Orjinal Araştırma **Van Sağ. Bil. Derg.** *2025; 18(1):14-19.*
Original Research *https://doi.org/10.52976/vansaglik.1478714*

Comparison of Bone Morphogenetic Protein-2 Secretion Capacity of Myoma, Myometrium and Endometrium

Myom, Myometriyum ve Endometriyum Dokularının Bone Morfogenetik Proteini -2 Salgılama Kapasitesinin Karşılaştırılması

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Cited: Aykanay Y, Aktun L, Sagır Gökdağlı F, Dündar B, Altuntai ŞL, Yiğitbaşı T. (2025). Comparison of Bone Morphogenetic Protein-2 Secretion Capacity of Myoma, Myometrium and Endometrium. *Van Sağlık Bilimleri Dergisi*, 18(1), 14-19.

ABSTRACT

Objective: Bone morphogenetic protein-2 (BMP-2) is a member of the transforming growth factor β3 (TGF-β3) family and plays a crucial role in decidualization and implantation. Myomas can adversely affect endometrial receptivity and embryo implantation. Recent studies have highlighted the importance of understanding the molecular mechanisms underlying endometrial receptivity. The objective of this study is to determine the BMP-2 secretion capacity of intramural, subserous, and submucous myomas, as well as their effects on BMP-2 levels in endometrial tissue.

Material and Method: Ninety-seven women who underwent hysterectomy or myomectomy because of symptomatic myomas were divided into three groups as submucous (SMM, n=26), intramural (IMM, n=34), and subserous (SSM, n=37). Myoma, myometrium and endometrial tissue samples were isolated and homogenized. BMP-2 concentrations of myoma, myometrium and endometrium tissue samples were evaluated with ELISA kits.

Results: BMP-2 levels showed no significant difference between myoma samples of SMM, IMM, SSM (p>0,05). Within the groups, endometrium of SMM have the lowest BMP-2 levels (2417±720 pg/ml, p<0.05). BMP-2 levels in endometrium of SSM and IMM groups were higher than myoma and myometrium of SSM and IMM groups. SMM group did not show any difference in BMP-2 levels in endometrium, myometrium and myoma.

Conclusion: Subserous, submucous and intramural myomas show different effect on BMP-2 expression in endometrium, myometrium and myoma. SSM and IMM have no impact on BMP-2 expression of endometrium. But SMM alter release of BMP-2 in endometrium. Decreased BMP-2 levels may be the responsible mechanism for impaired implantation and decreased pregnancy rates in this group of patients. Further investigations may lead to the development of novel therapeutics aimed at restoring BMP-2 levels.

Keywords: *Leiomyoma, BMP-2 protein, Infertility*

ÖZET

Giriş: Bone morfogenetik protein-2 (BMP-2), transforming growth faktörü β3 (TGF-β3) ailesinin bir üyesi olup, desidualizasyon ve implantasyonda kritik bir rol oynar. Miyomlar, endometrial reseptivite ve embriyo implantasyonunu olumsuz etkileyebilir. Son çalışmalar, endometrial reseptivitenin temelinde yatan moleküler mekanizmaların anlaşılmasının önemini vurgulamıştır. Bu çalışmanın amacı, intramural, subseröz, submuköz miyomların BMP-2 salgı kapasitesini ve endometrial dokuda BMP-2 seviyelerine olan etkilerini belirlemektir.

Materyal ve Metot: Semptomatik miyomlar nedeniyle histerektomi veya miyomektomi geçiren 97 kadın, submuköz (SMM, n=26), intramural (IMM, n=34) ve subseröz (SSM, n=37) olmak üzere üç gruba ayrıldı. Miyom, miyometrium ve endometrial doku örnekleri izole edilip homojenize edildi. Miyom, miyometrium ve endometrium doku örneklerinin BMP-2 konsantrasyonları ELISA kitleri kullanılarak değerlendirildi.

Bulgular: BMP-2 seviyeleri, SMM, IMM, ve SSM gruplarının miyom örnekleri arasında anlamlı bir farklılık göstermedi (p>0,05). Ancak, bu gruplar içinde, SMM grubunun endometriumunda en düşük BMP-2 seviyelerini saptandı (2417±720 pg/ml, p<0.05). SSM ve IMM gruplarının endometriumundaki BMP-2 seviyeleri, aynı gruplardaki miyom ve miyometrium örneklerinden daha yüksekti. SMM grubu, endometrium, miyometrium ve miyom dokuları arasında BMP-2 seviyelerinde farklılık göstermedi

Sonuç: Subseröz, submüköz ve intramural miyomlar endometriyum, miyometrium ve miyomdaki BMP-2 ekspresyonu üzerinde farklı etkiler gösterir. Çalışmamızda SSM ve IMM'ların endometriyumun BMP-2 ekspresyonu üzerine etkisi gösterilememiştir. SMM'lar ise endometriumda BMP-2 salınımını değiştirmiştir. Azalmış BMP-2 seviyeleri, bu hasta grubunda daha sık karşımıza çıkan implantasyonun bozulması ve azalmış gebelik oranlarından sorumlu mekanizma olabilir. BMP-2 seviyelerinin artırılmasını sağlayan tedaviler, gelecek çalışmalar için araştırma konusu olabilir.

Anahtar kelimeler: *Leiomiyom, BMP-2 protein, İnfertilite*

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Received: 05.05.2024, Accepted: 24.03.2025 and Publeshed:30.04.2025

INTRODUCTION

The role of uterine myomas in infertility has been hypothesized for many years. Several attempts have been made to demonstrate the relationship between these two topics seen common in reproductive-age women.

The incidence of myomas in unexplained infertility is approximately 1-2,4% and myomas are present in 10% of patients with infertility (Donnez and Jadoul, 2002). Studies evaluating the effects of myomas on infertility suggest that intramural myomas are related to decreased pregnancy rates while subserous myomas do not affect infertility (Bajekal and Li, 2000). Contrary to these, submucous myomas are found to be linked with infertility. And in these cases, myomectomy may increase pregnancy rates (Pritts et al., 2009). Despite the controversial data in the literature, submucous or cavity distorting myomas have more impact on pregnancy and implantation rates (Klatsky et al., 2008).

Data from many studies suggest that myomas decrease pregnancy rates by several different mechanisms. Distorted endometrial cavity, chronic endometrial inflammation, changes in uterine contractility, endometrial hormonal milieu and vascularization can be predisposing factors (Donnez and Jadoul, 2002; Rogers et al., 2008).

In a systematic review, Don et al revealed that fibroid-associated infertility could be potentially caused by alterations in the BMP- pathway in the endometrium, possibly leading to disturbed angiogenesis and reducing the success of implantation (Don et al., 2023)

Excessive production of extracellular matrix components can convert fibroid-related mechanical signals into biologic signals that affect the endometrium. Increased ECM stimulates the synthesis of transforming growth factor b (TGF-b) by altering intrafibroid microRNA production (Dokuzeylul Gungor et al., 2023)

Studies on underlying molecular mechanisms represent a growing field. Local growth factors secreted from myoma cells affect the myometrium and endometrium functions. TGF-β3 secreted from myomas impair expression of receptivity genes such as HomeoboxA10 (HOXA-10) and leukocyte inhibitory factor (LIF) (Arici and Sozen, 2000; Sinclair et al., 2011). Bone morphogenetic proteins (BMPs) are multifunctional growth factors that belong to the transforming growth factor β (TGF-β) superfamily. Studies report that BMP-2 expression in endometrium is necessary for successful homing, attachment and implantation of embryos (Li et al., 2007). TGF-β3 secreted from myomas inhibit endometrial BMP-2 activity by decreasing expression of BMP-2 receptors which results in decreased fertility rates.

In this study, we aimed to show BMP-2 secretion capacity of intramural, submucous and subserous myoma tissues and compare them with the BMP-2 levels of myometrium and endometrium.

MATERIAL and METHOD

Patient selection

We collected samples of myomas from 97 patients who were admitted to our hospital with symptoms such as pelvic pain, abnormal menstrual bleeding. These patients had undergone abdominal myomectomy or hysterectomy for these surgical indications and findings including endometrial hyperplasia, and endometrial polyp. We excluded patients who were using hormonal drugs or had evidence of other uterine, endometrial, or ovarian pathologies from the study groups.

Patients divided into 3 groups as submucous (SMM n=26), intramural (IMM n=34), and subserous myoma (SSM n=37) groups. Myoma, myometrium and endometrium tissue samples dissected from surgical specimens separately. Each surgical specimen was washed three times with sterile saline to remove blood, then transferred to RNAlater solution and stored at -80°C for future analysis.

Ethical approval

Ethical approval was issued by Istanbul Medipol University Ethical Committee with the registration number of 10840098-604.01-E.11889 in 2017. The procedures were in accordance with the ethical standards of the responsible local or national committee on human experimentation and with the Helsinki Declaration. Prior to specimen collection, all patients provided written informed consent.

Test principle of BMP 2

Myoma, endometrium and myometrium tissues minced to small pieces and rinsed in ice-cold Phosphate Buffered Saline (PBS) (1% mannitol, pH:7,4) to remove excess blood thoroughly. Tissue pieces weighed and then homogenized in PBS (tissue weight (g) / PBS volume (mL) = 1:9) with QIAGEN TissueLyser homogenizer. To further break the cells, samples sonicated with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates centrifuged for 5 min, at 5000 xg to get the supernatant. BMP-2 levels were measured in tissue samples by commercially available ELISA kits (E-lab science, E-EL-H0011, China) according to the manufacturer's specifications.

Statistical Analysis

The Statistical Package for Social Sciences, version 24.0 (SPSS Inc., Chicago, IL, USA) used for statistical analysis. BMP-2 and other individual group parameters assessed with one-sample Kolmogorov–Smirnov Z test and found abnormally distributed except age. Hence, statistical comparisons between groups performed by nonparametric Mann-Whitney

U test. ANOVA test is used for age. Kruskar wallis test used to compare three groups according to locations of myomas. Data presented as mean ± standard deviation (SD). For all comparisons, $p<0.05$ defined statistical significance.

RESULTS

A total of 97 patients included in the study. The study groups are divided into 3 groups according to localizations of myomas in the myometrium (submucosal myomas (SMM) n=26, intramural myomas (IMM) n=34 and subserous myomas (SSM) n=37). The mean age of patients was 44.09 ± 7.71 and did not show a significant difference between groups ($p>0.05$).

The results, as shown in Table 1, indicate that in SSM group BMP-2 levels were lowest in myoma (2524.84 ± 853.34 pg/ml). Endometrium showed higher BMP-2 levels than myoma and myometrium. There was a significant difference between endometrium and myoma samples (p=0.020).

Table 1. BMP-2 concentrations of myoma, endometrium and myometrium in subserous myoma group and comparisons between groups

	Subserous myomas	BMP-2 concentrations (pg/ml)
Group I	Myoma	2524.84± 853.34
Group II	Endometrium	3258.35± 458.19
Group III	Myometrium	3068.74± 400.44
	I vs II	0.020*
	I vs III	0.123
	II vs III	0.309

Mean ± SD, *p<0.05, BMP-2: Bone morphogenetic protein 2 (pg/ml)

Data from table 2 presents lower BMP-2 concentrations in intramural myoma samples compatible with subserous myoma group (2558.87±989.27 pg/ml). BMP-2 levels showed a significant difference between endometrium and myoma (p=0.039). As table 3 shows, endometrium and myometrium tissues did not show a significant difference in submucous myoma group.

Table 2. BMP-2 concentrations of myoma, endometrium and myometrium of IMM group and comparisons in between groups

	Intramural myomas	BMP-2 concentrations (pg/ml)
Group I	Myoma	2558.87 ± 989.27
Group II	Endometrium	3366.14 ± 345.35
Group III	Myometrium	3244.32 ± 325.47
	I vs II	0.039*
	I vs III	0.082
	II vs III	0.375

Mean ± SD, *p<0.05 BMP-2: Bone morphogenetic protein 2 (pg/ml).

Table 3. BMP-2 concentrations of myoma, endometrium and myometrium of SMM group and comparisons between groups.

	Submucous myomas	BMP-2 concentrations (pg/ml)
Group I	Myoma	2527.57 ± 990.13
Group II	Endometrium	2417.16 ± 719.89
Group III	Myometrium	2400.84 ± 803.06
	I vs II	0.655
	I vs III	0.565
	II vs III	0.749

Mean ± SD, BMP-2: Bone morphogenetic protein 2 (pg/ml)

Closer inspection of the table 4 presents that within the groups endometrial tissue BMP-2 concentrations showed a significant difference and SMM endometrium tissues have the lowest BMP-2 levels (2417±720 pg/ml, p<0.05). (Figure I).

Table 4. Comparisons of BMP-2 concentrations in myoma, endometrium and myometrium according to locations of myomas

BMP-2 (pg/ml)	IMM (n=34)	SSM (n=37)	SMM (n=26)	P value
Myoma	2524±853	2559±989	2528±990	0,892
Endometrium	3258±458	3366±345	2417±720	0,023*
Myometrium	3069±400	3244±325	2400±803	0,059

Mean ± SD, p<0.05, SS: Subserous myoma, SMM: Submucous myoma, IMM: Intramural Myoma, BMP-2: Bone morphogenetic protein 2 (pg/ml)

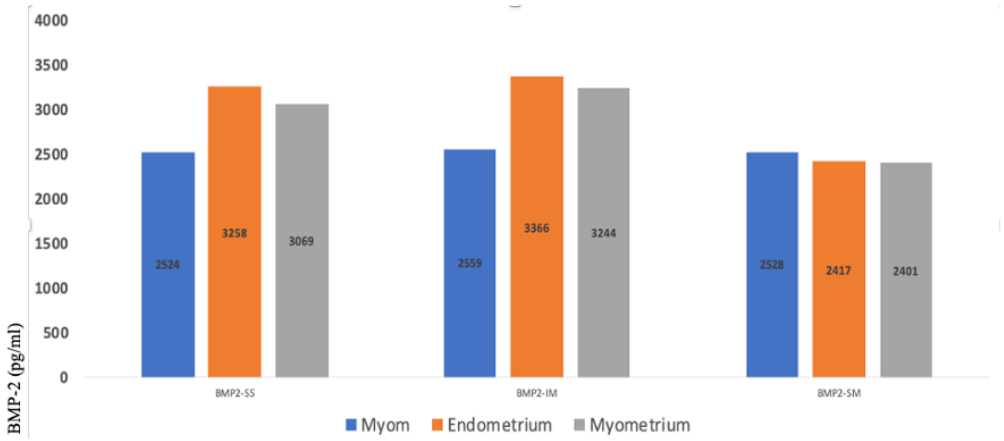

Figure 1. Comparisons of BMP-2 levels in myoma, endometrium and myometrium tissues according to the localization of myomas. BMP-2: Bone morphogenetic protein 2 SSM: Subserous myoma SMM: Submucous myoma, IMM: Intramural myoma

DISCUSSION

Uterine myomas are indolent benign tumors of women's reproductive system. Symptoms are usually associated with their size and location. It's well-known that submucous myomas can cause menorrhagia, abortion, pelvic pain even in small dimensions while subserous myomas are clinically asymptomatic. In this study, we aimed to show the difference among BMP-2 secretion levels of endometrium, myometrium and myoma tissues according to anatomic locations of myomas.

In our study, we showed that BMP-2 concentrations of myoma tissues in SSM-IMM groups are less than myometrium and endometrium of same patients. In SMM group, we showed no difference among myometrium, myoma and endometrium. Closer inspection of the results showed myomas secrete less BMP-2 and SSMs- IMMs do not affect BMP-2 concentrations of endometrium. In SMM group, BMP-2 concentrations showed no difference in endometrium, myometrium and myoma. With these results we concluded that submucous myomas, despite the small sample size, may influence BMP-2 concentrations of endometrium.

The literature on molecular effects of myomas on endometrium and endometrial receptivity have highlighted several mechanisms. Local growth factors secreted from myoma cells affect the myometrium and endometrium functions. Myomas are monoclonal tumors that secrete excess TGF-β3, which known to regulate transcription factors on endometrium. TGF-β3 secretion of myomas causes BMP-2 receptor resistance. In the implantation window, HOXA 10-HOXA 11 play a crucial role and BMP-2 receptor resistance causes down regulation of these proteins (Arici and Sozen, 2000; Sinclair et al., 2011) Likewise TGF-β3 secreted from myoma cells impair implantation promoting activity of BMP-2 signaling in the endometrium. BMP-2 expression in the endometrium is necessary for successful homing, attachment and implantation of embryos and decidualization (Zhao et al., 2018). Li et al. showed induction of BMP-2 during decidualization in vitro endometrial stromal culture (Li et al., 2007)

Endometrial tissues of patients with myomas have less BMP-2 levels compared to control groups (Sinclair et al., 2011) Doherty et al. showed in their study that myoma conditioned endometrial stromal cells present less BMP-2 response and concluded that TGF-β3 blockade could be a targeted therapy for myoma related infertility (Doherty and Taylor, 2015)

Many studies in the literature have emphasized that uterine structural anomalies like myomas, polyps and septums affect endometrial receptivity (Munro, 2019) Submucosal myoma samples expressed less endometrial HOXA 10 and HOXA 11 messenger RNA (mRNA) compared to IMM (Rackow and Taylor, 2010) Excision of submucous myomas is associated with increased clinical pregnancy and live-birth rates (Casini et al., 2006) A systematic review showed that patients with non-cavity distorting IMM have 15% decreased pregnancy rate in in vitro fertilization (IVF) cycles (Sunkara et al., 2010) A recent study by Unlu et al. reported that myomectomy of not cavity distorting intramural myomas improve endometrial receptivity by enhancing expression of homeobox genes (Unlu et al., 2016) On the other hand, a systematic review showed no difference in clinical pregnancy rate, live birth rate or miscarriage rate in patients with IMM and myomectomy of these tumors did not improve pregnancy rates (Metwally et al., 2011) In a review by Munro it was claimed that intramural myomas affect by paracrine ways and therefore the interspace between endometrium and myomas gain importance. Related with this it was concluded that myomas with less myometrial-endometrial distance most likely have negative effect on implantation (Munro, 2019)

Güngör et al. thought that fibroid derived- TGF-β3 diffusing into the endometrium and blocking BMP-2 receptors reduces LIF and HOXA levels. In their study, HOXA and LIF increased after performing myomectomy. They concluded that myomectomy reactivates endometrial BMP-2 receptors by eliminating TGF-β3-expressing cells, thus reinitiating HOXA and LIF production (Dokuzeylul Gungor et al., 2023).

This study has several potential limitations. The results from our small study need to be confirmed with larger studies. Endometrial receptivity and the implantation process are very complex and involve many factors working together. Because of this, it is hard to focus on the effect of just one factor and ignore the others. However, understanding how BMP2 affects implantation is an important step in exploring these processes.

Conclusion

Bone morphogenetic protein-2 (BMP-2) is part of the TGF-β3 family and plays a key role in implantation and the preparation of the endometrium. Myomas can negatively effect implantation. This study highlights that BMP-2 levels are lower in tissues with myomas, which may affect implantation and endometrial function. Understanding factors like BMP-2 could help create new treatments in the future, especially for patients with implantation failıres. Larger studies are needed, but knowing how BMP-2 and other pathways work can help in managing myoma cases and improving outcomes.

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

Ethical approval

Ethical approval was issued by Istanbul Medipol University Ethical Committee with the registration number of 10840098-604.01-E.11889 in 2017.

Funding

No funding.

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