Volume 65, Issue 5, 2021
Journal of Scientific Research
of
The Banaras Hindu University

Elevated Levels of CA-125, Estradiol and Cortisol as Prominent Markers to Diagnose Various Stages of Endometriosis

Ashish[1], Kusum[2], Sangeeta Rai[3], Bhupendra Kumar[2], Radha Chaube[2], Royana Singh[1]*

*[1]Department of Anatomy, Institute of Medical Science, Banaras Hindu University, Varanasi, India. ashish.ims2@bhu.ac.in, royanasingh@bhu.ac.in
[2]Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India. kusum.mhg.bhu13@gmail.com, bhupendrakumar@bhu.ac.in, chaubeyradhabhu@gmail.com
[3]Department of Obstetrics & Gynecology, Institute of Medical Science, Banaras Hindu University, Varanasi, India. drsangeeta1977@yahoo.co.in

Abstract: Endometriosis is a chronic inflammatory disease condition that affects an estimated 176 million women worldwide. The disease causes unbearable symptoms of pelvic pain and infertility that limits the quality of reproductive life of affected women. An attempt has been made to identify certain physiological and hormonal factors that may behave as reliable diagnostic markers to assess the various stages of endometriosis. It is a retrospective population-based study. Here a questionnaire-based study was conducted on females of Eastern Uttar Pradesh and Bihar that were suffering from endometriosis. Prevalent patients were characterized in terms of their socio-demographic and clinical characteristics, including the validated infertility and chronic disease registries. Progression of the disease was not found to be affected by age and body mass index (BMI) of subjects. Concentration of blood sugar (fasting and PP), and levels of serum luteinizing hormone (LH), follicle stimulating hormone (FSH), T4 and Thyroid stimulating hormone (TSH) did not change as disease progressed from stage 1 to 4. However, levels of serum estradiol, cortisol, prolactin, T3 and CA-125 increased as endometriosis progressed. There also existed a close association between CA-125, estradiol, cortisol and stages of endometriosis. Elevated levels of CA-125, estradiol and cortisol may behave as prominent diagnostic markers to assess progression of disease and to discriminate between various stages of endometriosis.

Index Terms: **Dysmenorrhea, Dyspareunia Endometriosis, Infertility, Pelvic Pain**

I. INTRODUCTION

Endometriosis is a chronic inflammatory disease condition in women, where tissues resembling endometrium, usually stromal or glandular, are located outside the uterine cavity (Klemmt and Starzinski-Powitz, 2017). Menorrhagia, dysmenorrhea, dyspareunia, dyschezia, dysuria, pelvic pain and infertility are the prominent symptoms seen in endometriosis suffering women. In addition, factors like environmental and dietary elements, immune system, viz. cytokines, interleukins, and intrinsic anomalies in endometrium are also associated with the disease (Saceanu et al., 2017, Adaji et al., 2017). Many previous studies have assessed the risk factors associated with endometriosis (Adaji et al., 2017). Age, race, alcohol usage, body mass index, cigarette smoking, and menstrual characteristics such as early age menarche, menstrual length, cycle regularity, dysmenorrhea, and menstrual flow intensity are all associated with the incidences of endometriosis (Kafaei-Atrian et al., 2019). Globally, one in ten women during their reproductive years (between puberty and menopause) are having endometriosis (de Almeida Asencio et al., 2019), which is about 176 million women population worldwide suffering from the disease (Zondervan et al., 2018a). Assessment of the endometriosis rate is difficult in general female population as the definitive diagnosis requires surgical visualization (Jacobs, 1988). Diagnosis of endometriosis is established through laparoscopy visualization at surgery. Endometrioma and deep endometriosis however, can be detected using imaging techniques (ultrasonography or MRI) (As-Sanie et al., 2019). CA-125 (cancer antigen 125 or carbohydrate antigen 125) is a protein secreted by normal human body in small quantities (Zondervan et al., 2018b), and its normal value is less than 35 U/mL ("Revised American Society for Reproductive Medicine Classification of Endometriosis: 1996.," 1997). It is produced from the endometrium and irritation of peritoneum by infection, surgery or during endometriosis (Taneja et al., 2017). CA-125 as a biomarker through serologic testing has been widely used for the

detection of endometriosis and monitoring of progressive disease (Cheng et al., 2002; Medl et al., 1997; "Revised American Society for Reproductive Medicine Classification of Endometriosis: 1996.," 1997). Now-a-days, endocrine therapies are also providing effective palliation with relatively little toxicity in many hormone-sensitive cancers (Bowman et al., 2002). However, the response to endocrine therapy in case of endometriosis is yet to be explored. Despite causing debilitating symptoms of pelvic pain and infertility, and restraining the quality of life of affected women, the causative factors of endometriosis are not widely studied in Indian populations belonging to different ethnic groups. In the present study, an attempt has been made to identify certain physiological and/or hormonal factors that may behave as reliable diagnostic markers to assess the various stages of endometriosis.

II. MATERIALS AND METHODS

A. Classification Systems of Endometriosis

Numerous proposed systems to classify various forms of endometriosis exist mainly in the American Society of Reproductive Medicine (ASRM) ("Classification of Endometriosis," 1979), which is modified and renamed into Revised American Society for Reproductive Medicine classification of endometriosis (rASRM) ("Revised American Society for Reproductive Medicine Classification of Endometriosis: 1996.," 1997). All of these classifications divide endometriosis into four stages related to the increasing severity of the ovaries lesions, particularly the number of endometrial implants, their depth, and adhesions (Canis et al., 1997); Stage I: 1-5 points indicates minimal disease i.e., few superficial implants (Rock, 1995), Stage II: 6-15 points score indicates mild disease which includes more and deeper implants , Stage III: 16-40 points for moderate disease having many deep implants, small cysts on one or both ovaries and Stage IV: >40 points indicate severe condition with many deep implants, large cysts on one or both ovaries with dense adhesions (Parasar et al., 2017).

B. Study design

The present retrospective study was conducted at Department of Obstetrics and Gynecology, Sir Sunder Lal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India (25°20'N, 83°0'E) from September 2017 to September 2019. The study was questionnaire-based and the details of the lifestyle, habits, and familial history of patients were recorded. All subjects were of Indian ethnicity from eastern Uttar Pradesh and Bihar, the two states of northern India. The study was approved by Institutional Medical Ethical Committee (No. Dean /2018/ EC/936). All subjects were informed about the study and their consents were taken prior to the start of study.

1) Inclusion criteria

Female patients of 18-50-year age group, and were diagnosed to have endometriosis and BMI less than 32 kg/m2 were included in the study.

2) Exclusion criteria

Patients with other causes of chronic pelvic pain, including infectious diseases, Pelvic inflammatory disease (PID), adhesions due to previous surgeries or infections were excluded from the study.

C. Clinical characteristic

Points noted were the age of subjects, residence, physical and socioeconomic status, type of infertility, duration of infertility, menstrual cycle-age of onset, frequency, and its flow, an association of symptoms like dysmenorrhea, dyspareunia, chronic pelvic pain, urinary symptoms and their correlation to the stage of endometriosis.

D. Physical Examination

Findings were analyzed concerning BMI, abdominal/adnexal masses, mobility of uterus, and the presence of adnexal tenderness.

1) TVS and MRI finding

Advance technology of imaging methods was suggested for the detection of deep-infiltrating lesions. MRI and computed tomography, including ultrasound were evaluated.

2) Laparoscopic findings

Diagnostic laparoscopy, a gold standard tool for direct visualization of the pelvis, which helps in identifying the etiology of the patients' pain, was advised and evaluated. The laparoscopic staging was done based on the revised AFS scoring system (Taneja et al., 2017), which categorizes the finding into four stages.

III. STATISTICAL ANALYSIS

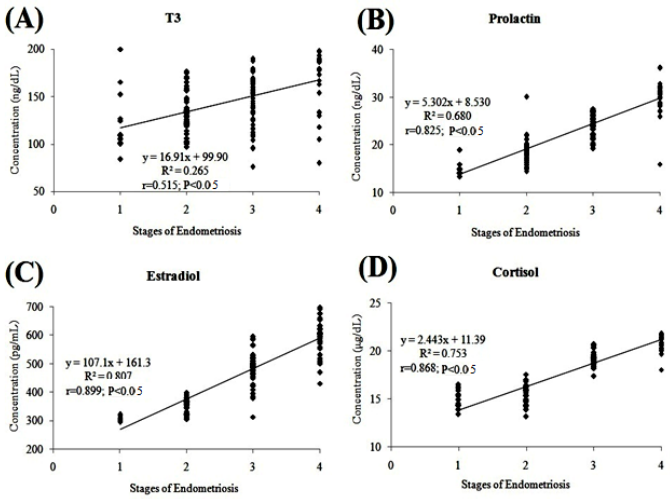
The distributions of data sets obtained in this study were checked for normality using Kolmogorov-Smirnoff test. Means were separated using Tukey's test when data were normally distributed and variances were homogeneous (Bartlett's test for equal variances). All dependent variables, viz. age, body mass index (BMI), CA-125, sugar concentrations (fasting and PP), and hormonal profiling including Luteinizing hormone (LH), Follicle stimulating hormone (FSH), estradiol, cortisol, prolactin, Triiodothyronine (T3), Tetraiodothyronine (T4) and Thyroid stimulating hormone (TSH) were subjected to one-way ANOVA considering different stages of endometriosis (viz. stage 1, 2, 3 and 4) as independent variables. Dependent variables (i.e., age, BMI, CA125, sugar concentrations (fasting and PP), LH, FSH, estradiol, cortisol, prolactin, T3, T4 and TSH) were also regressed against different stages (viz. stage 1, 2, 3 and 4) of endometriosis (independent variable), and graphs

displaying significant effects (P<0.05) were extrapolated. In addition, dependent variables were regressed amongst each other and graphs showing significant outcomes (P<0.05) were drawn. All statistical analyses were performed using MINITAB 16 (Minitab Inc., State College, Pennsylvania, United States of America).

IV. RESULTS AND DISCUSSION

Results revealed significant effects of CA-125, estradiol, cortisol, prolactin and T3 on different stages (i.e., stage 1, 2, 3 and 4) of endometriosis **(Table 1).** In contrast, the effects of age, BMI, sugar concentrations (fasting and PP), LH, FSH, T4 and TSH on different stages of endometriosis were insignificant. Regression graphs revealed that with progression in the stages of endometriosis from stage 1 to stage 4, the concentration/ levels of CA-125, estradiol, cortisol, prolactin and T3 increased. However, the association of estradiol and cortisol to different stages of endometriosis was much higher than those of prolactin and T3 **(Figure 1 (A-D); Figure 2-A).** This was further visualized from the obtained positive correlation coefficients between estradiol/ cortisol and the concentrations of CA-125 as the disease progressed **(Figure 2-B and 2-C).** In the present study, progression of the disease was not affected by the age and BMI of the female patients. Similarly, concentration of blood sugar (fasting and PP), and the levels of

Fig. 1. Scatter-plot regression graphs showing the effects of **(A)** T3, **(B)** Prolactin, **(C)** Estradiol, and **(D)** Cortisol on different stages of endometriosis (R2-values and r-values are significant at P<0.05).



serum LH, FSH, T4 and TSH did not show any change as the disease progressed from stage 1 to stage 4. However, the levels of serum estradiol, cortisol, prolactin, T3 and the concentration of molecular marker CA-125 increased as endometriosis progressed from stage 1 to stage 4. Higher levels of serum cortisol and prolactin in women suffering from advanced stages of endometriosis may be associated with stress. (Dugan et al., 2004) have also reported that under prolonged stress conditions the level of serum cortisol increases. It is probable that stress may be directly related to the development of endometriosis and its, further, progression to advanced stages (Cunha-Filho et al., 2001; Wang et al., 2009).

Earlier studies have also shown that endometriosis is a progressive estrogen-dependent disease affecting women during their reproductive years (Bulun, 2019; Bulun et al., 2012). Biologically active estrogen, i.e., estradiol intensifies the pathological processes like inflammation and growth, and the symptoms associated with endometriosis. The availability of abundant quantities of estradiol for endometriotic tissue may probably be due to several known and unknown mechanisms. One of them is the availability of local aromatase expression (Bulun et al., 2012; Trukhacheva et al., 2009).Although previous studies have shown that women with endometriosis have no increased risk of thyroid dysfunction (Petta et al., 2007); however, our study has recorded the contradictory findings. We have found increased serum T3 levels with the progressions of endometriosis from stage 1 to stage 4. Similar to our finding, it has been reported that (Peyneau et al., 2019) that thyroid disorders are associated with severe forms of endometriosis. They found that proteins involved in thyroid metabolism were dysregulated in eutopic and ectopic endometrium of endometriotic patients, leading to resistance of ectopic endometrium T3 action (Peyneau et al., 2019). However, few others (Parasar et al., 2017) found that women suffering from endometriosis had higher rates of hypothyroidism.

Cancer antigen 125 (CA-125) is a biological marker for epithelial cell ovarian cancer. There is an increase in CA 125 in endometriosis through the stimulation of coelomic epithelia. Increased serum CA-125 level in the present study may be associated with the ovarian endometriomas. Increased levels of serum CA-125 with grade of endometriosis, as recorded in our study are also consistent with the findings of many previous studies (Barbieri et al., 1986, Weisheng et al., 2019, Alio et al., 2019).

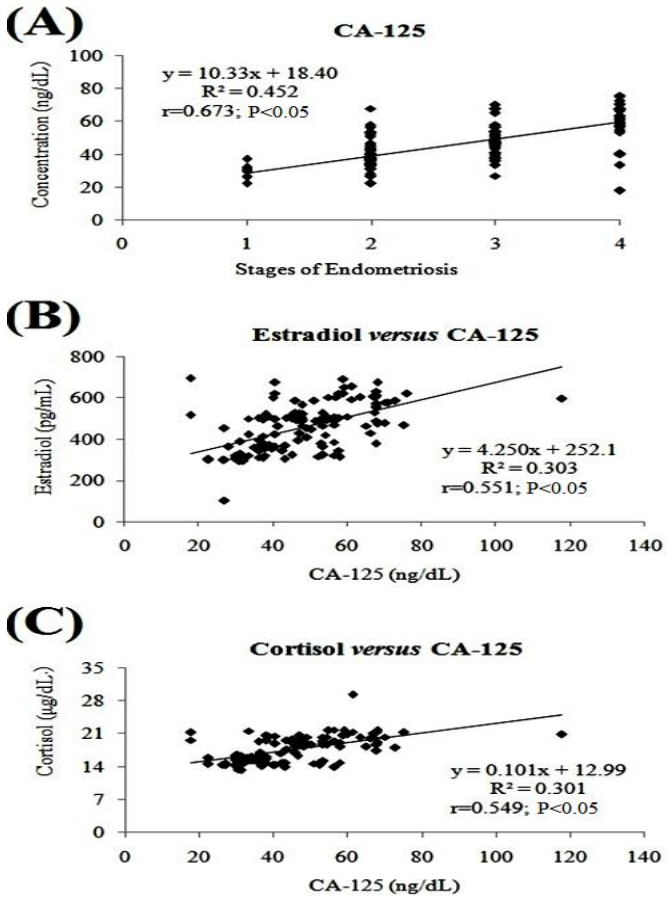


Fig. 2. Scatter-plot regression graphs showing relationships between (A) CA-125 and progression of endometriosis, (B) Estradiol and CA-125, and (C) Cortisol and CA-125 during endometriosis (R2-values and r-values are significant at P<0.05).

Results of our present study have also revealed close associations between the levels of CA-125, estradiol or cortisol and the different stages of endometriosis than those between the disease and the levels of prolactin and T3. This close association between CA-125, estradiol, cortisol and endometriosis are further affirmed by the increasing levels of estradiol or cortisol and increasing concentrations of molecular marker, CA-125 as the disease progressed from stage 1 to stage 4. These findings further reveal that besides CA-125, estradiol and cortisol may also behave as prominent diagnostic markers to assess the progression of the disease and to discriminate the different stages of endometriosis. However, more clinical-based research studies are still needed to validate the present findings.

Table 1. One-way ANOVA table showing effect of different stages of endometriosis on various physiological/ hormonal parameters of female patients (Values are Mean±SD; F-values significant at P<0.05).

Variable	Stage 1 (N=19)	Stage 2 (N=80)	Stage 3 (N=75)	Stage 4 (N=47)	F-value; P-value; df
Age	32.10 ± 8.14	32.05±6.91	32.53±8.79	31.44±8.91	F=0.62; P=0.605; df=3, 193
BMI (kg/m^2)	22.18± 1.39	21.54±2.48	22.02±2.34	21.41±2.28	F=0.96; P=0.413; df=3, 193
LH	7.20 ± (4.90 - 8.90)	5.10± (4.20-7.55)	5.80± (4.63-9.24)	5.60± (3.58-7.90)	F=1.17; P=0.323; df=3, 193
FSH	14.03 ± 6.88	12.82±6.64	11.74±6.63	13.51±6.86	F=1.68; P=0.172; df=3, 193

Prolactin	15.47 ± 7.02	18.31±2.28	24.37±2.44	30.23±3.03	F=143.73; P<0.0001; df=3, 193
T_3	122.78 ± 33.20	134.07±22.15	147.54±25.37	166.40±32.66	F=23.91; P<0.0001; df=3, 193
T_4	9.09 ± 2.23	10.06±2.80	9.27±2.42	9.59±2.62	F=01.13; P=0.340; df=3, 192
TSH	2.90± (1.0-3.87)	2.72± (1.51-3.90)	2.44± (1.34-3.90)	2.60± (1.73-4.22)	F=0.90; P=0.441; df=3, 193
CA-125	29.95 ± 3.00	38.74±8.15	49.10±8.76	60.24±16.68	F=52.66; P<0.0001; df=3, 193
Sugar (FA)	100.96± 15.82	97.91±15.72	98.59±14.61	92.53±16.36	F=1.96; P=0.121; df=3, 212
Sugar (PP)	143.25± 26.47	126.21±23.70	137.022±27.32	122.49±19.87	F=2.61; P=0.055; df=3, 106
Estradiol	295.24± 47.79	352.03±26.93	486.03±50.50	594.00±60.70	F=230.07; P<0.0001; df=3, 148
Cortisol	14.89±0.89	15.37±0.94	19.22±0.75	21.20±1.96	F=177.77; P<0.0001; df=3, 116

CONCLUSION

Since no association was found between endometriosis and age, BMI, sugar concentration (fasting and PP), LH, FSH, T4 or TSH levels in the present study; hence, screening of the patients based purely on these parameters and severity of the disease may not be suggested. Moreover, these days, endocrine therapies are providing effective results in hormone-sensitive cancers. Future studies may therefore be designed to assess the response of estradiol or cortisol based endocrine therapies and endometriosis.

REFERENCES

1. Klemmt PA, Starzinski-Powitz A. Molecular and Cellular Pathogenesis of Endometriosis. Curr Women s Heal Rev. 2017;13(999):1–11.
2. Riazi H, Tehranian N, Ziaei S, Mohammadi E, Hajizadeh E, Montazeri A. Clinical diagnosis of pelvic endometriosis: A scoping review. BMC Womens Health. 2015;15(1):01.
3. Saceanu SM, Patrascu S, Patrascu A, Surlin V. Endometriosis: When and How We Treat. In: Fertility-oriented Female Reproductive Surgery. 2017.
4. Adaji E, Ahankari A, Myles P. An investigation to identify potential risk factors associated with common chronic diseases among the older population in India. Indian J Community Med. 2017;42(1):46–52.
5. Kafaei-Atrian M, Mohebbi-Dehnavi Z, Sayadi L, Asghari-Jafarabadi M, Karimian-Taheri Z, Afshar M. The relationship between the duration of menstrual bleeding and obesity-related anthropometric indices in students. J Educ Health Promot. 2019 Apr 24;8(18):81.
6. de Almeida Asencio F, Ribeiro HA, Ayrosa Ribeiro P, Malzoni M, Adamyan L, Ussia A, et al. Symptomatic endometriosis developing several years after menopause in the absence of increased circulating estrogen concentrations: a systematic review and seven case reports. Gynecol Surg. 2019;16(1):1–11.
7. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. Vol. 4, Nature Reviews Disease Primers. Nature Publishing Group; 2018. p. 1–25.
8. Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, et al. Clinical diagnosis of endometriosis: a call to action. Am J Obstet Gynecol. 2019;220(4):354.e1-354.e12.
9. As-Sanie S, Black R, Giudice LC, Gray Valbrun T, Gupta J, Jones B, et al. Assessing research gaps and unmet needs in endometriosis. Vol. 221, American Journal of Obstetrics and Gynecology. 2019. p. 86–94.
10. Taneja D, Gupta S, Bansal A. High CA – 125 in Patients with Endometrioma. J Case Reports. 2017;7(1):97–9.
11. Koninckx PR, Meuleman C, Oosterlynck D, Cornillie FJ. Diagnosis of deep endometriosis by clinical examination during menstruation and plasma CA-125 concentration.

Fertil Steril. 1996;65(2):280–7.
12. Medl M, Ogris E, Peters-Engl C, Mierau M, Buxbaum P, Leodolter S. Serum levels of the tumour-associated trypsin inhibitor in patients with endometriosis. BJOG An Int J Obstet Gynaecol. 1997;104(1):78–81.
13. Cheng YM, Wang ST, Chou CY. Serum CA-125 in preoperative patients at high risk for endometriosis. Obstet Gynecol. 2002;99(3):375–80.
14. Bowman A, Gabra H, Langdon SP, Lessells A, Stewart M, Young A, et al. CA125 response is associated with estrogen receptor expression in a phase II trial of letrozole in ovarian cancer: Identification of an endocrine-sensitive subgroup. Clin Cancer Res. 2002;8(7):2233–9.
15. Classification of Endometriosis. Fertil Steril. 1979;32(6):633–4.
16. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67(5):817–21.
17. Canis M, Donnez JG, Guzick DS, Halme JK, Rock JA, Schenken RS, et al. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67(5):817–21.
18. Rock JA. The revised American Fertility Society classification of endometriosis: reproducibility of scoring. ZOLADEX Endometriosis Study Group. Fertil Steril. 1995;63(5):1108–10.
19. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. Vol. 4, Nature Reviews Disease Primers. 2018. p. 1–25.
20. Lima AP, Moura MD, Rosa e Silva AAM. Prolactin and cortisol levels in women with endometriosis. Brazilian J Med Biol Res. 2006;39(8):1121–7.
21. Machida T, Taga M, Minaguchi H. Prolactin secretion in endometriotic patients. Eur J Obstet Gynecol Reprod Biol. 1997;72(1):89–92.
22. Cunha-Filho JSL, Gross JL, Lemos NA, Brandelli A, Castillos M, Passos EP. Hyperprolactinemia and luteal insufficiency in infertile patients with mild and minimal endometriosis. Horm Metab Res. 2001;33(4):216–20.
23. Wang G, Tokushige N, Markham R, Fraser IS. Rich innervation of deep infiltrating endometriosis. Hum Reprod. 2009;24(4):827–34.
24. Bulun SE. Endometriosis. N Engl J Med. 2009;360(3):268–79.
25. Bulun SE, Monsavais D, Pavone ME, Dyson M, Xue Q, Attar E, et al. Role of estrogen receptor-β in endometriosis. Semin Reprod Med. 2012;30(1):39–45.
26. Trukhacheva E, Lin Z, Reierstad S, Cheng YH, Milad M, Bulun SE. Estrogen receptor (ER) β regulates ERα expression in stromal cells derived from ovarian endometriosis. J Clin Endocrinol Metab. 2009;94(2):615–22.
27. Petta CA, Arruda MS, Zantut-Wittmann DE, Benetti-Pinto CL. Thyroid autoimmunity and thyroid dysfunction in women with endometriosis. Hum Reprod. 2007;22(10):2693–7.
28. Peyneau M, Kavian N, Chouzenoux S, Nicco C, Jeljeli M, Toullec L, et al. Role of thyroid dysimmunity and thyroid hormones in endometriosis. Proc Natl Acad Sci U S A. 2019;116(24):11894–9.
29. Sinaii N, Plumb K, Cotton L, Lambert A, Kennedy S, Zondervan K, et al. Differences in characteristics among 1,000 women with endometriosis based on extent of disease. Fertil Steril. 2008;89(3):538–45.
30. Barbieri RL, Niloff JM, Bast RC, Scaetzl E, Kistner RW, Knapp RC. Elevated serum concentrations of CA-125 in patients with advanced endometriosis. Fertil Steril. 1986;45(5):630–4.
31. Pittaway DE, Fayez JA. The use of CA-125 in the diagnosis and management of endometriosis. Fertil Steril. 1986;46(5):790–5.
32. Brown J, Pan A, Hart RJ. Gonadotrophin-releasing hormone analogues for pain associated with endometriosis. Cochrane Database Syst Rev. 2010;(12).

***