Running title

Pre- and post-operative serum AMH levels

Correspondence

Title:

Changes in serum anti-Müllerian hormone levels may predict damage to residual normal ovarian tissue after laparoscopic surgery for women with ovarian endometrioma

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Capsule

Post-operative changes in serum AMH levels correlate with surgical damage to normal ovarian tissue after cystectomy for women with ovarian endometrioma. Measuring serum AMH levels may be clinically useful to identify the efficacy of surgical procedure in terms of fertility preservation.

Abstract

We measured serum AMH levels before and after surgery in women undergoing unilateral and monolocular cystectomy for benign ovarian diseases. Comparing to control benign cysts, we found a significant decline in serum AMH levels with consequent depletion of follicles in tissue specimens after surgery for women with ovarian endometrioma.

Key words: Ovarian endometrioma; cystectomy; ovarian damage; serum AMH

Therapeutic approach for women with ovarian endometrioma may vary according to the age of women, size of the cyst, symptoms, and desire for future conception. Laparoscopic cystectomy for endometrioma is common and seems to be feasible in terms of post-operative fecundability and recurrence rate comparing to that of fenestration and coagulation of cyst wall (1, 2). However, the safety of this technique with respect to residual ovarian damage has been questioned. On the other hand, there had been conflicting reports on ovarian response to ovulation induction in infertile women with previous surgical excision of endometrioma (3, 4).

Even skilled hands show excellent results, cystectomy for endometrioma may cause unavoidable risk of surgical injury to residual normal ovarian tissue. This may cause loss of ovarian follicles in women had operated on for endometrioma (5, 6). According to a recent report, absence of follicular growth was observed in 13% of operated ovaries although this event never occurred in the contralateral gonad (7). Therefore, an objective clinical parameter(s) is needed to evaluate damaging efficacy of surgical procedure in terms of preserving one’s fertility potential.

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein, belongs to the

transforming growth factor-β family and is produced solely by the granulosa cells of the recruited follicles until they become sensitive to follicle-stimulating hormone (8, 9). Serum level of AMH declines with age, is menstrual cycle independent, and is unaffected by gonadotrophin or GnRH agonist administration (10). Therefore, it may represent the most reliable serum marker to indicate the number of growing follicles and reflect ovarian follicular reserve.

Hence, it is worthy to elucidate the changes in serum AMH levels after ovarian surgical procedure to evaluate its efficacy (11). One recent report found an insignificant association between post-surgical serum AMH levels and size of endometrioma (12) and another study demonstrated variable time-dependent changes in serum AMH levels after ovarian surgery (13). However, information regarding association between post-cystectomy serum AMH levels and presence of normal ovarian tissue containing follicles within cyst wall is lacking. Here, we report changes in serum AMH levels and their possible relationship with depletion of follicles in tissue specimens removed during cystectomy in women with ovarian endometrioma.

During the period between 2007 and 2009, we prospectively recruited

thirty-two women aged <40 years, had unilateral laparoscopic cystectomy for endometriotic cyst (study group, endometrioma, n=19) and other benign ovarian cysts (control group, n=13; mature cystic taratoma, n=8; serous or mucinous cystadenoma, n=5) for pelvic pain, infertility and persistent ovarian cystic mass. These cysts were all >4cm in size, monolocular and diagnosed pre-operatively by trans-vaginal ultrasonography/MRI and subsequently confirmed by histology. Women had previous surgery with ovarian involvement were excluded. Ten women with endometrioma had GnRHa therapy for 3-5 month before surgery. Approval for the study was obtained by the local Institutional Review Board. All patients gave informed consent for this study.

Blood samples were collected from all women at one month before surgery and within three month after the surgery (mean 2.2 ± 1.0 month) and sera were stored at -80℃ until assayed. Pre- and post-operative serum AMH concentrations were measured by an enzyme immunoassay kit according to the manufacture's instructions (EIA AMH/MIS; Immunotech, Beckman Coulter, Marseille, France). All assays were performed in duplicate and mean values were used for data analysis. The intra- and inter-assay coefficients of variation for AMH assay were below 10% with a detection limit of

0.14ng/mL. The percentage (%) change between pre- and post-operative serum AMH values was calculated as follows:

% AMH change = (pre-operative AMH value minus post-operative AMH value) / pre-operative AMH value X 100

After careful adhesiolysis and determination of tentative cleavage plane, stripping of the endometrioma cyst wall was performed by gentle counter traction by grasping forceps. Operated ovaries were left in place without suturing. For non-endometriotic ovarian cysts, extracorporeal manual stripping with double-balloon suction catheter (S.A.N.D. balloon catheter, Hakko, Nagano, Japan) or intra-corporeal stripping was performed according to the size and mobility of the cyst. Surface of the ovary was then sutured with absorbable surgical strings. Laparoscopic surgery was performed by two authors (K.H./A.F.).

Presence of follicles and normal ovarian tissue in enucleated cyst wall were examined by studying the routine hematoxyline-eosin stained histological preparations. We evaluated the presence of healthy ovarian tissue (nested primordial and primary follicles within densely packed ovarian stroma) in enucleated cyst wall by examining two

5μm sections derived from each of endometriotic and non-endometriotic cyst wall. Histological analysis was performed by one investigator (M.K.) without knowledge of pre- and postoperative AMH results in a blind fashion.

Data were analyzed by student's *t*-test for continuous variables and by Mann-Whitney *U*-test when there was skewed distribution. Multivariate analysis of variance (ANOVA) was performed with some possible confounding variables, such as tumor type, cyst wall with healthy ovarian tissue, age of the patient, size of the cyst, pre-operative AMH levels, and difference in operator. A value of $P<0.05$ was considered to be significant.

Although women with endometrioma were significantly older than women with non-endometriotic control cyst [29.8 ± 5.4 (mean ± SD) vs. 25.9 ± 5.6 years old, P=0.046], we did not find any significant difference in pre-operative AMH levels between these two groups [4.27 ± 3.00 (mean ± SD) vs. 3.98 ± 2.16 ng/mL]. The mean diameter of the cyst was comparable between two groups [6.7 ± 1.9cm (mean ± SD) vs. 6.8 ± 2.1cm].

Percentage changes in serum AMH level were significantly larger in women

with endometrioma than in control women [-24.6 ± 29.3% (mean ± SD) vs. 3.3 ± 34.5%, P=0.02]. Presence of normal ovarian tissue was confirmed in 13 women (nine in endometiroma and four in control). In women with endometrioma, % change in AMH levels was significantly larger in women with cyst that contains normal ovarian tissue comparing to that of women without healthy ovarian tissue [-42.0 ± 32.9% (mean ± SD) vs. –8.9 ± 13.4%, P=0.01, Figure 1]. On the other hand, we did not find any significant difference in AMH levels between control women having cyst walls with and without normal ovarian tissue (Figure 1).

After analyzing several confounding variables, ANOVA revealed that the type of the cyst and the presence of healthy ovarian tissue were significantly associated with the post-operative decline in serum AMH levels (Table 1). However, when we analyzed the model with these two variables together, the statistical significance for the type of the cyst was lost (Table 1).

We report here for the first time that comparing to control benign cysts, a significant decline in serum AMH levels occurred within three months with consequent depletion of follicles in tissue specimens removed during excision surgery for women

with ovarian endometrioma. Each woman had first ovarian surgery and had single cyst excision. Although over the years, various tests and markers of ovarian reserve have been reported, the clinical value of testing for basal FSH and inhibin-B is limited (14, 15). Again, ultrasonogrphic markers such as antral follicle count and ovarian volume can be used as indicators of ovarian reserve. However, it is difficult to assess the exact number of antral follicles and ovarian volume of the cystic ovary before cystectomy (16). Therefore, we believe that as the most reliable and easily measurable test, post-operative serum AMH levels could be clinical useful to assess the status of ovarian reserve after excision surgery for endometrioma and also for the selection of patients who wish to preserve future fertility.

We measured post-operative serum AMH levels at variable time within three months after surgery. Issues regarding recovery of these values within this time frame need to be addressed. Recently, Chang, et al. (13) reported time-course follow-up of serum AMH levels after ovarian cystectomy. They found largest decline in serum AMH levels one week after surgery and variable recovery during follow-up periods. Indeed, complete recovery of serum AMH levels was not observed at three month after surgery

especially in women with endometrioma (12). Women with endometrioma may need longer duration for recovery in serum AMH levels, because surgical injury itself and inflammatory reaction could be more detrimental to the surrounding ovarian cortex in these women. Prolonged follow-up studies with larger subjects are needed to strengthen our findings of post-operative AMH levels and examine its pattern of recovery.

Age is a major determinant of serum AMH levels (8, 10). Age may affect the duration of recovery from surgery-related decline in serum AMH levels. Even a significant difference in the age of women between study and control groups was observed, we did not find any significant difference in pre- or post-operative AMH levels in these two groups of women. Moreover, multivariate analysis indicated that loss of normal ovarian tissue and the type of the cyst were more significant confounding factors related to change in AMH levels after surgery. Finally, Our current findings may indicate the importance of measuring pre- and post-operative serum AMH levels as a marker of ovarian reserve to evaluate the efficacy of surgical procedure in terms of fertility preservation.

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Figure Legend:

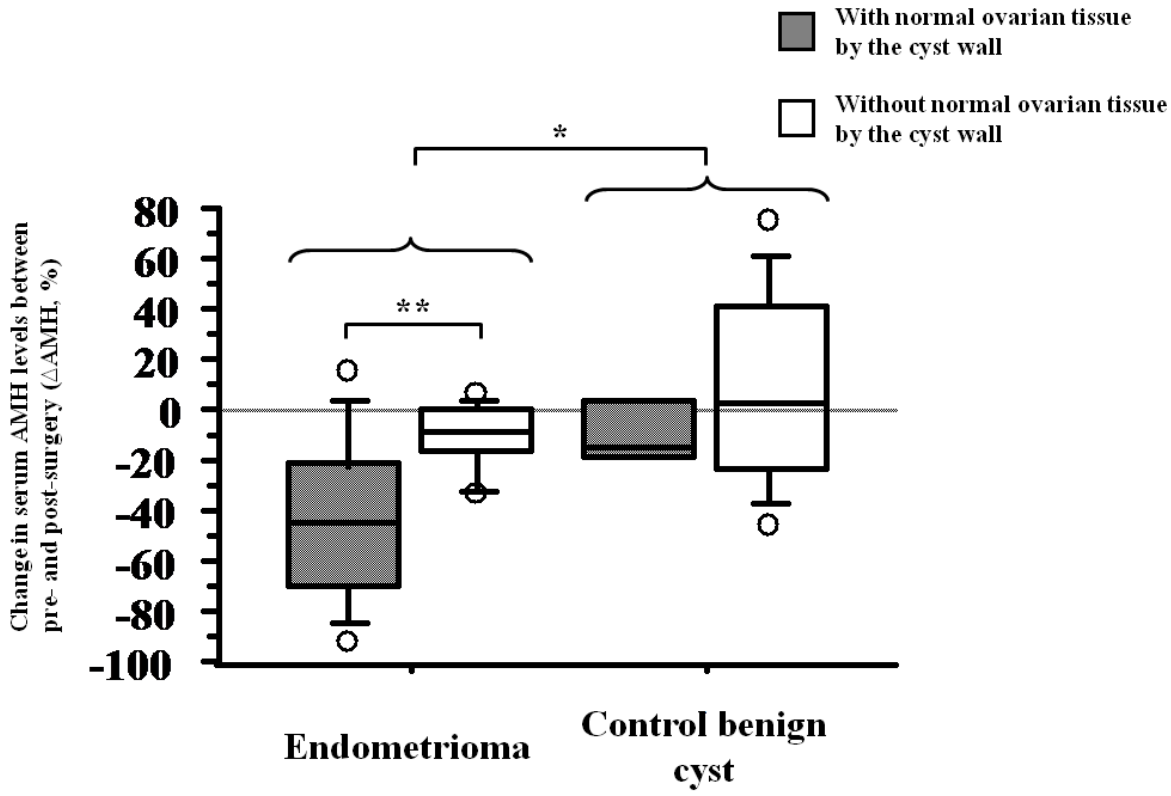

Figure 1. Shows % changes in serum AMH levels after post-operative cystectomy according to the type of the cyst and presence or absence of normal ovarian tissue within cyst wall. Woman had unilateral cystectomy for endometrioma showed significant decline in post-operative serum AMH levels than in control women with benign cyst (*P=0.02, Student's *t*-test). In women with endometrioma, % change in serum AMH levels was significantly higher in women with cyst wall containing normal ovarian tissue comparing to that of women containing similar cysts without normal ovarian tissue (**P=0.01, Mann-Whitney *U*-test). No significant difference between pre- and post-operative serum AMH levels was observed in control women having these two types of cysts. Boxes represent the distance (interquartile range) between the first (25%) and third (75%) quartiles, and horizontal lines in the boxes represent median values.

Table 1. Multivariate analysis of variance (ANOVA) utilizing possible confounders to relative change in post-operative serum AMH levels

Confounding variables	F value	P value	F value*	P value*
Diameters of the cyst (cm)	0.0005	0.98	0.03	0.86
Difference in primary operator	0.41	0.53	1.46	0.24
Age (years)	1.55	0.23	0.43	0.52
Preoperative serum AMH value (ng/mL)	1.88	0.18	3.01	0.09
The type of cyst	2.20	0.15	4.55	0.04
Presence of healthy ovarian tissue	4.91	0.04	•	•

*The values when “the presence of healthy ovarian tissue” were omitted from the model.