

Significance of Serum Soluble Fas (sFas/CD95) and Tumor Necrosis Factor- Alpha (TNF- α) in Radiologically Diagnosed Patients with Uterine Leiomyoma

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Abstract:

Leiomyoma is a common benign tumor of female reproductive system arising from the smooth muscle cells of uterus. About 17.8% of females in 40s age group and 77% of hysterectomies found to have leiomyoma. It affects females in reproductive age and cause pelvic pain, abortion, infertility, menorrhagia which in some cases ends by hysterectomy. In this study the extrinsic apoptotic pathway was evaluated in patients with uterine leiomyoma by measuring serum soluble Fas (sFas) and Tumor Necrosis Factor Alpha (TNF α) in both patients and control healthy women. Forty patients with leiomyoma from 22-52 years old were involved in this study. Forty apparently healthy, their ages matched volunteers were selected as a control. Four ml of venous blood sample was obtained from each, serum was separated and serum concentration of sFas and TNF α measured using ELISA technique. There was a highly significant increase in serum concentration of TNF- α in patients with leiomyoma (mean 0.579pg/ml \pm SE 0.039) than control group (mean 0.120pg/ml \pm SE 0.007), also there was a highly significant increase in serum concentration of sFas of patient (mean 0.326pg/ml \pm SE 0.028) compared to control healthy subjects (mean 0.126pg/ml \pm 0.025). Receiver Operator Characteristic (ROC) curve for determination of TNF α and sFas cutoff value shows that TNF α has cutoff value of \geq 0.220pg/ml with 97.5% sensitivity and 100% specificity for leiomyoma and accuracy rate of 99.6%. The sFas cutoff value is \geq 0.154pg/ml with 80% sensitivity and 82.5% specificity for leiomyoma and accuracy rate of 87.4%.

اهمية تركيز الرابط الذائب فاس (sFas/CD95) وعامل نخر الورم نوع الفا (TNF- α) لمرضى الورم الليفي الرحمي المشخصين شعاعيا.

الخلاصة:

الورم الليفي الرحمي هو من الاورام الحميدة الشائعة التي تصيب الجهاز التناسلي الانثوي والذي ينشأ من طبقة العضلات الملساء المكونه للرحم، وهذا المرض يصيب النساء في عمر الانجاب وتشير الدراسات الى ان نسبة (17.8) % من النساء تعاني من الورم الليفي الرحمي في سن الاربعين، و انه موجود بنسبة (77) % من عمليات رفع الرحم والتي اجريت لاسباب عدة. ومن اهم اعراضه الم في منطقة الحوض، الاسقاط، العقم والنزف الرحمي الذي قد ينتهي بعملية رفع الرحم كليا. استهدفت هذه الدراسة تقييم عمل فعالية الاستماتة في النساء المصابات بالورم الليفي الرحمي وذلك بقياس تركيز كل من عامل نخر الورم الفا والرابط الذائب فاس في مصل الدم بتقنية قياس الممتز المناعي المرتبط بالانزيم في كل من مجموعتي المرضى والسيطرة. اختير 40 مريضا باعمار بين 22-52 سنة مثلو عينة الاختبار وكذلك اختير 40 شخص يعمر مقارب لمجموعة المرضى من الاصحاء ظاهريا ليكونوا مجموعة السيطرة. تم ملاحظة ارتفاع معنوي في تركيز عامل نخر الورم الفا لدى المرضى (0.579 بيكاغرام/مل \pm 0.039) مقارنة بمجموعة السيطرة (0.120 بيكاغرام/مل \pm 0.007). بالإضافة الى ارتفاع معنوي في تركيز الرابط الذائب فاس لدى المرضى (0.326 بيكاغرام/مل \pm 0.028) مقارنة بمجموعة السيطرة (0.126 بيكاغرام/مل \pm 0.025). (باستخدام منحنى صفة العامل المستلم Receiver Operator

Characteristic (ROC) curve فان مستوى القطع (cutoff value) لعامل نخر الورم الفا (TNF- α) هو ≤ 0.220 بيكاغرام بنسبة حساسية (sensitivity) 97.5% ونسبة تحديد (specificity) 100% للمرض مع معدل صحه (accuracy) 99.6%. مستوى القطع للرابط الذائب فاس (sFas) هو ≤ 0.154 بيكاغرام مع نسبة حساسية (sensitivity) 80% ونسبة تحديد (specificity) 82.0% للمرض مع معدل صحه..87.4%.(accuracy).

Introduction:

Leiomyoma is common monoclonal benign tumors of the female reproductive system arising from the smooth muscle layer of uterus (1). It could be single or multiple with variable size in the same patient (2). Prevalence data range from 5% to 21% and white women races showed lower prevalence than black one (3). Patients may complain heavy or prolonged menstrual periods, pelvic pain, frequent urination, dyspareunia and low back pain (4). **Genetic changes, race** (Black women), **hormones** (Estrogen, progesterone, various growth factors) and **cytokines** are the main risk factors (5). Hormonal medical treatment is the 1st choice to control patient symptoms (6) but (in many cases) the surgical treatment is the choice if the medical treatment fail and the patient ends by hysterectomy (7)

Apoptosis (greek word means falling of the leaves from a tree) a process by which cells in a multicellular organism commit suicide. It is a form of death that the cell itself initiates, regulates, and excutes the process using its cellular and molecular machinery (8). It is highly regulated process (also called programmed cell death) and plays a fundamental role in development of the body and tissue homeostasis (9). The cells that undergo apoptosis will be disintegrated into membrane bound particles eliminated by phagocytosis (10). Apoptosis considered the most common form of physiological cell death that occurs during embryonic development, tissue remodeling, and tumor regression (11).

There are two apoptotic pathways **extrinsic** (also called receptor- mediated or Fas/Fas Ligand (FasL) mediated) and **intrinsic** (or mitochondrial) (12). Induction of either pathways result in the activation of caspases (a class of intracellular cystein proteases) that are

responsible for the cleavage of a variety of cellular substrates and the morphological changes attributed to apoptosis (13).

When Fas, TNF and other Tumor necrosis factor **Related Apoptosis Inducing Ligand (TRAIL)** interact with their corresponding **Death Receptors** e.g. FasL, activation of the extrinsic pathway will occur. The reduction in apoptosis or apoptosis resistance is pivotal for tumor development (14), this disable of apoptotic responses also thought to be a major contributor to cancer treatment resistance (15).

TNF- α is a multifunctional cytokine playing roles in apoptosis, cell survival, inflammation, and immunity (16). It is expressed mostly by cells of myeloid lineage (17). Monocytes and macrophages are considered the main producers for TNF- α , even though there are conditions of infections and autoimmunity where the TNF- α can be expressed by other cell types including T and B cells, natural killer cells, and neutrophils (18). TNF- α acts by binding to two different TNF- α receptors which are widely distributed on most cells throughout the body, these are receptor-1, also called P55, and receptor-2, also known as P75 (19,20). TNF- α in small amount has beneficial effects this include inflammation (vasodilatation and increase vascular permeability), adhesions of neutrophils to endothelium, enhance the microbicidal activity of neutrophils, activation and adhesion of platelets, and increase expression of class-I and II MHC proteins (21). In the other side, large amount of TNF- α has harmful or detrimental effect e.g. septic shock (hypotension and high fever), disseminated intravascular coagulopathy DIC, and inflammatory symptoms of some autoimmune diseases (22).

Fas/Fas ligand (FasL) receptor interaction plays a pivotal regulatory role via immune homeostasis, immune privilege, and self-

tolerance (23). Fas and Fas ligand are members of the TNF receptors family that widely expressed on the surface of different kinds of cells especially activated lymphocytes, this family also includes the death receptor TNF- α , TNF- α related apoptosis-inducing ligand (Apo 2L) and TNF weak inducer of apoptosis (Apo 3L) (24).

Fas is a 45 KDa, type 1 cell surface protein (25) and the mature Fas protein is a 319 amino acid structure of three domains: an extracellular domain with (157 amino acid long) that bind to FasL, transmembrane domain (17 amino acid long), and a cytoplasmic domain (145 amino acid long) that transduce the death signal (26). It is found on the surface of cells as a monomer, upon T-cell activation trimerization is induced and the signal can be transduced to kill the target cell (27).

FasL was identified by Nagata's laboratory in 1993. It is a 40 KDa type II cell surface protein of 280 amino acids that is inducibly expressed in lymphocytes, particularly T-cells (28) and constitutively expressed in cells present in immune privileged organs e.g. Sertoli cells of the testis and epithelial cells of the eye (29). Inducible FasL expression by T-cells is tightly controlled at the transcriptional level through intricate interactions among various positive and negative transcriptional regulators (30). FasL protein is expressed in three distinct forms: (1) membranous form on the cell surface, (2) membranous form stored in intracellular microvesicles which are excreted into the intracellular milieu in response to various physiological stimuli, and (3) soluble form (sFasL). (30,31).

Soluble Fas and Fas ligand theory in tumor development:

There is a link between soluble Fas receptors that antagonize the proapoptotic protein (Fas, sFas ligand) and tumor development (32). It appears that tumor development and progression may represent a continuum where Fas expression and/or function are

progressively lost on the malignant cell (33). Fas loss-of-function has been shown to enhance tumor frequency, decrease tumor latency, and increase spontaneous tumor metastasis (34). Production of sFas in patients with tumor may be a key mechanism to inhibit Fas-mediated apoptosis (35). The identification of sFas levels as a predictor of outcome in malignant disease further establishes a connection between Fas loss-of-function and tumor progression (36).

Transduction of the Fas apoptotic signal requires trimerization of membrane-associated Fas, an intact DISC signaling complex, and the absence of high levels of cell-associated inhibitory or antiapoptotic proteins (26). Trimerization of Fas receptor can be inhibited by soluble receptors (sFas) that act as decoys, binding FasL and preventing association with transmembrane Fas (35). Two distinct soluble receptors, designated sFas and DcR3, have been shown to bind FasL and competitively antagonize Fas signaling (37). sFas is the designation given to multiple soluble isoforms of the Fas protein lacking the transmembrane region of Fas (38). DcR3 is a decoy receptor unrelated to the Fas protein that can bind to FasL (39). Little is known about the DcR3 receptor, although it appears to be amplified in lung and colon tumors (40). Unlike other soluble receptors in the tumor necrosis factor receptor superfamily, sFas is generated from alternative mRNA splicing instead of proteolytic cleavage of membrane-associated protein (41). Five distinct isoforms of sFas have been described, The most predominant isoform results from an in-frame deletion of the transmembrane domain, resulting in a loss of membrane anchoring (42). The molecular controls that regulate alternative sFas splicing have not been elucidated (43). Understanding these controls may be especially important in manipulating sFas levels in malignant disease (44).

Aim of the study

- Measuring serum sFas and TNF- α in patients with uterine leiomyoma and apparently healthy women (controls).
- Evaluation of receptor mediated apoptosis (extrinsic pathway) in patients with uterine leiomyoma and controls.
- Evaluation of sensitivity and specificity of serum sFas and TNF- α in patients with uterine leiomyoma.

Materials and methods:

This study was carried out at gynecology consultation clinic Baghdad Teaching Hospital during the period from July 2015 to November 2016. A total of 40 patients that have been selected were diagnosed as cases of leiomyoma by the following ultrasonic criteria: well demarcated regular hypoechoic or anechoic mass located in the wall of the uterus (subendometrial, intramural or subserous) at different sizes with peripheral vascularity seen by Doppler study. Any lesions with irregular out line, increased vascularity or located outside of uterus were excluded to decrease error of the diagnosis. Patients registered and subjected to direct supervision and follow up by the specialists.

The control group consisted of 40 apparently healthy women, their age matched to the patient group. They have no leiomyoma by ultrasound study and non-significant past medical history.

Four ml of venous blood sample was obtained from each subject and collected in a non-heparinized plain tube, then centrifuged at 1800 Xg for 10 minutes to separate serum. Serum concentration of

TNF- α was detected by TNF- α Enzyme Linked Immunosorbant Assay (ELISA) kits for quantitative determination of TNF- α from R&D SYSTEM (USA). Serum concentration of sFas was detected by Human sFas ELISA kit, for quantitative determination of sFas concentration in serum by R&D SYSTEM (USA). All the laboratory procedures were done at Immunology Department/Teaching Laboratories

Data obtained in the present study were summarized, presented and analyzed using two software programs; these were statistical package for the social sciences (SPSS) version 16 and Microsoft Office Excel 2010. For purpose of presentation numeric variables were expressed in the form of mean \pm SD (standard deviation), while categorical variables were expressed in the form of number and percentage. Mean values were compared using independent samples t-test. Chi-square test was used to study association between any two categorical variables. Correlation coefficient was used to evaluate correlation between numeric variables. Receiver operator characteristic (ROC) curve analysis was performed to find the cutoff value for TNF α and sFAS. P-value was considered significant when it was equal to or less than 0.05.

Results:

There was a highly significant increase in serum concentration of TNF- α in patients with leiomyoma (mean 0.579pg/ml \pm SE 0.039) than control group (mean 0.120pg/ml \pm SE 0.007). P value= <0.001, Fig -1

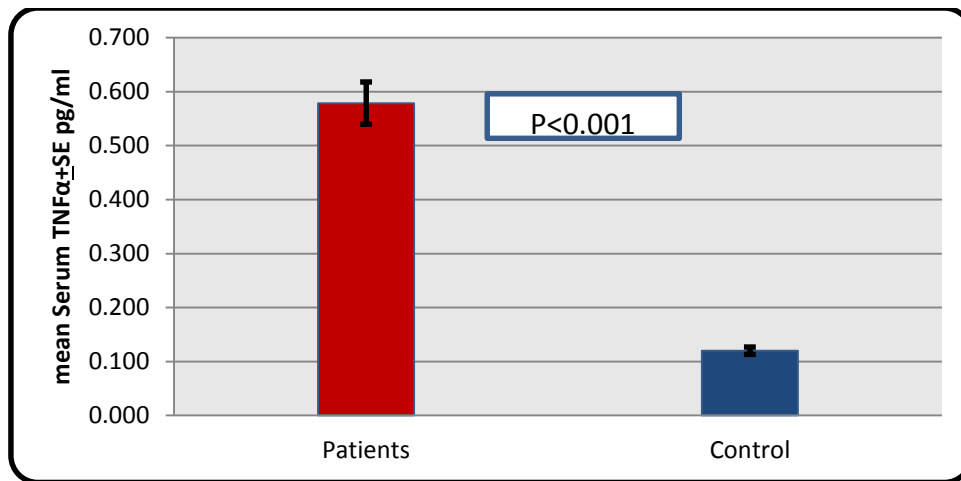


Figure-1: Comparison of mean serum TNF-α (pg/ml) between patients group and control group. P<0.001

The serum sFas level in patient and control groups was shown in figure-2. The figure showed a highly significant increase in serum concentration of sFas of patients

(mean 0.326pg/ml ± SE 0.028) compared to control healthy subjects (mean 0.126pg/ml ± 0.025). P value= <math>< 0.001.</math>

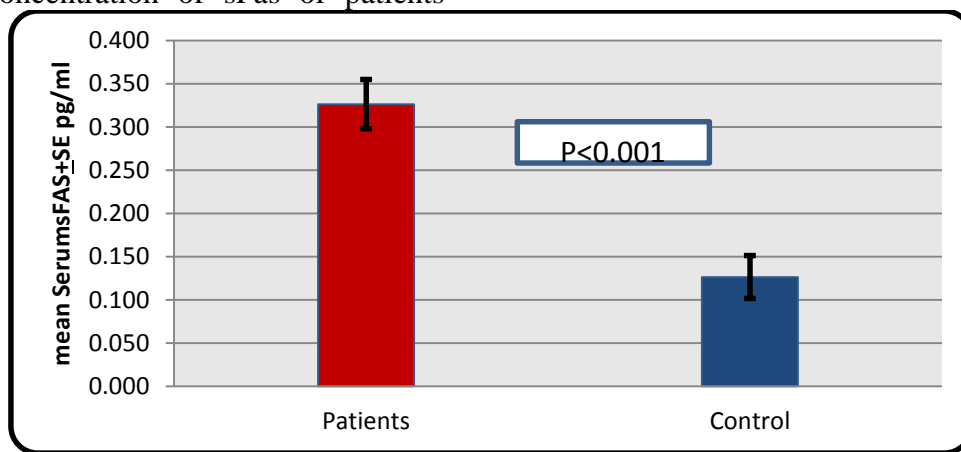


Figure-2: Comparison of mean serum sFas concentration between patient group and control group. P<0.001

Determination of TNF-α and sFas cutoff value:

Figure-3 and table-1 showed the Receiver Operator Characteristic (ROC) curve for determination of TNF-α and sFas cutoff value. TNF-α has cutoff value of

≥0.220pg/ml with 97.5% sensitivity and 100% specificity for leiomyoma and accuracy rate of 99.6%. The sFas cutoff value is ≥0.154pg/ml with 80% sensitivity and 82.5% specificity for leiomyoma with accuracy rate of 87.4%.

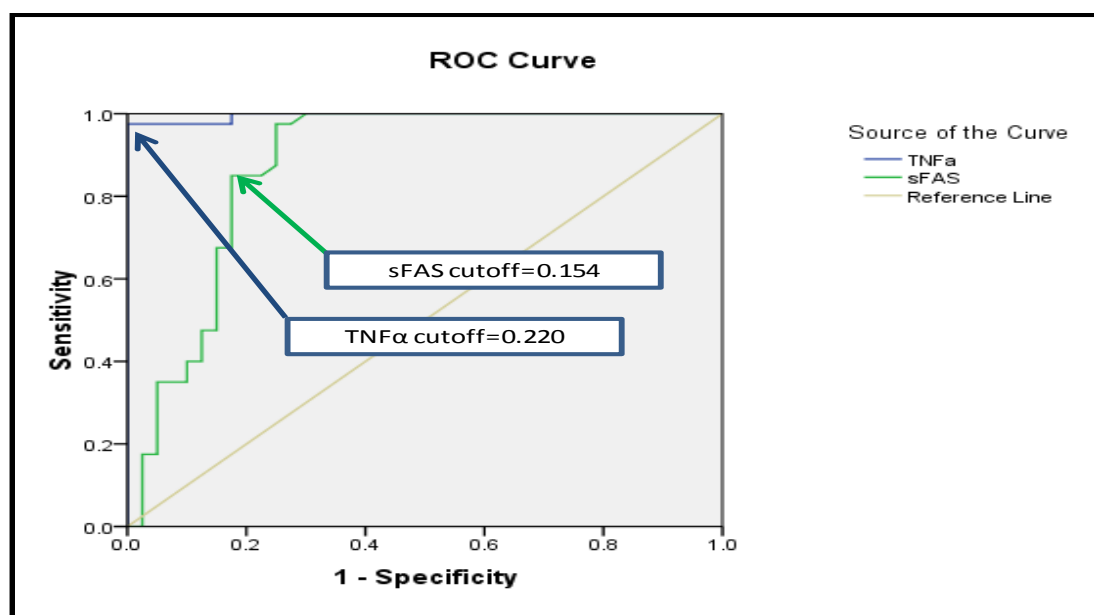


Figure-3: Receiver Operator Characteristic (ROC) curve for determination of TNF- α and sFas cutoff value.

Table-1: Parameters of ROC curve for determination of TNF- α and sFas cutoff value

Parameter	Cutoff value	AUC (accuracy)	Specificity	Sensitivity	P-value
TNF- α	≥ 0.220	0.996 (99.6%)	100%	97.5%	<0.001
sFas	≥ 0.154	0.874 (87.4%)	82.5%	80%	<0.001

Discussion:

Many studies referred to different changes in cell biology that may deviate it from normal growth and replication. At the same time a defect in the body defense mechanisms against this deviation being another factor ends by tumor growth and existence (7,8). It is known that leiomyoma cells had an increase in sex hormone receptors than normal myometrium (9) this will increase the growth hormone receptors in these cells to mediate the proliferative action of the sex hormones (10). This increment in growth hormone receptors renders the cells more liable for proliferation by being more sensitive to growth factors (11). Against this abnormal high rate of proliferation, apoptosis defence mechanism act through increase the level of TNF- α to enhance the extrinsic pathway in response to higher concentration of sex hormones especially progesterone and other growth factors (12,13), and this explain the highly significant increase in serum TNF- α in

patients of this study compared to healthy controls with cutoff value of ≥ 0.220 pg/ml, 97.5% sensitivity and 100% specificity for leiomyoma with accuracy rate of 99.6%.

In this study there is a significant increase in serum sFas in patients compared to controls. The cutoff value is ≥ 0.154 pg/ml with 80% sensitivity and 82.5% specificity for leiomyoma and accuracy rate of 87.4%. The increase in serum sFas concentration can be considered the way by which leiomyoma tumor evade apoptosis:

The TNF- α enhance the extrinsic apoptosis pathway when the Fas, TNF and other Tumor necrosis factor Related Apoptosis Inducing Ligand (TRAIL) interact with their corresponding death receptors (DR) (14). Fas is a potent mediator for extrinsic pathway of apoptosis (15). Fas need FasL to start apoptosis, Fas/FasL receptor interaction is the key to start the extrinsic apoptotic pathway (16). Impaired death receptor signalling is one of mechanisms by which tumour evade immune system (36). Impairment of death receptor

signalling by tumour cells can be achieved by production of sFas that act as a decoy for the FasL.(37) The level of FasL affected by the presence of soluble Fas (sFas) that capture FasL so the trimerization of Fas receptor can be inhibited (36,38) and the tumour cells protect themselves against apoptosis (17).

Conclusions:

- ❖ There is a high significant increase in serum concentration of TNF- α in patients with leiomyoma compared to healthy controls. This finding might be one of important mediators responsible for local and systemic manifestation in patients with leiomyoma.
- ❖ There is a significant increase in serum soluble Fas (sFas) concentration, and may be responsible for tumor growth and evasion of immune system by blocking TNF- α and Fas- FasL apoptotic pathway.

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