of several growth factors and cytokines from ECM. Such reliance may also have the effect of a self-reinforcing dependency cycle; TNF-, which produces a variety of cytokines, also releases more TNF- molecules; eventually, this may develop into a cascade that is difficult to regulate.[19] In the case of obesity, having an ideal state of health may be hampered by an abundance of body fat. Obesity is mostly brought on by excessive calorie consumption, inactivity, and genetic predisposition.[18] In contrast to Ciebiera M, et al which disagreed with our findings, increase BMI is deemed negligible for increasing serum TNF-a levels in patients with UFs. This may be because our patient's BMI was between (22.3-29.9). Chronic inflammation, which is a feature of obesity, may contribute to aberrant tissue regeneration, as it does in UFs. Many inflammatory mediators may be released by excessive adipose tissue. Reactive oxygen species (ROS) levels that impede cell death and enhance ECM deposition are also elevated in excess fat formation.[18] TNF- is secreted by human adipose tissue, which may provide a solid justification for the link between obesity and inflammation. Ciebiera M, et al. 2018.[16] reported that serum TNF-a levels did not rise with age, which is consistent with our study's conclusion that age has no effect on TNF-a levels. Wang et al. 2015[18] disagreed with our study, which found that serum TNF-a levels were not elevated in women with leiomyoma. They claimed that women with a history of infertility had significantly higher serum TNF-a concentrations than fertile controls, and that TNF-a could potentially serve as an infertility marker. When we compared symptomatic patients, we discovered a very substantial correlation between dysmenorrhea and TNF-a. Ma et al. (2013) provided support for this study by discovering that pro-inflammatory cytokines could promote the synthesis or release of PGF2a, which causes uterine hypercontractility, decreases endometrial blood flow (blood vessel constriction), increases procoagulant activity, and intensifies pain, particularly in patients with von Willbrand disease.[19] Recent research has shown that vigorous aerobic exercise reduces levels of prostaglandins and their metabolite, as well as levels of TNF and the severity of discomfort related to dysmenorrhea.[20] In Govorov, et al. 2019[21], it was found that inflammatory markers, including TNF-a, were related to heavy menstrual bleeding. This finding is consistent with our own research, which found that patients who complained of heavy menstrual bleeding had higher serum TNF-a levels. This may be because

TNF-a is a well-known multifunctional cytokine that participates in both pro- and anti-inflammatory processes. TNF-a was shown to be at its maximum preovulatory and at its lowest during the luteal phase, suggesting that it may be progesterone-dependent and responsible for vaginal bleeding.[21]

CONCLUSION

There was a highly significant correlation between the presence of uterine fibroids and the high serum levels of TNF-a. Levels of TNF –a are significantly correlated with heavy menstrual bleeding and dysmenorrhea in women with uterine fibroid. There was no significant correlation between TNF –a and age, BMI of the study group. Site and size of fibroid have insignificant correlation with TNF –a levels.

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