Bcl-2 Antigen and TNF-alpha Expression in Myomas

Miyomlarda Bcl-2 Antijen ve TNF-alfa Ekspresyonu

Yıldız UYAR, MD,[a]
Nalan NEŞE, MD,[b]
Yeşim BAYTUR, MD,[a]
Hasan YILDIZ, MD,[a]
Ümit İNCEBOZ, MD,[a]
Ali Rıza KANDİLOĞLU, MD[b]

Departments of
[a]Obstetrics and Gynecology,
[b]Pathology,
Celal Bayar University
Faculty of Medicine, Manisa

Geliş Tarihi/*Received:* 22.12.2008
Kabul Tarihi/*Accepted:* 23.06.2009

Yazışma Adresi/*Correspondence:*
Yıldız UYAR, MD
Celal Bayar University
Faculty of Medicine,
Department of Obstetrics and
Gynecology, Manisa,
TÜRKİYE/TURKEY
yildizuyar@ttmail.com

ABSTRACT Objective: Myomas are the most frequently observed benign smooth muscle tumors of the uterus; however, their etiopathogenesis has not been fully clarified yet. In the present study, our aim was to examine Bcl-2 antigen and tumor necrosis factor-alpha (TNF-α) expression in myomas and explore their possible roles in the development and pathogenesis of myoma. **Material and Methods:** Thirty cases (ages 41-50) were included in the study. None of the cases were given hormone treatment and all were histopathologically diagnosed with "myoma uteri" after surgery. Our groups were myoma tissue (group 1), normal myometrium tissue (group 2) and myometrium tissue adjacent to myoma (group 3). In group 1, 2 and 3, the levels of TNF-α and Bcl-2 antigens were evaluated by calculating the immunohistochemical staining ratios. Statistical analyses were carried out using Mann-Whitney U test and Kruskal Wallis test. $p<0.05$ was considered statistically significant. **Results:** The age interval of the 30 patients was 41-50 while the average age was (±SD): 45.83 (±2.97). It was found that, regarding TNF-α and Bcl-2 expression, myomas showed a higher positive histochemical staining rate compared to myometrium and myometrium tissue adjacent to myoma. Such a difference was not found between myometrium and myometrium tissue adjacent to myoma. **Conclusion:** The data obtained shows that TNF-α, whose regulatory mechanism in uterus has not been clearly understood yet, may have a role in the development of myoma. Likewise, showing Bcl-2 expression supports that inhibition of apoptosis may have a role in the pathogenesis.

Key Words: Myoma; Tumor necrosis factor-alpha; apoptosis regulatory proteins; growth differentiation factors

ÖZET Amaç: Miyomlar, uterusun en sık rastlanan benign düz kas tümörleridir, ancak hala etiyopatogenezleri tam olarak aydınlatılamamıştır. Bu çalışmada, miyomlarda Bcl-2 antijeni ile tümör nekroz faktörü-alfa (TNF-α) ekspresyonunu araştırmak ve miyom gelişimi ve patogenezindeki olası rollerini aydınlatmayı amaçladık. **Gereç ve Yöntemler:** Otuz olgu çalışmaya dahil edildi. Olguların hiçbiri hormon tedavisi almadı ve cerrahi sonrası tümüne histopatolojik olarak 'miyoma uteri' tanısı kondu. Miyom dokusu (grup 1), normal miyometriyum dokusu (grup 2) ve miyoma komşu miyometriyum dokusu (grup 3) gruplarımızı oluşturdu. Grup 1, 2 ve 3'te TNF-α ve Bcl-2 antijenleri düzeyleri immünohistokimyasal boyanma oranları hesaplanarak değerlendirildi. İstatistiksel değerlendirmeler Mann-Whitney U test ve Kruskal Wallis testi kullanılarak yapıldı. $P<0.05$ istatistiksel olarak anlamlı kabul edildi. **Bulgular:** Toplam 30 olgunun yaş aralığı: 41-50, yaş ortalaması (±SD): 45.83 (±2.97) idi. Miyomların, miyometriyum ve miyoma komşu miyometriyum dokusu ile karşılaştırıldığında TNF-α ve Bcl-2 ekspresyonu için immünohistokimyasal boyanma derecesi açısından yüksek oranda pozitiflik gösterdiği saptandı. Miyometriyum ve miyoma komşu miyometriyal doku arasında böyle bir farklılık saptanmadı. **Sonuç:** Elde edilen bulgular, günümüzde uterusta düzenleyici mekanizmasının net olarak anlaşılamadığı TNF-α'nın miyom gelişiminde rolü olabileceğini göstermekte, aynı şekilde Bcl-2 ekspresyonunun gösterilmesi de patogenezde apoptozis inhibisyonunun rolü olabileceği görüşünü desteklemektedir.

Anahtar Kelimeler: Miyom; tümör nekroz faktörü-alfa; apoptozis regulatuar proteinler; büyüme faktörleri

Turkiye Klinikleri J Gynecol Obst 2009;19(5):249-54

Myomas, the most frequently observed benign tumors during reproductive age, are also shown as the most important cause of menstrual bleeding complaints experienced during reproductive age.[1] The pathogenesis of myoma has not been fully clarified yet. Up to date, some differences have been shown between myomas and myometriums surrounding myomas.[2]

As myomas grow during reproductive age and shrink during postmenopausal period, it is thought that steroids may have the most important role in the etiology of myomas. Estrogen receptor has been found to be more intensive in myomas, and PR-A, a progesterone receptor, has been reported to have increased levels in myomas.[3,4] However, there exist controversial views on whether menstrual cycle phases cause significant difference on mitotic activity in myomas. Dokmeci et al showed that physiological hormonal changes in the cycle do not cause a difference in mitotic activity in myomas while some researchers studying the relation between menstrual cycle phases and mitotic activity in myoma found an increase in mitotic activity at the secretory phase due to the effect of progesterone.[5-7] Along with steroids, various growth factors have been shown to have effects on the development of myoma. Steroids were found to stimulate growth factor expression, and growth factors were found to increase the gene transcription regulated by steroids. It has been reported that epidermal growth factor (EGF) and insulin-like growth factors (IGF-1 and IGF-2) are more intensive in myomas compared to myometrium, and binding proteins (IGF-BP 1, 2 and 3) are found in myomas.[8,9] Although vascular endothelial growth factor (VGEF) expression in myoma is not regulated by steroids, VGEF has also been reported to be connected to steroids in myometrium surrounding myoma.[10]

Bcl-2 is a protein that is the physiological inhibitor of programmed cell death, namely apoptosis, and it is accepted as "generalized cell death suppresor".[11] While Bcl-2 expression is high in endometrial hyperplasia, it is known that its expression is low in endometrial carcinoma. Programmed cell death prolonged as a result of the inhibition of apoptosis by Bcl-2 is thought to give rise to the development and continuity of hyperplastic lesion.[12]

TNF-α, which is a molecule that stimulates catabolism in the whole body, is also called "cachectin" as it is liable from cachexin observed in malignant diseases and chronic infection.[13] At the beginning of inflammation, TNF-α activates endothelium along with various complements, histamine and thrombin. Various adhesion molecules produced from activated endothelium cause endothelium to become more active. Thus, inflammation is maintained by a chronic development.[14] As TNF-α mRNA synthesis is inhibited by estrogen in endometrium, its rise close to menstruation has been linked with estrogen withdrawal. The mechanism of its steroid mediated regulation and the regulatory mechanism of TNF-α expression in uterus have not been fully understood yet.[15] Biological functions of TNF-α change based on its concentration. In low concentrations, it acts both as a vasodilator and as a fibroblast factor. In high concentrations, an endocrine effect is observed and this causes fatal changes.[16] Along with helping immune system, TNF-α, with all these characteristics, is also effective in the development of many pathologies.

In our study, our aim was to evaluate Bcl-2 antigen, which is a physiological inhibitor of apoptosis, and TNF-α expression, which is thought to have a role in cell growth and differentiation, in myoma, myometrium and myometrium tissue adjacent to myoma of each patient based on their immunohistochemical staining ratio and to explore their possible roles in the development and pathogenesis of myoma.

MATERIAL AND METHODS

CASES

This project was supported by Celal Bayar University, Medical Faculty, Scientific Research Fund and the study was initiated after the ethics committee of Celal Bayar University, Medical Faculty Hospital approved the study protocol. 30 cases (ages 41-50) applying to our Obstetrics and Gynecology outpatient clinic and having diagnosed with

myoma uteri were included in the study. All the patients were asked to sign an informed consent form that includes detailed information about the study. All the cases were in perimenopausal period and none had received hormone treatment. All the cases underwent hysterectomy during proliferation phase of menstruation, and the diagnosis of "myoma uteri" were diagnosed histopathologically in all the patients. Our groups were myoma tissue (group 1), myometrium tissue (group 2) and myometrium tissue adjacent to myoma (group 3).

IMMUNOHISTOCHEMICAL METHOD

In 30 cases, myometrium samples adjacent to leiomyoma and myoma were taken while in randomly selected 10 cases myometrial tissue samples were taken. These myometrial tissue samples were taken from areas far away from myoma. The samples were fixed in 10% buffered formaline and embedded in paraffin. Using immunohistochemical method, TNF-α and Bcl-2 were studied on sections obtained from the paraffin blocks. Leiomyoma samples were taken from undegenerated areas and from the myoma having the biggest diameter if the case has multiple myomas.

5 μ sections were placed on slides coated with Poly-L-Lysine. Slides were incubated at 60°C for 16 hours. After deparafination with xylene and rehydration with alcohol, they were kept in 10% hydrogen peroxide for 10 minutes. Then, they were washed 4 times with tris buffered saline (TBS) solution, subjected to antigen retrieval in 10 mM sodium citrate solution (pH 6.0) and kept in microwave (home type) 4 times at maximum energy (720 W) with 5 minutes intervals. They were then cooled in room temperature for 20 minutes. All the sections were washed 4 times with TBS. In order to prevent non-specific background staining, samples were subjected to protein blockage for 5 minutes in room temperature before primary antibody application.

For 30 minutes, sections were incubated using monoclonal mice antibody directed against TNF-α (Histopathology Ltd., 10026.0.5, 0.1 mg/0.5 mL, 1:75) and monoclonal mice antibody directed against Bcl-2 (Neomarkers, MS-123-P, 1 mg/1 mL, 1:25). After washed by TBS for 4 times, sections were first kept in biotinylated anti-immunoglobuline (Lab Vision biotinylated antipolyvalent immunoglobuline TP-125-BN) solution for 20 minutes. Then, washed with TBS for 4 times and kept in streptavidine-peroxidase (Lab Vision Streptavidine Peroxidase, TS-125-HR) for 20 minutes and washed with TBS for 4 times again. Liquid diamino benzidine (DAB) (Lab Vision, K3468) was applied for 20 minutes as a chromogene. After the sections were washed with distilled water, a contrast staining was performed using acid-free and alcohol-free Mayer's hematoxylene, then sections were washed under tap water and rehydrated through alcohol series. Slides were dried and coverslipped with Entellan. All the incubations were carried out at room temperature in humid and covered environment. pH 7.6, 0.05 M TBS was used in all the washings.

IMMUNOHISTOLOGICAL EVALUATION

Immune stainings were semiquantitatively scored based on the staining ratio and intensities of the positive cells in all the section. Granulomatous inflammatory lymph node and tonsil were used as positive controls for TNF-α and Bcl-2, respectively. Scoring system was degreed as no staining in smooth muscle cells (0), 1-25% staining (+1), 26-50% staining (+2), 51-75% staning (+3) and 76-100% staining (+4).

Statistical analyses were carried out using Mann-Whitney U test for comparisons between two groups and using Kruskal Wallis test for comparisons among three groups. $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the patients was (±SD): 45.83 (±2.97) while the age interval was 41-50. In 74% of the patients, myomas were 2-6 cm and the mean myoma diameter of the patients was 4.86 cm.16 cases had applied to our clinic with menorrhagia, 5 with low back pain, 1 with abdominal distention and 8 for routine controls (Figure 1).

Regarding TNF-α and Bcl-2, myomas showed a higher positive histochemical staining ratios com-

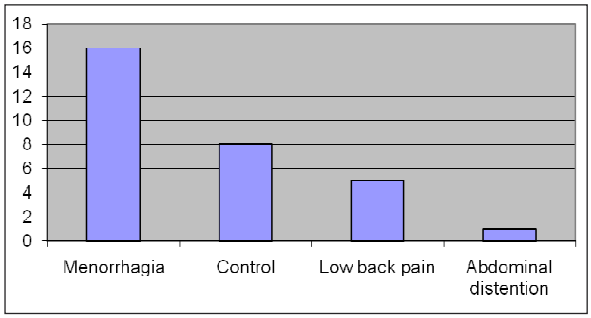

FIGURE 1: Complaints and their distribution.

pared to myometrium and myometrium tissue adjacent to myoma. Such a difference was not found between myometrium and myometrium tissue adjacent to myoma (Figure 2 and Figure 3).

In myomas, the mean staining ratio was 60.75% (+3) for TNF-α and 68.25% (+3) for Bcl-2. In myometrium, the mean staining ratio was 37.5(+2) for TNF-α and 45% (+2) for Bcl-2. In myometrium adjacent to myoma, the mean staining ratio was 38.25% (+2) for TNF-α and 37.5% (+2) for Bcl-2.

In Kruskal-Wallis test, statistically significant differences were found among the mean staining ratios of myoma, myometrium and myometrium tissue adjacent to myoma for TNF-α and Bcl-2 (p= 0.001, p= 0.000 respectively).

In Mann-Whitney Test, when the mean staining ratios of myoma, myometrium and myometrium tissue adjacent to myoma were compared for TNF-α and Bcl-2, myoma mean staining ratio was higher than myometrium and myometrium tissue adjacent to myoma mean staining ratios. The differences were statistically significant [p= 0.016, p= 0.001 (TNF-α); p= 0.019, p= 0.009 (Bcl-2) respectively] (Table 1). Hovewer, when the mean staining ratios of myometrium and myometrium tissue adjacent to myoma were compared for TNF-α and Bcl-2, no significant differences were found between the mean staining ratios (p= 0.669, 0.158 respectively) (Table 1).

DISCUSSION

Uterine leiomyomas are diagnosed frequently in about 20-25% of the women over 30.[9] In the development of myomas, effects of growth factors and sex steroids and factors effecting apoptosis are intermingled.[17]

The activity of estrogen is mediated through local growth factors such as IGF-1 and EGF.[18] Progesterone increases EGF and Bcl-2 expression and decreases IGF-1 and TNF-α expression.[17] Progesterone contributes to the growth of myoma cells by increasing the expression of Bcl-2 protein and estrogen contributes the same by stimulating the proliferative potential of myoma cells.[19] Bcl-2 increases cell replication by decreasing the growth factor demand of the cells. It is thought that Bcl-2 plays an

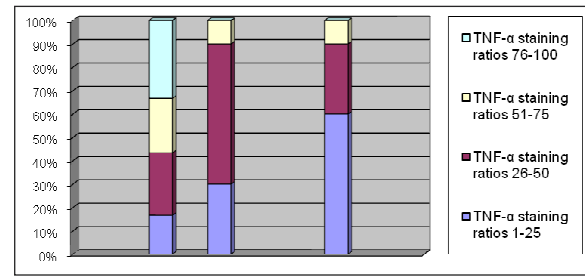

FIGURE 2: TNF-α staining ratios in myoma, myometrium and myometrium tissue adjacent to myoma.

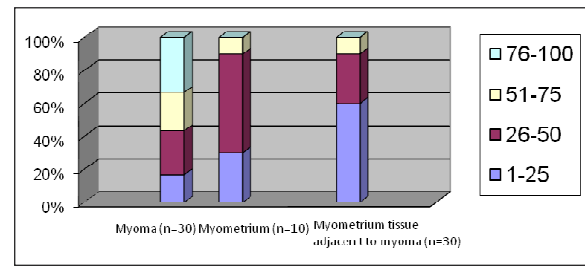

FIGURE 3: Bcl-2 staining ratios in myoma, myometrium and myometrium tissue adjacent to myoma.

TABLE 1: Mann-Whitney test p values obtained with comparison of the mean staining ratios of myoma, myometrium and myometrium tissue adjacent to myoma for TNF-α and Bcl-2.

	Myoma-myometrium	Myoma- myometrium tissue adjacent to myoma	Myometrium-myometrium tissue adjacent to myoma
TNF-α	0.016	0.001	0.669
Bcl-2	0.019	0.009	0.158

important role in tumor development as is extends the life of the cells and allows mutation accumulation.[20]

In the literature, there are many studies examining Bcl-2 expression in myoma growth. Zhang et al showed that Bcl-2 expression is doubled in myomas compared to myometrium, and found that this expression is stronger in the secretory phase.[21] Kawaguchi et al. observed that the biggest proliferative activity in myomas takes place during secretory phase and pregnancy.[22] Based on these findings, progesterone was shown to have a role in Bcl-2 expression. In a study on cases with myoma, leiomyosarcoma and uterine smooth muscle tumor of uncertain malignant potential, Bodner et al, showed that Bcl-2 expression was significantly higher in myomas and thus Bcl-2 expression could be used as a good prognostic marker in patients with uterus smooth muscle tumor.[20] Vu et al found that Bcl-2 expression was not different in never-treated cases compared to cases treated with GnRH analogs for 2-6 months.[23] However, Bcl-2 was higher in both groups when compared to controls.

In our study, a statistically significant difference was found between Bcl-2 staining ratios of myomas and Bcl-2 staining ratios of myometrium and myometrium tissue adjacent to myoma. However, there was no statistically significant difference between the staining ratios of myometrium and adjacent myometrium. This shows us that Bcl-2 expression is increased in myomas and this could have an effect on myoma growth, which is consistent with the literature.

TNF-α, which is a pluripotent proinflammatory cytokine, plays an important role in many autoimmune and inflammatory diseases (systemic lupus erythematosus, systemic cyclerosis, ankylosing spondylitis, diabetes mellitus).[24,25] Although TNF-α has been shown to inhibit proliferation in different cells and induce apoptosis, there is few knowledge on its expression in myoma cells.[24]

Kurachi et al. found that TNF-α expression is much more in myoma cells compared to myometrium cells and that this expression is more significant during proliferative phase.[16] Similarly, they emphasized that progesterone could have an inhibitory effect on TNF-α as TNF-α expression was less in the myoma cells in the uterus of pregnant women. As a result, they thought that TNF-α expression could be a basic molecular characteristic in myomas and progesterone decreases TNF-α levels, thus it may has an important role on development of myomas. Some researcher indicated that progesterone has a dual effect on myoma and it inhibits myoma by down-regulating IGF-1 expression and induced development of myoma by up-regulating Bcl-2 and EGF secretion on one hand and down-regulating TNF-α secretion on the other hand.[26]

Posaci et al. compared TNF-α levels of 20 cases undergoing hysterectomy for myoma and 20 cases having endometriosis diagnosed by laparoscopy to the TNF-α levels of a control group, and they found that neither the endometriosis nor the myoma group showed a significant difference with respect to TNF-α levels when compared to the control group.[27] Moreover, they measured peritoneal liquid TNF-α levels of 9 cases with endometriosis and found no correlation between peritoneal liquid and serum TNF-α levels in these cases. Sikorski et al also compared TNF-α, IL-1 and IL-6 serum levels in cases with and without myoma and found that the only significant increase in myoma cases was in IL-1 levels.[28] On the contrary, they stated that peritoneal liquid TNF-α levels could be used as an indicator of angiogenic activity in peritoneal myomas growth. Therefore, they reached to the idea that more studies had to be conducted with proinflammatory cytokines to study pathogenesis of proliferative diseases of uterus.

In the literature, there are few studies exploring the effects of TNF-α on myoma development. In the present study, we found that TNF-α staining ratio was higher in myomas compared to myometrium and myometrium tissue adjacent to myoma. There were no statistically significant difference between the staining ratios of myometrium and myometrium tissue adjacent to myoma. This shows us that TNF-α may has an inductive role in

myoma development. In our study, all the cases were operated at proliferative period and both Bcl-2 and TNF-α secretion were shown to be higher in myomas In cases with myoma, Bcl-2 secretion was higher during secretion period while TNF-α secretion was higher during proliferation phase which, together with the literature data, indicates that TNF-α is more important in the development of myoma that is known to be sex hormone dependant disease. Future studies on TNF-α expression and the effect mechanism of TNF-α on myomas may clarify this issue.

Determining different cytokines and growth factors in myoma development and exploring their effect mechanisms may allow establishing different and more effective treatment choices for these benign tumors that are observed in women in reproductive age and diminish the quality of life.

REFERENCES

1. Belaisch J.[Leiomyomas. Epidemiology and physiopathologic hypothesis]. Gynécologie 1989;40(3):169-74.
2. Cramer DW. Epidemiology of myomas. Semin Reprod Endocrinol 1992;10(4):320-4.
3. Wilson EA, Yang F, Rees ED. Estradiol and progesterone binding in uterine leiomyomata and in normal uterine tissues. Obstet Gynecol 1980;55(1):20-4.
4. Tamaya T, Fujimoto J, Okada H. Comparison of cellular levels of steroid receptors in uterine leiomyoma and myometrium. Acta Obstet Gynecol Scand 1985;64(4):307-9.
5. Dökmeci F, Ensari A, Telli E, Özbay S, Tulunay O, Dinçer Cengiz S. [The relation ship between mitotic activity and menstrual cyclus phases in uterus myoma]. Turkiye Klinikleri J Gynecol Obst 1993;3(2):158-61.
6. Kawaguchi K, Fujii S, Konishi I, Nanbu Y, Nonogaki H, Mori T. Mitotic activity in uterine leiomyomas during the menstrual cycle. Am J Obstet Gynecol 1989;160(3):637-41.
7. Pavlovich SV, Volkov NI, Burlev VA. Proliferative activity and level of steroid hormone receptors in the myometrium and myoma nodes in different phases of menstrual cycle. Bull Exp Biol Med 2003;136(4):396-8.
8. Lumbsden MA, West CP, Bramley T, Rumgay L, Baird T. The binding of epidermal growth factor to the human uterus and leiomyomata in woman rendered hypo-oestrogenic by continuous administration of an LHRH agonist. Br J Obstet Gynaceol 1988;95(12):1299-304.
9. Vollenhoven BJ, Herington AC, Healy DL. Messenger ribonucleic acid expression of the insulin-like growth factors and their binding proteins in uterine fibroids and myometrium. J Clin Endocrinol Metab 1993;76(5):1106-10.
10. Harrison-Woolrych ML, Sharkey AM, Charnock-Jones DS, Smith SK. Localization and quantification of vascular endothelial growth factor messenger ribonucleic acid in human myometrium and leiomyomata. J Clin Endocrinol Metab 1995;80(6):1853-8.
11. Vinatier D, Dufour P, Subtil D. Apoptosis: a programmed cell death involved in ovarian and uterine physiology. Eur J Obstet Gynecol Reprod Biol 1996;67(2):85-102.
12. Amezcua CA, Zheng W, Muderspach LI, Felix JC. Down-regulation of bcl-2 is a potential marker of the efficacy of progestin therapy in the treatment of endometrial hyperplasia. Gynecol Oncol 1999;73(1):126-36.
13. Mitchell MD, Trautman MS, Dudley DJ. Cytokine networking in the placenta. Placenta 1993;14(3):249-75.
14. Saygılı Ö, Gültekin F. [Intercellular adhesion molecules]. Turkiye Klinikleri J Med Sci 1999;19(6):362-5.
15. Benyo DF, Miles TM, Conrad KP. Hypoxia stimulates cytokine production by villous explants from the human placenta. J Clin Endocrinol Metab 1997;82(5):1582-8.
16. Kurachi O, Matsuo H, Samoto T, Maruo T. Tumor necrosis factor-alpha expression in human uterine leiomyoma and its down-regulation by progesterone. J Clin Endocrinol Metab 2001;86(5):2275-80.
17. Maruo T, Ohara N, Wang J, Matsuo H. Sex steroidal regulation of uterine leiomyoma growth and apoptosis. Hum Reprod Update 2004;10(3):207-20.
18. Murphy LJ, Ghahary A. Uterine insulin-like growth factor-1: regulation of expression and its role in estrogen-induced uterine proliferation. Endocr Rev 1990;11(3):443-53.
19. Maruo T, Matsuo H, Shimomura Y, Kurachi O, Gao Z, Nakago S, et al. Effects of progesterone on growth factor expression in human uterine leiomyoma. Steroids 2003;68(10-13): 817-24.
20. Bodner K, Bodner-Adler B, Kimberger O, Czerwenka K, Mayerhofer K. Bcl-2 receptor expression in patients with uterine smooth muscle tumors: an immunohistochemical analysis comparing leiomyoma, uterine smooth muscle tumor of uncertain malignant potential, and leiomyosarcoma. J Soc Gynecol Investig 2004;11(3):187-91.
21. Zhang ZL, Zhang Y, Xu BQ. [Expression of bcl-2 protein in uterine leiomyomas and normal myometrium]. Hunan Yi Ke Da Xue Xue Bao 2001;26(4):363-5.
22. Kawaguchi K, Fujii S, Konishi I, Iwai T, Nanbu Y, Nonogaki H, et al. Immunohistochemical analysis of oestrogen receptors, progesterone receptors and Ki-67 in leiomyoma and myometrium during the menstrual cycle and pregnancy. Virchows Arch A Pathol Anat Histopathol 1991;419(4):309-15.
23. Vu K, Greenspan DL, Wu TC, Zacur HA, Kurman RJ. Cellular proliferation, estrogen receptor, progesterone receptor, and bcl-2 expression in GnRH agonist-treated uterine leiomyomas. Hum Pathol 1998;29(4):359-63.
24. Hoffstedt J, Eriksson P, Hellström L, Rössner S, Rydén M, Arner P. Excessive fat accumulation is associated with the TNF alpha-308 G/A promoter polymorphism in women but not in men. Diabetologia 2000;43(1):117-20.
25. Pociot F, Briant L, Jongeneel CV, Mölvig J, Worsaae H, Abbal M, et al. Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-alpha and TNF-beta by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 1993;23(1):224-31.
26. Maruo T, Ohara N, Matsuo H, Xu Q, Chen W, Sitruk-Ware R, et al. Effects of levonorgestrel-releasing IUS and progesterone receptor modulator PRM CDB-2914 on uterine leiomyomas. Contraception 2007;75(6 Suppl):S99-103.
27. Posaci C, Acar B, Güçlü S, Kırkali G, Altunyurt S, Önvural A, Uslu T. [The importance of tumor necrosis factor-α (TNF-α) in the diagnosis of endometriosis and uterin fibroids]. Turkiye Klinikleri J Gynecol Obst 2000;10(1):45-8.
28. Sikorski R, Kapeć E, Zaleska W. [Serum levels of proinflammatory cytokines in women with uterine myomas] Ginekol Pol 2001; 72(12A):1485-8.