

Original Article

Effect of anti-mullerian hormone, FSH and LH in evaluating ovarian reserve after laparoscopy surgery

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Abstract: Laparoscopy has become the primary choice for common ovarian surgery. This study recruited patients undergone ovarian laparoscopy surgery in our hospital, and measured their anti-mullerian hormone (AMH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels, in order to analyze their correlation with ovarian reserve functions. Ovarian cysts patients, including chocolate cysts group and non-chocolate cysts were recruited as the experimental group, in addition to healthy individuals as the control group. At different time points (2 days before surgery, 2 days after surgery, and 3 months after surgery), AMH, FSH and LH levels were quantified. The ovarian volume (V), peak systolic velocity (PSV) of ovarian intestinal artery, and antral follicles count (AFC) were measured by colored ultrasonography 3 months after surgery. AMH, FSH and LH levels were higher in disease group at all time points compared to control group ($P < 0.05$). The levels of AMH, FSH and LH were decreased in patient group 2 days after surgery compared to those before surgery, and were slightly increased 3 months later. No significant difference has been observed between unilateral chocolate cyst and non-chocolate cyst patients regarding hormone levels ($P > 0.05$) while bilateral chocolate cyst patients had more significant changes ($P < 0.05$). After surgery, V and PSV were similar against control group while AFC was remarkably decreased ($P < 0.05$). Levels of AMH, FSH and LH were decreased after ovarian laparoscopy, accompanied with decreased AFC, all of which indicated injury on ovarian reserve.

Keywords: Anti-mullerian hormone, follicle stimulating hormone, luteinizing hormone, ovarian laparoscopy

Introduction

Anti-mullerian hormone (AMH) plays a crucial role during male fetus development as it can induce the degeneration of mullerian tube [1]. In females, mullerian tube differentiates into uterus, ovarian duct and primary vaginal cells [2]. AMH is expressed in females from pregnant 36 weeks until menopausal period. AMH is not expressed in primordial follicle but is abundantly expressed in granulosa cells in preantral follicle and small antral follicles of ovary. In those follicles with diameter between 4 mm and 8 mm, AMH is down-regulated. In those larger antral follicles (larger than 8 mm) and atretic follicles, AMH expression is even silenced [3]. AMH thus is secreted from ovarian follicle cells and can negatively regulate follicle growth [4].

Ovarian cyst is one common disease in child-bearing women with peak ovarian functions. Classical treatment includes open abdominal surgery, but recently has been replaced by laparotomy surgery with higher efficiency, less trauma and short recovery period. However, due to the frequent use of electric coagulation during laparotomy surgery, ovarian function may be compromised, causing lower number of newborn oocytes with decreased quality, namely, lower ovarian reserve. Further aggravation of ovarian reserve injury may develop into functional failure of ovarian [5]. Multiple static and dynamic factors can be used to evaluate ovarian reserve in clinics. Among those indexes, AMH has been chosen due to its feasibility for assay. However, single indicator may not reflect all aspects of ovarian function [6]. This study

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Table 1. General information of patients

Group	N	Age (year)	Average age (year)	Menstrual cycle (day)
Disease	20	20-40	28.4±3.2	28.5±2.2
Ovarian chocolate cyst	12	23-40	29.2±3.1	29.1±1.7
Non-chocolate cyst	8	20-38	26.8±3.4	28.9±1.9
Control	20	21-39	27.1±3.4	29.1±1.2

thus investigated levels of AMH, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in patients undergone ovarian surgery to evaluate the dynamic ovarian function, in an attempt to analyze the effect on modulating ovarian function.

Materials and methods

General information of patients

A total of 20 ovarian cyst patients (aging between 20 and 40 years, average = 28.4±3.2 years old) from January 2014 to January 2015 were recruited as the disease group. All patients have undergone ovarian cyst removal surgery under laparotomy. Based on pathological features of the cyst, patients were further divided into chocolate cyst (N = 12) and non-chocolate cyst (N = 8) group. Another cohort of 20 healthy individuals were recruited as the control group. No significant difference existed between disease and control groups regarding age or menstrual cycle ($P > 0.05$, **Table 1**).

This study has been pre-approved by the ethical committee of our hospital. All details including disease condition, severity, surgical approaches and procedures, possible complications, lab tests before/after surgery and ovarian reserve function assays were instructed with patients. Post-operative follow-ups by the means of telephone, out-patient clinics or dropping-in visits were performed. All participants have signed written consents before enrolling.

Inclusive criteria: (1) Normal menstrual cycle and sex hormone level; (2) No open-abdominal surgery required; (3) No history of steroid drug use; (4) No previous ovarian surgery; (5) No other diseases such as polycystic ovarian syndrome or pre-failure of ovarian function; (6) No pregnancy during the follow-up.

Exclusive criteria: (1) Complicated with malignant tumors; (2) With endocrine disorders or

dysfunction of major organs; (3) Having pelvic surgery previously; (4) With history of auto-immune disease or infection diseases.

Surgical method

Laparoscopy surgery was applied in all patients. Via a 10 mm trocar at umbilical skin, artificial pneumoperitoneum was generated by CO₂ perfusion and was maintained at 12 mmHg. During ovarian cystectomy, the ovarian cortex was cut to make one incision, on which one pair of grasping forceps were used to strip the cyst from normal tissues. 3-0 vicryl sutures were used to close the incision, followed by homeostasis.

Lab indexes

Three different time points were set (T1, 2 days before surgery; T2, 2 days after surgery; T3, 3 months after surgery). At all three time points, serum AMH, FSH and LH levels were observed in patients and control individuals.

Three months after surgery, transvaginal color ultrasound was performed to detect ovarian volume (V), peak systolic velocity (PSV) of ovarian intestinal artery, and antral follicles count (AFC). V (in cm³) was calculated as length (in cm) × width (in cm) × height (in cm) × 0.5.

Sample collection

10 mL venous blood samples were collected and centrifuged immediately at 3 000 g for 10 min to separate serum, which was kept at -80°C for further use.

Serum assay

Enzyme-linked immunosorbent assay (ELISA) was used to detect serum AMH level using test kit (Kaiji Biotech, Nanjing, China). In brief, samples were diluted in gradients and were added into 96-well plate (N = 5 in each). After adding reaction buffer, washing and chromogenic substrates, the reaction was quenched and the plate was observed under a microplate reader for measuring absorbance value at 450 nm. The linear regression function was plotted to calculate sample concentration.

Chemiluminescence immunoassay was employed to detect serum levels of FSH and LH using test kits (Beckman Coulter, US).

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Table 2. AMH, FSH and LH levels at different time points

Index	T1		T2		T3	
	Disease	Control	Disease	Control	Disease	Control
AMH (ng/ml)	2.56±0.93*	1.21±0.47	1.98±0.78*	1.21±0.46	2.31±0.56*#	1.07±0.13
FSH (ng/ml)	9.96±3.26*	5.58±2.35	7.07±3.04*	9.29±3.13	5.45±2.21*#	7.12±2.81
LH (ng/ml)	9.27±6.91*	5.78±3.29	7.02±4.16*	9.32±5.31	5.35±3.08*#	7.56±4.12

Note: *, P<0.05 compared to T1 values; #, P<0.05 compared to T2 values.

Table 3. AMH levels and cyst types

Group	AMH		
	T1	T2	T3
Ovarian chocolate cyst			
Unilateral	2.04±0.87	1.96±0.46	2.00±0.51
Bilateral	2.44±1.03	1.98±0.57*,&	2.12±0.48#,&
Non-chocolate cyst			
Unilateral	2.19±0.97	1.86±0.58	2.13±0.32
Bilateral	2.68±1.06	1.92±0.63*	2.24±0.55#

Note: *, P<0.05 compared to T1 values; #, P<0.05 compared to T2 values; &, P<0.05 compared to unilateral chocolate cyst patients.

Statistical analysis

SPSS17.0 software was used to process all collected data, of which enumeration data were compared by chi-square test while measurement data were compared by analysis of variance (ANOVA) and were presented as mean ± standard deviation (SD). A statistical significance was defined when P<0.05.

Results

AMH, FSH and LH levels

We firstly compared levels of AMH, FSH and LH across different time points in all patients. Results showed suppressed hormone levels at 2 days after surgery compared to those before surgery (P<0.05, **Table 2**), and slightly increase at 3 month afterwards, but were still lower than pre-operative levels. When compared to control group, patients had remarkably increase of all hormones at all time points (P<0.05, **Table 2**).

AMH level across different cyst types

AMH level at T2 was lowered in bilateral chocolate and non-chocolate cyst patients compared to T1 (pre-operative) values, and was slightly elevated at T3 (**Table 3**). Those patients with bilateral chocolate cysts had more significant alternation of AMH level compared to unilateral

chocolate cyst patients (P>0.05, **Table 3**).

FSH and LH levels across cyst types

Further analysis focusing on FSH and LH levels in cyst patients found those with bilateral chocolate or non-chocolate cyst had lowered T2 values compared to T1, and slightly elevated levels at T3 (but still lower than T1). Unilateral chocolate cyst or non-chocolate cyst patients had no significant difference of FSH and LH levels at all time points (P>0.05, **Table 4**). Bilateral chocolate cyst patients had more significant change of FSH and LH levels (P<0.05, **Table 4**).

Levels of V, PSC and AFC

Three months after surgery, color ultrasound was employed to measure V, PSV and AFC of ovarian tissues. Results showed no significant change of V or PSV after surgery in patients when compared to control group (P>0.05, **Table 5**). The AFC values were 6.21±0.96 and 6.88±1.32 in chocolate ovarian cyst and non-chocolate cyst patients, respectively. Both of those values were lower than control group (P<0.05, **Table 5**).

Discussion

Ovarian cyst is one common disease in gynecology. Primary treatment for ovarian cyst is still surgery, which can fix multiple subtypes of ovarian cysts including ovarian epithelial tumor, pure cyst or chocolate cyst [7]. Ovarian chocolate cyst is one common type of endometrium, and accounts for around 17% to 44% of total cases. It is one common reason for female infertility and requires assisted reproduction [8]. Laparotomy surgery has multiple advantages including minimal wounds, fast recovery and less adverse effects, and has become the primary choice for treatment pure ovarian cyst,

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Table 4. FSH and LH levels in different sub-groups

Group	FSH			LH		
	T1	T2	T3	T1	T2	T3
Chocolate cyst						
Unilateral	8.24±3.07	8.06±2.26	8.10±2.41	8.27±6.71	8.04±6.96	8.13±7.02
Bilateral	8.94±3.73	5.18±2.07*,&	6.42±2.18*,&	8.81±5.82	5.12±3.01*,&	6.47±4.31*,&
Non-chocolate						
Unilateral	8.29±3.97	8.06±2.28	8.13±2.32	8.22±6.13	8.02±6.56	8.11±6.84
Bilateral	8.78±4.96	5.12±2.03*,&	6.54±2.35*,&	8.85±5.94	5.19±3.15*,&	6.45±4.38*,&

Note: *, P<0.05 compared to T1 values; #, P<0.05 compared to T2 values; &, P<0.05 compared to unilateral chocolate cyst patients.

Table 5. Values of V, PSV and AFC

Index	Ovarian chocolate cyst	Non-chocolate cyst	Control
V (cm ³)	5.81±0.32	5.83±0.13	5.84±0.21
PSV (cm/s)	9.81±0.11	9.83±0.12	9.83±0.14
AFC	6.21±0.96*,&	6.88±1.32*	7.27±1.87

Note: *, P<0.05 compared to control group; #, P<0.05 compared to non-chocolate cyst group.

benign mature teratoma and parovarian cyst [9]. However, scholars have reported possible injury on ovarian reserve by ovarian cystectomy under laparotomy. However, whether the surgery is the direct reason causing such injury is difficult to demonstrate. Previous studies have been performed to monitor estrogen and ovarian hyperstimulation hormone in real-time, in combined with imaging examination but obtained inconclusive results [10]. As one novel evaluating index for ovarian reserve function, AMH can also exert critical role in measuring ovarian response during artificially assisted reproduction [11].

In this study, we examined AMH, FSH and LH levels in ovarian cyst patients who received laparotomy surgery in our hospital at different time points. We found decreased levels 2 days post-surgery and slightly elevation 3 months later, but were still lowered than pre-operative levels. Bilateral chocolate cyst and non-chocolate cyst patients had consistent patterns of hormone levels. Unilateral chocolate cyst patients, however, had no significant change of AMH levels. Bilateral ovarian chocolate cyst patients had more significant changes of AMH, FSH and LH levels. These results suggested the ovarian surgery decreased AMH, FSH and LH, especially in bilateral chocolate cyst patients. As one poly-peptide-like hormone, AMH belongs

to transformation growth factor β superfamily. Granule cells in ovary do not express AMH at fetal stage, but starts to gradually secrete AMH until child-bearing period. When the size of antral follicle is less than 4 mm, AMH has the highest expression level. Its expression, however, suddenly disappears in antral follicles between 4 mm and 8 mm, and not in preantral or atrophic follicles [12]. A correlation analysis found lowered AMH level in endometrium patients [13]. As chocolate cyst normally belongs to endometrium at moderate or late stage, the serum AMH level is lowered than non-chocolate cyst patients [14]. After surgery, AMH level in chocolate cyst patients was also decreased more significantly than non-chocolate cyst ones. Moreover, non-chocolate cyst patients can have gradual recovery of AMH level after surgery within 6 months, while chocolate cyst patients only reached about 80% of preoperative AMH level [15]. AMH exerts critical functions as it can inhibit the proliferation of ovarian granule cells and follicles, decrease FSH sensitivity of follicles and impedes the selection of advantageous follicles [16]. Meanwhile, another analysis revealed better efficacy of AMH in reflecting the alternation of ovarian reserve function due to its close correlation with follicle number [17]. In one study about AMH stability, its level remains relatively stable without significant fluctuation between two menstrual cycles, and is not affected by exogenous hormone [18].

This study also measured V, PSC and AFC of ovary three months after surgery, and found no significant change of V or PSV between disease and control group. AFC level, however, was lowered in cyst group compared to normal ones. These results suggested decreased AFC after ovarian surgery, indicating compromised ovari-

an reserve function. Previous studies suggested no significant difference regarding Kupperman score, FSH, LH or ultrasonic indexes by laparotomy ovarian surgery [19]. Due to the occurrence of lower ovulation rate in chocolate cyst patients as a result of decreased AFC, the activity of ovarian tissue near cyst is severely compromised, causing the insensitivity against FSH or LH and significantly decreased number of advantageous follicle number [20], as consistent with our results.

In summary, levels of AMH, FSH and LH were decreased after ovarian surgery, leading to decreased secretion of antral follicle but no change of ovarian size. The ovarian surgery under laparotomy thus may cause ovarian reserve injury, especially in chocolate cyst patients. AMH may work to reflect such adverse effects, thus providing evidences for clinical application such as the choice of optimal surgery plan.

Disclosure of conflict of interest

None.

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