ORIGINAL RESEARCH ORİJİNAL ARAŞTIRMA

Clinical Value of Serum MMP 9 Levels for Fertility Prognosis in Women with Endometriosis

Endometriozisli Kadınlarda Serum MMP 9 Düzeylerinin İnfertilite Prognozu İçin Klinik Değeri

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ABSTRACT

Objectives: The prevalence of endometriosis ranges between 2-10% among reproductive age women. For cases suffering from endometriosis and infertility, the surgical assessment of pelvis and reproductive organs by r-ASRM scoring does not guide the clinicians to an appropriate management strategy after surgery. This study aims to reveal the value of Matrix Metalloproteinase 9 (MMP 9) levels in infertile women who have histo-pathologically confirmed endometriosis and its correlation with EFI scores and prediction of good fertility prognosis. **Material and Methods:** This cross-sectional study conducted in the university hospital settingThirty-four infertile women with histo-pathologically confirmed endometriosis were included. Age, body mass index, duration of infertility, and levels of Ca-125, MMP 9 and, revised American Society of Reproductive Medicine(r-AFS) and Endometriosis Fertility Index (EFI) scoresof each patient were recorded. The good fertility prognosis was defined as an EFI score ≥7. **Results:** EFI scores were correlated with, age, duration of infertility, severity of the disease, Ca-125 and MMP 9 levels. The multiple linear regression analysis revealed that MMP 9is an independent predictor for severe endometriosis andfor EFI scores. The multivariate logistic regression analysis revealed that MMP 9 was the sole non-surgical factor predicting an EFI score ≥7. **Conclusion:** If validated by larger prospective trials, MMP 9 seems to be a promising serum marker for prediction of good fertility prognosis for women with endometriosis.

Keywords: Endometriosis; infertility; Matrix Metalloproteinase 9

ÖZET

Amaç: Endometriozis prevalansı üreme çağındaki kadınlarda %2-10 arasında değişmektedir. Endometriozis ve infertiliteden muzdarip vakalarda, r-ASRM skorlaması ile pelvis ve üreme organlarının cerrahi değerlendirmesi, klinisyenleri ameliyattan sonra uygun bir yönetim stratejisine yönlendirmez. Bu çalışma, histopatolojik olarak doğrulanmış endometriozisi olan infertil kadınlarda Matriks Metalloproteinaz 9 (MMP 9) düzeylerinin değerini ve EFI skorları ve fertilite prognozunun tahmini ile ilişkisini ortaya koymayı amaçlamaktadır. **Gereç ve Yöntemler:** Üniversite hastanesi ortamında yürütülen bu kesitsel çalışmaya histopatolojik olarak doğrulanmış endometriozisi olan otuz dört infertil kadın dahil edildi. Her hastanın yaşı, vücut kitle indeksi, infertilite süresi ve Ca-125, MMP 9 düzeyleri ve revize edilmiş Amerikan Üreme Tıbbı Derneği (r-AFS) ve Endometriozis Fertilite İndeksi (EFI) puanları kaydedildi. İyi fertilite prognozu, EFI puanı ≥7 olarak tanımlandı. **Bulgular:** EFI puanları, yaş, infertilite süresi, hastalığın şiddeti, Ca-125 ve MMP 9 düzeyleri ile ilişkiliydi. Çoklu doğrusal regresyon analizi, MMP 9'un şiddetli endometriozis ve EFI puanları için bağımsız bir öngörücü olduğunu ortaya koydu. Çok değişkenli lojistik regresyon analizi, MMP 9'un EFI skoru ≥7'yi öngören tek cerrahi olmayan faktör olduğunu ortaya koydu. **Sonuç:** Daha geniş prospektif çalışmalarla doğrulanırsa, MMP 9 endometriozisli kadınlar için fertilite prognozunun tahmini için umut verici bir serum belirteci gibi görünüyor.

Anahtar Kelimeler: Endometriozis; kısırlık; Matris Metalloproteinaz 9

The prevalence of endometriosis ranges between 2-10% among reproductive age women.[1] It is one of the major causes of infertility and the prevalence reaches about 20-40% among infertile women.[2] The gold standard diagnostic test for diagnosis of endometriosis is laparoscopycombined with histopathological confirmation of ectopic endometrial glands and/or stroma.[1] The diagnosis by laparoscopy concurrently includes scoring according to revised American Society of Reproductive Medicine (r-ASRM).[3] However, for cases suffering from endometriosis and infertility, the surgical assessment of pelvis and reproductive organs by r-ASRM scoring does not guide the clinicians to an appropriate management strategy after surgery.

In order to predict their fertility outcomes, 'Endometriosis Fertility Index (EFI)' has been developed for infertile women with endometriosis as a new scoring system.[4] EFI, includes all the components of the r-ASRM score, but also a detailed assessment of the functions of fallopian tubes (connecting the ovaries to the uterus), fimbria (finger extensions at the end of the fallopian tubes) and ovaries. The score, calculated according to the history and surgical findings of the patient, surpassing r-ASRM score, predicts the chance of non-ART pregnancy and guides the clinical management regarding infertility related with endometriosis in the presence of functional gametes and the uterus.[4]

Matrix metalloproteinases (MMPs) are extracellular endopeptidases, which have a significant role in degradationand remodelling of extracellular matrix for cellular migration and invasion.[5] MMP2 and MMP 9 constitute gelatinase subgroup of MMPs, secreted frommacrophages and neutrophil leukocytes.[6] Especially MMP 9, have been shown to be elevated in eutopic/ ectopic endometrial tissue in women with endometriosis andboth have been suggested to have a role in pathogenesis of endometriosis by promoting invasionof the endometriotic lesions.[7-9] Moreover, MMP 9 has been shown to be higher in women with endometriosis but also correlated with the severity of the disease.[10]

The current study aims to reveal the value of MMP 9 levels in infertile women who have histopathologically confirmed endometriosis and its correlation with EFI scores and prediction of good fertility prognosis.

MATERIAL AND METHODS

STUDY DESIGN

This cross- sectional study was conducted between November 2016 and July 2017 in aUniversity Hospital setting and the study was approved by the local ethical committee (No: 15112016-6). This study was conducted in accordance with the Principles of the Declaration of Helsinki. Written informed consents were taken from all participantssome of whomhave already contributed to another study performed during the same period.[10] During the study period, 34 patients who were undergoing laparoscopy for infertility and endometriosiswere eligible for the currentstudy. Exclusion criteria were body mass index (BMI)<19 kg/m² and >30 kg/m², conversion to laparotomy, any medical treatment in the last six months and any additional etiologic factor for infertility other than endometriosis.

All the patients were operated via laparoscopy under general anesthesia. The (r-AFS) classification system was used todetermine the stage of the disease by point scoring followed by staging.[3,10] Additionally, EFI score for each patient was calculated by the surgeon who performed the operation.[4] Good prognosis for fertility was taken as EFI scores ≥7 (women with a high probability of non-ART conception).[11]

SERUM AND URINE SAMPLES

A venous blood sample for MMP-9 and CA-125 measurements was taken in the theater room before any intervention. The serum samples were taken into flat tubes and were stored at -80 C until assayed. Serum MMP 9 levels were analyzed by commercially available enzyme linkedimmunosorbent assay (ELISA) kit (Chemwell 2900 Aurenesness, Cloud-CloneCorp., Palm City, Florida, 34990, USA). Intra-assay and inter-assay coefficients for MMP 9 were <10% and <12%, respectively. CA-125 levels were measured CMIA (Chemiluminesans Microparticle Immunoassay) by Abbott Architect *i* 2000 (Abbott Park, IL 60064, USA) autoanalyzer. Intra-assay and inter-assay coefficient variations for3.2 % and 3.9%, respectively.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM® SPSS® Statistics 20 software (IBM Corp., Armonk, NY). Continuous variables were expressed as mean, standard deviation, median, and range where appropriate; binary variables were reported as counts and percentages. Relationships of each clinical, laboratory and surgical findingswith EFI score wereevaluated with a bivariate correlation analysis. Afterwards, a multiple linear regression analysis was performed to demonstrate the independent impact of those factors on EFI score.

Factors predicting an EFI score ≥7 (women with a high probability of non-ART conception), were evaluated by abinary logistic regression analysis. Variables with a P value <0.05 in univariate analysis were included into multivariate analysis. The effects of variables on prediction of non-ART conception were reported as adjusted odds ratios (OR) and 95% confidential intervals (CI).

To determine the diagnostic performances and the threshold values of variables found significant, a receiver operating characteristic (ROC) curve analysis was performed by plotting every possible cut-off score's sensitivity on the y-axis against 1-specificity on the x-axis. An area under the curve (AUC) value of >0.70 was considered satisfactory. For the ROC curve, the point with the largest sum of specificity and sensitivity was chosen asa threshold. In presenting the results, sensitivity, specificity,positive predictive value (PPV), negative predictivevalue (NPV), and accuracy were all reported.

RESULTS

The demographic characteristics of the participants, staging of endometriosis by r-ASRM and EFI scores, Ca-125 and MMP 9 levels aregiven in Table 1.

Nineteen women (55.9%) had stage I-II endometriosis, and 15 (44.1%) had stage III-IV endometriosis. The median EFI score was 6.09 (3-9). The median levels of, MMP 9 and Ca-125 were, 27.99 (7-124) pg/mL and 56.66 (3.0-217) U/mL, respectively.

The relationships of clinical, laboratory and surgical findings with EFI score were shown in Table 2.

The bivariate correlation analysis revealed that the age (r=-0.300), duration of infertility (r=0.417),

TABLE 1: Characteristics of patients (n=34)	
Variables	**Value**
Age (year) ($\bar{X}\pm SD$)	32.79±3.98
Body mass index (kg/m²) ($\bar{X}\pm SD$)	26.42±2.83
Gravidity (Median min-max)	0.5 (0-5)
Parity (Median min-max)	0(0-4)
Duration of infertility (years) (Median min-max)	4(1-16)
Endometriosis (n) (%)	
Stage I-II	19(%55.9)
Stage III-IV	15(%44.1)
EFI score (Mean±SD)	6.12±1.63
EFI score groups	
0-3	3(8.8)
4-6	13(38.3)
7-10	18(52.9)
Ca-125 (U/mL) (Median min-max)	23(3-217)
MMP 9 (pg/mL) (Median min-max)	14(7-124)

TABLE 2: Relationships of clinical, laboratory and surgical findings with EFI score					
	Bivariate Correlation Analysis[a]		**Multiple Linear Regression Analysis**		
Variables	**r**	**p**	**Coefficients**	**95% CI[b]**	**p**
Age (years)	-0.300	**0.039**	-0.057	-0.126 to-0.073	0.593
Body mass index (kg/m²)	-0.063	0.672	-0.160	-0.214 to-0.024	0.116
Gravidity	0.109	0.460	0.024	-0.345 to-0.422	0.840
Parity	0.085	0.566	0.172	-0.107 to-0.771	0.134
Duration of infertility (years)	-0.417	**0.003***	-0.163	-0.212 to-0.035	0.157
Ca-125 (U/mL)	-0.545	**<0.001***	-0.016	-0.009 to-0.008	0.903
MMP-9[c] (pg/mL)	-0.605	**<0.001***	-0.302	-0.037 to-0.004	**0.016***
Severity of endometriosis	-0.672	**<0.001***	-0.488	-1.620 to-0.501	**<0.001***

[a]Spearman's rho; [b]CI, confidence interval; [c]MMP-9, matrix metalloproteinase-9, *p<0.05

TABLE 3: Non-surgical (Clinical and Laboratory) factors predicting an EFI score of ≥7 (Women with a High Probability of Non-ART Conception)						
	Univariate Analysis			Multivariate Logistic Regression Analysis		
Variables	OR[a]	95% CI[b]	p	OR[a]	95% CI[b]	p
Age (years)	0.809	0.673-0.972	**0.024***	0.860	0.670-1.106	0.240
Body mass index (kg/m^2)	0.971	0.792-1.191	0.780	-	-	-
Previous pregnancy	1.429	0.442-4.622	0.552	-	-	-
Duration of infertility (years)	0.770	0.615-0.964	**0.023***	0.858	0.621-1.185	0.352
Ca-125 (U/mL)	0.973	0.955-0.992	**0.006***	0.975	0.954-0.996	0.055
MMP-9[c] (pg/mL)	0.757	0.599-0.958	**0.020***	0.684	0.479-0.978	**0.037***

[a]OR, Odds Ratio; [b]CI, confidence interval; [c]MMP-9, matrix metalloproteinase-9, *p<0.05

levels of Ca-125 (r=-0.545), and MMP 9 (r=-0.605), and the presence and severity of endometriosis (r=-0.672) were significantly correlated with EFI scores. However, multiple linear regression analysis revealed that only MMP 9 (p=0.016), and the presence and severity of endometriosis (p<0.001) had independent relationships with EFI scores.

The multivariate binary logistic regression analysis revealed that MMP 9 (p=0.037) was the sole non-surgical (clinical and laboratory) factor predicting an EFI score of ≥7 (Table 3).

The cut-off value for MMP 9 was found to be 13.50 pg/mL (AUC=0.852, CI: 0.735-0.968, p<0.001), with a 76.7% sensitivity, 83.3% specificity, 82.1% PPV, 78.1% NPV, and 80% accuracy.

DISCUSSION

EFI is the first scoring system validated for prediction of fertility in infertile women with endometriosis.[4] This scoring system enables the clinician to select the cases that are unlikely to conceive naturally and those who will benefit from assisted reproductive technologies. When counseling the endometriosis patients about their fertility prognosis after surgery, EFI scores are highly reasonable for achievement of non-ART treatments, especially for the 1st-2nd years.[2,9] An EFI score of ≥7 corresponds to 37.4-56.4% per year (3.1-4.7% per month) fecundity rate in the first year after surgery, decreasing to 10.7-27.5% per year (0.9-2.3 per month) for the third postoperative year.[9] Likewise, with a median EFI score of 7.6, 48.2% of the patients concieved spontaneous-

lywithin a mean of 4.2 months (range 1-12 months).[12] The current preliminary study indicates that, MMP 9 is a promising non-invasive marker for fertility prognosis correlated with EFI scores. However, the value of MMP 9as an applicable variable for EFI score parameters needs to be evaluated. If validated by future research, MMP 9 might increase the accuracy and discriminative performance of this system.

When consulting the endometriosis cases suffering from infertility, a staging surgery is sometimes refused by the couple or is impractical. For such cases, a simple blood test, MMP 9, might reveal valuable information. The significant correlation between MMP 9 and EFI scores might help to discriminate good prognosis cases for fertility.Those with high levels of MMP 9 (>13.50pg/mL) might be offered infertility treatment strategies rather than expectant management for spontaneous pregnancy. However, the limited number of subjects included in the current study disables us to draw strong conclusions.

A surgical procedure for EFI score includes not only scoring but also interventions for the treatment of the disease in the same session. This is why, fecundity rates based on EFI scores are most probably affected by these surgical interventions. Eventually, further studies involving patients who are not surgically staged are needed to validate the predictive value of MMP 9 for good fertility prognosis in endometriosis. Our future study, regarding the MMP 9 levels of untreated cases, will reveal spontaneous pregnancy rates in a two-year follow-up.

CONCLUSION

If validated by larger prospective trials, MMP 9 seems to be a promising serum marker for prediction of good fertility prognosis for women with endometriosis.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that pro- *vides or produces medical instruments and materials which may negatively affect the evaluation process of this study.*

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

All authors contributed equally while this study preparing.

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