Effects of Combined Laparoscopic Cystectomy and Leuprolide Acetate Therapy on Anti-Mullerian Hormone Level and Antral Follicle Count Profile in Endometriosis

Fatimah Usman,[1] Bagus Hilmawan,[1] Kemas Yusuf,[1] Heriyadi Manan,[1] Theodorus,[2] Hana Andrina[3]

[1]Division of Reproductive, Endocrinology & Infertility, Department of Obstetrics and Gynecology, dr. Mohammad Hoesin General Hospita Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatera, Indonesia

[2]Health and Medical Research Unit, Faculty of Medicine, Universitas Sriwijaya

[3]Department of Obstetrics and Gynecology, dr. Mohammad Hoesin General Hospital, Faculty of Medicine, Universitas Sriwijaya, Palembang, South Sumatera, Indonesia

Correspondence: Fatimah Usman, Email: titimusman@yahoo.com

Abstract

Objective: This study examines the effectiveness of combined laparoscopic cystectomy and leuprolide acetate therapy on AMH level and AFC profile in endometriosis.

Method: This study is a randomized controlled clinical trial with open label form. The subjects were divided into control and therapy groups, with the therapy group receiving leuprolide acetate injection following laparoscopic cystectomy. In both groups, the AMH levels and AFC were examined prior to the surgery and six weeks after before they were compared.

Results: There were significant pre-post differences of AMH levels(p=0.000) and AFC (p=0.000) in the therapy group but not in the control group. In the therapy group, the mean increases of AMH level and AFC were 0.94 (95% CI: 0.63-1.25) and 9.53 (95% CI: 6.83-12.24), respectively. Linear regression model found that AFC level prior to intervention was a significant predictor of endometriosis ASRM grade (p=0.001) with an R-value of 0.633, suggesting strong correlation.

Conclusion: The combined therapy of laparoscopic cystectomy and leuprolide acetate injection for endometriosis provided better outcome concerning ovarian reserve, as opposed to the therapy with laparoscopic cystectomy alone.

Key words: Endometriosis; leuprolide acetate; cystectomy; anti-mullerian hormone; antral follicle count

Peran Kombinasi Laparoskopi Kistektomi dan Terapi Leuprolide Asetat Terhadap Kadar Hormon Anti-Mullerian dan Profil Jumlah Folikel Antral pada Penderita Endometriosis

Abstrak

Tujuan:Studi ini bertujuan menguji keefektifan kombinasi kistektomi laparoskopi dan terapi *leuprolide acetate* terhadap kadar AMH dan profil AFC pasien endometriosis.

Metode: Penelitian ini merupakan uji klinis terkontrol acak dengan bentuk *open label*. Subjek dibagi menjadi kelompok kontrol dan terapi, dengan kelompok terapi menerima injeksi *leuprolide acetate* setelah kistektomi laparoskopi. Pada kedua kelompok, kadar AMH dan AFC diperiksa sebelum pembedahan dan enam minggu setelahnya sebelum kemudian dibandingkan.

Hasil: Terdapat perbedaan bermakna kadar AMH (p=0,000) dan AFC (p=0,000) sebelum dan sesudah pada kelompok terapi tetapi tidak pada kelompok kontrol. Pada kelompok terapi, rata-rata peningkatan kadar AMH dan AFC adalah masing-masing 0,94 (95% CI: 0,63-1,25) dan 9,53 (95% CI: 6,83-12,24). Model regresi linier menemukan bahwa kadar AFC sebelum intervensi merupakan prediktor signifikan derajat endometriosis sesuai ASRM (p=0,001) dengan nilai R sebesar 0,633 yang menunjukkan korelasi yang kuat.

Kesimpulan: Terapi kombinasi kistektomi laparoskopi dan injeksi *leuprolide acetate* untuk endometriosis memberikan hasil yang lebih baik terhadap cadangan ovarium, dibandingkan dengan terapi dengan kistektomi laparoskopi saja.

Kata kunci: Endometriosis; *leuprolide acetate*; kistektomi; hormon anti-mullerian; jumlah folikel antral

Introduction

Endometriosis is a gynecological disease causing pain and fertility issues in women of reproductive age. Its detriments are furthered by its varying complications, evasiveness in diagnosis, and high recurrence rate.[1,2] Several methods of diagnosis and treatment have been attempted but their results lie short of satisfactory. Among those methods, laparoscopy has been designated as a gold standard, though as an invasive approach, it has its own setbacks including damaging and lowering patients' ovarian reserve.[3, 4]

The ovarian reserve offers a glimpse of patients' fertility potential – which is the quality and quantity of oocytes, patients' remaining reproductive capacity, and also the success of assisted reproductive technologies.[3] Without a definite and direct measurement method currently available, ovarian reserve can only be estimated through evaluations of physical, biochemical, and ovarian morphometric markers as well as stimulation tests. Anti-Mullerian Hormone (AMH) is one such biochemical marker while Antral Follicle Count (AFC) measured during early follicular phase of menstrual cycle is an ovarian morphometric marker.[5]

Laparoscopic cystectomy has been shown to reduce ovarian reserve, possibly through damaging the healthy ovarian tissue and primordial follicle during excision. Bipolar coagulation technique may also cause inappropriate ablation depth causing damage to ovary cortex.[5,6]

Recent research found that administration of a GnRH analogue between two surgical procedures had a positive effect on reducing the cyst size, glandular activity, stroma vascularization, and also hastened endometriotic cells apoptosis. There was no significant disturbance on functional ovarian tissue as measured through AMH level and AFC.[7] There are also reports on the efficacy of GnRH agonist on managing the symptoms such as pelvic pain and even increasing pregnancy rate.[8,9]

Therefore, this research was conducted to examine the effectiveness of combined laparoscopic cystectomy and leuprolide acetate (a GnRH agonist) therapy on Anti-Mullerian Hormone (AMH) level and Antral Follicle Count (AFC) profile. This research also sought to determine the correlation between AMH level and AFC before intervention with the grade of endometriosis as later determined during laparoscopy.

Method

This research was designed as a randomized controlled trial with open label form and was conducted from July to December 2019 at RSUP Dr. Moh. Hoesin Palembang. Patients diagnosed with endometriosis by mean of ultrasound with no prior history of ovarian surgery were eligible to enroll. The patients who had history of smoking, chemotherapy, hormonal treatment, and other internal medical diseases such as hepatic, respiratory, renal, hematological, or endocrine dysfunction were excluded.

After acquiring informed consent as well as history taking and basic physical examination, each of the 30 patients included as samples were examined for AMH level and AFC at the third day of their menstrual cycle. All samples received leuprolide acetate injection (Tapros® depot suspension once a month) for a month before finally underwent laparoscopic cystectomy. Only patients with ASRM grade III or IV endometriosis were included in further study and they were randomly assigned to either control or therapy group. The therapy group received leuprolide acetate injection immediately after surgery and it was continued for three months; their AMH levels and AFC were checked six weeks after surgery. The leuprolide acetate injection for the control group was withheld for six weeks after surgery until their AMH levels

and AFC were also checked, after which they would also receive leuprolide acetate injection for three months. The data collected were analyzed using SPSS 20. Homogeneity test was performed to determine the normality of data distribution. Baseline AMH levels and AFC prior to intervention were compared between groups using independent T test for normally distributed data and Mann-Whitney U test for abnormally distributed data. The AMH levels and AFC before and after intervention were compared in each group using paired T test for normally distributed data and Wilcoxon signed-rank test for abnormally distributed data. The AMH levels and AFC after intervention were compared between groups using independent T test for normally distributed data and Mann-Whitney U test for abnormally distributed data. Linear regression model was used to find correlation between AMH level, AFC, and endometriosis ASRM grade. Significance level was set at 0.05.

Results

The average age of subjects was 30 years old in the therapy group and 29 years old in the control group. Both the therapy and control groups had more patients who were nulliparous (80% and 86.67%, respectively) and had primary infertility (80% and 86.67%, respectively). The AMH levels and AFC before and after intervention were initially tested for homogeneity using Shapiro-Wilk test, showing that only AFC in the control group was not normally distributed.

The mean AMH level prior to intervention was found to be higher in the control group (2.91 ± 1.13) than in the therapy group (1.93 ± 0.98), a difference that was statistically significant (p = 0.017). Meanwhile, there was no statistically significant difference of AFC prior to intervention (p = 0.436).

There was a statistically significant difference of AMH levels before and after intervention in the therapy group (p = 0.000). The mean AMH level in the therapy group prior to intervention was 1.93 ± 0.98, while after intervention, it increased to 2.87 ± 0.91; showing a difference of 0.94 (95% CI: 0.63–1.25). Meanwhile, the difference of the mean AMH levels before and after intervention in the control group of 0.26 (95% CI: -0.13–0.64) was found to be statistically insignificant (p = 0.179).

There was also a statistically significant difference of AFC before and after intervention in the therapy group (p = 0.000). The mean AFC in therapy group prior to intervention was 6.80 ± 1.61, while after intervention, it increased to 16.33 ± 5.46; showing a difference of 9.53 (95% CI: 6.83–12.24). Meanwhile, the difference of the median AFC before and after intervention in the control group was found to be statistically insignificant (p = 0.298).

Comparison of AMH levels and AFC of both groups after intervention showed that the difference of mean AMH levels was 0.29 (95% CI: -0.50–1.08) while the difference of median AFC was 9.00. However, the differences found in AMH levels (p = 0.463) and AFC (p = 0.161) were not statistically significant.

The linear regression model analysis showed that prior to intervention, AFC was a statistically significant variable to be used as a predictor of endometriosis ASRM grade (p = 0.001). The correlation coefficient or R-value of the regression model was 0.633, indicating strong correlation. The formula to predict endometriosis ASRM grade was as follow: endometriosis ASRM grade = 4.618 – (0.242 x (AFC)).

Discussion

This research found a significant increase of AFC pre- and post-intervention within the therapy group treated with laparoscopic cystectomy and leuprolide acetate; a result

Table 1 Baseline Differences of AMHLevels and AFC Prior To Intervention

Parameter	Mean AMH level and median AFC prior to intervention		P-value
	Laparoscopic Cystectomy and Leuprolide Acetate Injection	Laparoscopic Cystectomy	
AMH Level	1.93 ± 0.98	2.91 ± 1.13	0.017
AFC	7.00	7.00	0.436

Table 2 Comparison of AMH Levels before and after Intervention

Parameter	Mean AMH Level		Mean Differences	P-value
	Before Intervention	After Intervention		
Laparoscopic Cystectomy and Leuprolide Acetate Injection	1.93 ± 0.98	2.87 ± 0.91	0.94 ± 0.55	0.000
Laparoscopic Cystectomy	2.91 ± 1.13	3.16 ± 1.18	0.25 ± 0.69	0.179

Table 3 Comparison of AFC before and after Intervention

Parameter	Mean and median of AFC		Mean or median differences	P-value
	Before intervention	After intervention		
Laparoscopic Cystectomy and Leuprolide Acetate Injection (Mean)	6.80 ± 1.61	16.33 ± 5.46	9.53 ± 4.88	0.000
Laparoscopic Cystectomy (Median)	7.00	7.00	0	0.298

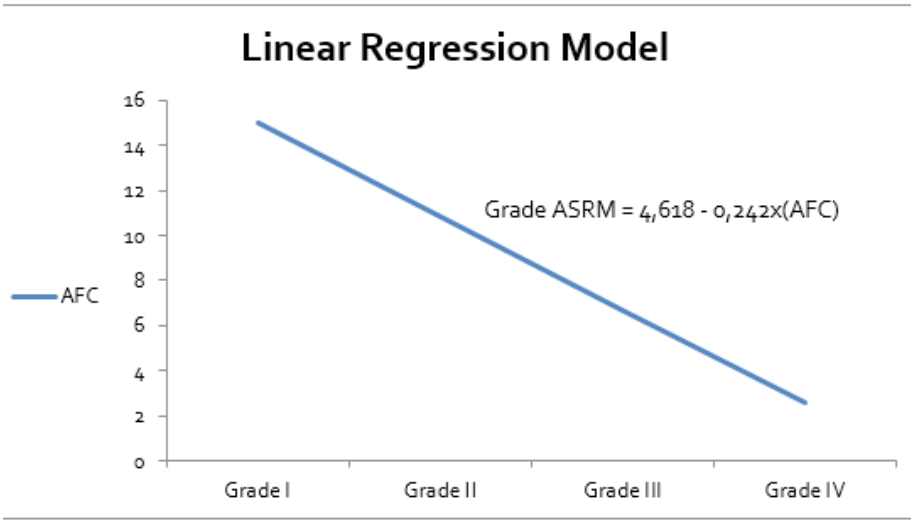

Figure 1 Linear Regression Model

not found on the control group treated with only laparoscopic cystectomy. Though there were several studies stating that there was no indication or benefit in systemic medical therapy, both pre- or post-operative ones, our findings seem to disagree.[10,11] Our research findings seem to be more in line with ones by Tsolakidis et al. who found that leuprolide acetate injection, when given as part of 'three-step procedure', might increase AFC at six months postoperative.[12]

Various articles have stated that laparoscopic cystectomy procedure itself may cause damage to ovarian tissue which negatively impacts ovarian reserve.[13,14,15] The damage was thought to be caused either by accidental removal of healthy ovarian tissue, vascular trauma after electric cauterization, or postoperative inflammatory reaction.[12] However, this supposed decrease in AFC was not demonstrated in both our groups. This could probably due to a significant rebound already happened at three months after surgery, although Georgievska et al. and Salihoglu et al. reported a rebound in AFC respectively three and two months postoperative.[16,17] Nevertheless, the significant increase of AFC seen within the therapy group supported the argument of beneficial effects of leuprolide acetate injection combined with laparoscopic cystectomy for endometriosis patients.

The level of anti-mullerian hormone, however, was stated to reach its preoperative levels only after six[15,18] or twelve[19] months after surgery, which was very different in time period compared to this research where the levels were analyzed six weeks after surgery. Significant difference of AMH levels was found within the therapy group but not within the control group, also supporting the argument of leuprolide acetate injection benefit when combined with laparoscopic cystectomy for endometriosis patients.

Although there were no significant differences in both groups' AMH levels and AFC after surgery, it didn't necessarily mean that there was no effect of leuprolide acetate injection on patient outcome. The AMH level prior to intervention was found to be statistically different with the control group having a greater level than that of the therapy group. This difference would surely make post-intervention comparison biased. Meanwhile, the lack of difference in AFC was most possibly caused by abnormally distributed data.

Analysis using linear regression model showed that AFC level prior to intervention had significant correlation with endometriosis ASRM grade. The ASRM grade according to the model can be predicted using the endometriosis ASRM grade formula = 4.618 – (0.242 x (AFC)). The linear regression model had an R-value of 0.633, indicating strong correlation. This model could be used to predict endometriosis ASRM grade before surgery, which could potentially help stratify patients' risk of needing one. Higher AFC prior to surgery indicates lower ASRM grade, possibly due to lesser degree of destruction or disturbance to healthy ovarian tissue.

This research was able to confirm the beneficial effect of leuprolide acetate injection when combined with laparoscopic cystectomy on ovarian reserve compared to laparoscopic cystectomy alone. The timeframe for examinations was able to exclude the probable bias caused by a natural rebound of AMH level and AFC. The positive impact on ovarian reserve, however, might not be readily translated into fertility outcome, which was not studied in this research.

Conclusion

The combination of laparoscopic cystectomy and leuprolide acetate injection for endometriosis seems to offer an advantage compared to laparoscopic cystectomy alone on patients' ovarian reserve, as reflected by AMH level and AFC. Another study is needed to confirm the effect of such combined

approach on fertility outcome.

Conflict of interest

All authors declare no conflict of interest.

Funding

This study did not receive any specific grants from public, commercial, or non-profit funding agencies.

Author Contribution

Each of the authors made contributions to all processes in this research, including preparation, data collection and analysis, drafting, and approval for the publication of this manuscript.

References

1. Speroff L, Glass RH, Kase NG. Clinical gynecologic endocrinology and infertility. 6th ed. Philladelphia: Lippincott Williams & Wilkins; 1999.
2. Ferrero S, Remorgida V, Venturini PL, Bizzarri N. Endometriosis: The effects of dienogest. BMJ Clin Evid. 2015; 0802: 1-8.
3. Bellelis P, Dias JR, Podgaec S, Gonzales M, Baracat EC, Abrao MS. Epidemiological and clinical aspects of pelvic endometriosis–a case series. Rev Assoc Med Bras. 2010; 56(4): 467─71.
4. HIFERI Indonesia. Panduan nasional pelayanan kedokteran: Konsensus nyeri endometriosis. Perkumpulan Obstetri dan Ginekologi Indonesia; 2013.
5. Verma S. Evidence linked treatment for endometriosis associated infertility. Apollo medicine. 2012; 9(3): 184─92.
6. Adnyana P. Endometriosis: Masalah klinis dan penanganannya, subbagian fertilitas dan endokrinologi reproduksi, makalah pertemuan ilmiah tahunan. Bali: Bagian Obstetri dan Ginekologi FKUNUD/RS Sanglah; 2005.
7. Bhat RG, Dhulked S, Ramachandran A, Bhaktha A, Vasudeva A, Kumar P, Rao ACK. Laparoscopic cystectomy of endometrioma: Good surgical technique does not adversely affect ovarian reserve. J Hum Reprod Sci. 2014; 7(2):125─9.
8. Schorge JO, Schaffer JI, Halvorson LM, Hoffman BL, Bradshaw KD, Corton MM. Williams gynecology. Chapter 10 : Endometriosis. California: McGraw-Hill; 2010
9. Depalo R, Jayakrishan K, Garruti G, Totaro I, Panzarino M, Giorgino F, et al. GnRH agonist versus GnRH antagonist in in vitro fertilization and embryo transfer. Reprod Bio Endocrinol. 2012; 10:26.
10. Yadav A, Patel S. A review article on ovarian cystectomy in endometriomas by laparoscopy. Int J Reprod Contracept Obstet Gynecol. 2017; 6(11): 4754─8.
11. Busacca M, Somigliana E, Bianchi S, De Marinis S, Calia C, Candiani M, et al. Post-operative GnRH analogue treatment after conservative surgery for symptomatic endometriosis stage III-IV: A randomized controlled trial. Hum Reprod. 2001; 16(11): 2399─402.
12. Oral E, Demir B, Inceboz U. Endometriosis and ovarian reserve. Womens Health (Lond). 2015; 11(5): 671─5.
13. Ergun B, Ozsurmeli M, Dundar O, Comba C, Kuru O, Bodur S. Changes in markers of ovarian reserve following laparoscopic ovarian cystectomy. J Minim Invasive Gynecol. 2015;22:997─1003.
14. Cagnacci A, Bellafronte M, Xholli A, Palma F, Carbone MM, Di Carlo C, et al. Impact of laparoscopic cystectomy of endometriotic and non-endometriotic cysts on ovarian volume, antral follicle count, and ovarian doppler velocimetry. Gynecol Endocrinol. 2016; 32(4):298─301.
15. Ding Y, Yuan Y, Ding J, Chen Y, Zhang

X, Hua K. Comprehensive assessment of the impact of laparoscopic ovarian cystectomy on ovarian reserve. J Minim Invasive Gynecol. 2015; 22(7):1252─9.
16. Salihoglu KN, Dilbaz B, Cirik DA, Ozelci R, Ozkaya E, Mollamahmutoglu L. The short-term impact of laparoscopic cystectomy on ovarian reserve tests in bilateral and unilateral endometiotic and non-endometriotic cysts. J Minim Invasive Gynecol. 2016; 23(5):719─25.
17. Georgievska J, Sapunov S, Cekovska S, Vasilevska K. Ovarian reserve after laparoscopic treatment of unilateral ovarian endometrioma. Acta Inform Med. 2014; 22(6): 371─3
18. Goodman LR, Goldberg JM, Flyckt RL, Gupta M, Harwalker J, Falcone T. Effect of surgery on ovarian reserve in women with endometriomas, endometriosis and controls. Am J Obstet Gynecol. 2016; 215(5): 589.e1─6.
19. Vignali M, Mabrouk M, Ciocca E, Alabiso G, di Prun AB, Gentilini D, Busacca M. Surgical excision of ovarian endometriomas: Does it truly impair ovarian reserve? Long term anti-Mullerian hormone (AMH) changes after surgery. J Obstet Gynaecol Res. 2015; 41(11): 1773─8.