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Anti mullarian hormone level in non endometriotic benign ovarian cyst before and after laparoscopic ovarian cystectomy

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Abstract

Objective :*This study aimed tothe comparison of anti-mullerian hormone level in benign ovarian cyst Before and after laparoscopic ovarian cystectomy.* ***Methods:****This was clinical trial studyincluded 34 women undergoing Laparoscopic Ovarian Cystectomy for benign ovarian cyst to evaluate ovarian reserve by using AMH level determination, and at one and 3 months after laparoscopic cystectomy. Serum AMH levels were compared before and after the surgery and between various types of ovarian cyst.****Results:*** *There was significant higher reduction among younger than olderage groups (49.7% versus 41.5%), bigger than smaller cyst size (51.5% versus 39.2%), higher reduction among patients with higher pre-operative Anti-Mullerian Hormone (≥ 5 ng/ml) than<5 ng/ml (50.6% versus 40.7%).Serum AMH was significantly decreased after ovarian cystectomy in the next 3 months post-operative.****Conclusions:*** *Decreased serum AMH may be contributed to decreased ovarian reserve after laparoscopic ovarian cystectomy. This can result from thermo-coagulation used for hemostasis during the operation.*

Key words:*Anti-mullerian hormone, Ovarian cyst, Laparoscopic*

I. INTRODUCTION

An ovarian cyst is a fluid containing sac that is formed in the ovaries. In the United States, almost all of the women in reproductive age and about 18 percent of those who are in post-menopausal age have ovarian cysts**[1].**

Most of the ovarian cysts are benign in nature with very low chance of malignant transformation. Most common symptoms of these cysts such as chronic pelvic pain, dyspareunia, gastrointestinal and urinary symptoms are resulted from the pressure they apply to the nearby structures such as the GI tract and bladder **[2]**.

Management of these cysts is mandated when there is chance for malignancy (cysts larger than 10cm and abnormal serum CA-125 levels) and intolerable symptoms. Surgical removal of the cysts is required for

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persistent ones measuring 5-10 cm which have not shown any shrinkage after expectant management for several cycles should be considered **[3]**.

The operation of resecting an ovarian cyst (ovarian cystectomy) can be performed either by laparotomy or laparoscopy. Recent studies have reported significant decrease in ovarian reserve, estimated by measurement of serum anti-mullerian hormone (AMH) levels drops significantly after ovarian cystectomy**[4]**.

This reduction was partially reversible three months after operation. Some other studies have reported no decrease in the serum level of AMH or damage to ovarian reserve after ovarian cystectomy**[5]**. The aim of this study is the comparison of anti-mullerian hormone level in benign ovarian cyst Before and after laparoscopic ovarian cystectomy

II. METHODS:

This was clinical trial study performed in Obstetrics and Gynecology Department, Zagazig University Hospital during the period from March 2020 to August 2020. Included 34 women with diagnosis of benign ovarian cyst whounderwent laparoscopic ovarian cystectomy and diagnosis of ovarian cyst was made by ultrasound imaging and CA125. The age of the studied group was (30.3±4.9) ranged from (23 to 38), (52.9%) of them was in the age group more than 31 years, their parity was (0.7±0.9) ranged from (nulliparous to para 3), (58.8%) of them was nulliparous, (41.2%) were multiparous and (11.8%) of the studied group were infertile. Before enrollment, all participants were requested to sign informed consent forms. Study protocol was approved by the ethics committee of Faculty of Medicine, Zagazig University. The work was carried out for studies involving humans in accordance with the World Medical Association's Code of Ethics (Helsinki Declaration).

Inclusion criteria were: Cases of benign ovariancyst diagnosed by ultrasound imaging. Exclusion criteria: History of suspected or proved ovarian malignancy. Previous adnexal surgery, or surgery of ectopic pregnancy. Polycystic ovarian syndrome.Evidence of premature ovarian failure or premature menopause which is detected by FSH & LH assay.History of infertility due to endometriosis.Size of the cyst more than 15x15x15cm.Patients who had sonographic malignant criteria (The presence of a solid component and detectable flow by Doppler imaging within a cystic ovarian mass).All participants underwent a detailed history, general examinations.

Before the laparoscopy (investigations)

Diagnosis of ovarian cyst was made by ultrasound imaging(SIMENS ACUSON X300 medical systems,: ultrasound machine with 2D endovaginal proper with frequency 7MHz). Criteria of benign cysts like (low echogenicity, a thin cyst wall, unilocular , and absence of internal papillary excrescences).Cases of benign ovarian cyst including dermoid cyst, mucinous and serous cystadenoma were included . The most important morphologic features on ultrasound that are of concern for malignancy Participants were followed by scheduled outpatient visits and ultrasonography imaging. No medical treatment trial was prescribed to eliminate any possible effect of GnRH therapy on pre-operative measures of AMH levels. Those who had persistent cysts measuring 5-10 cm or intolerable symptoms of adnexal mass were candidate for laparoscopic ovarian

cystectomy and were included in this study. Participants scheduled for laparoscopic ovarian cystectomy were examined and serum level of AMH , CEA , CA 125 were measured before the operations.

Preoperative and postoperative follow up ultrasound evaluations were performed by the same gynecology resident using ultrasound device (7.5 MHz trans-vaginal probe, Ultasonix OP machine; British Columbia, Canada). Pre and postoperative measurements of AMH was performed by DSL active mullerian inhibiting substance/ AMH ELISA kit (Diagnostic Systems Laboratories, Webster, Texas, United States) the same reference lab and reported in ng/ml values and detection limit of 0.006 ng/ml.

Laparoscopic maneuver:

Participants underwent laparoscopic ovarian cystectomy under general anesthesia. And complete aseptic techniques and on supine (supine position with abduction of lower limbs and with flexion of the thighs onto the pelvis of about 20°) position and pelvic laparoscopic operative design using a Verres needle passed through a 1cm umbilical incision, pneumoperitoneum was induced by CO2 insufflation to maintain an intra-abdominal pressure of 12 -15mmHg.The initial laparoscopic port was placed at the umbilicus with a 5–12 mm port. The pelvis was carefully surveyed, and the ovarian cyst was examined for any signs that may be suggestive of malignancy such as ascites, excrescences on the surface of the ovary, and implants noted on peritoneal, liver, or diaphragm surfaces. Five millimeter ports were then placed laterally. The surgeon obtained cell washings at this time for cytology. All cysts that were appeared benign cystectomy was performed.Cystectomy was then performed by incising the capsule of the ovary with scissor. The cyst is then enucleated carefully with traction and countertraction and dissection as needed. In case of intraoperative rupture occurs, particularly with a dermoid cyst, the peritoneal cavity was liberally be rinsed with normal saline or Ringer's lactate that has been shown to be safe. After the cyst was removed from the ovary, an endoscopic bag was then advanced through the umbilical 10–12 mm port, and the cyst is placed in the bag. The bag was then advanced up to the umbilical incision, and the port was removed while advancing the edges of the bag through the skin incision. The bag was then opened and triangulated to facilitate removal of the cyst intact and those which were small enough or with morcellation carefully avoiding any spillage out of the bag. Prior to removal, the cyst was drained while in the bag. Once the specimen was small enough, the bag was removed with the specimen through the incision. After removal of the bag and specimen, the 10–12 mm laparoscopic port was replaced through the umbilical incision and the camera advanced through the port. The ovary was then carefully inspected for hemostasis. Bleeding from the bed of the cyst in the ovary in some patient required measures to obtain hemostasis. Hemostasis in the bed of the ovary at the site of the cystectomy has traditionally been accomplished with bipolar cautery. The edges of the ovarian capsule did not need to be reapproximated as is traditionally done with an open ovarian cystectomy. The procedure was completed by irrigation of the pelvis, and a careful survey of the pelvis for hemostasis with the intraperitoneal pressure decreased to 5 mmHg. The port sites were then inspected for hemostasis. After all port sites are inspected for hemostasis, the C02 peritoneum was then allowed to escape through the umbilical port. Care was taken to ensure that as much gas is expressed as possible to minimize postoperative discomfort for the patient and to avoid the bowel being pushed into the incision sites as residual gas escapes. The incisions were then closed with suture, Steri-Strips, or a skin adhesive. Fascial closure was recommended for ports 10 mm or greater in size prior to skin closure to prevent subsequent development of an incisional hernia .

After the surgery, participants were observed in hospital ward for 24 hours to avoid surgical complications or those associated with anesthesia. For all participants, operative and post-operative course were successful with no specific complication. Participants were evaluated at outpatient clinic at 1 month and 3 months after the surgery for measuring serum level of AMH and ultrasound evaluation of surgical outcome and recurrence of the primary cyst.

Statistical Analysis:

Data were checked, entered and analyzed using SPSS version 23 for data processing. The following statistical methods were used for analysis of results of the present study.

III. RESULTS:

Table (1), showed that the ovarian cyst size in the study group was (69±1.6) ranged from (48 to 103) mm, divided equally to two groups less and more than 69 mm, most cysts were unilateral (70.6%) and (41.2%) of the studied group were dermoid cyst type.

Table (1): Characteristics of the ovarian cyst among the studied group:

Variable	**The studied group(34)** **mean ± SD** **(Range)** **Median**	
The ovarian cyst size (mm)*:*	69±1.6 (48-103) 69	
Variable	**NO(34)**	**%**
The ovarian cyst size:		
*< **69** mm*	17	50.0%
*≥ **69** mm*	17	50.0%
The ovarian cyst side:		
Unilateral	24	70.6%
Bilateral	10	29.4%
The ovarian cyst type:		

Serous	10	29.4%
Dermoid	14	41.2%
Mucinous	10	29.4%

There was highly statistically significant reduction in AMH post-intervention **Figure (1)**.

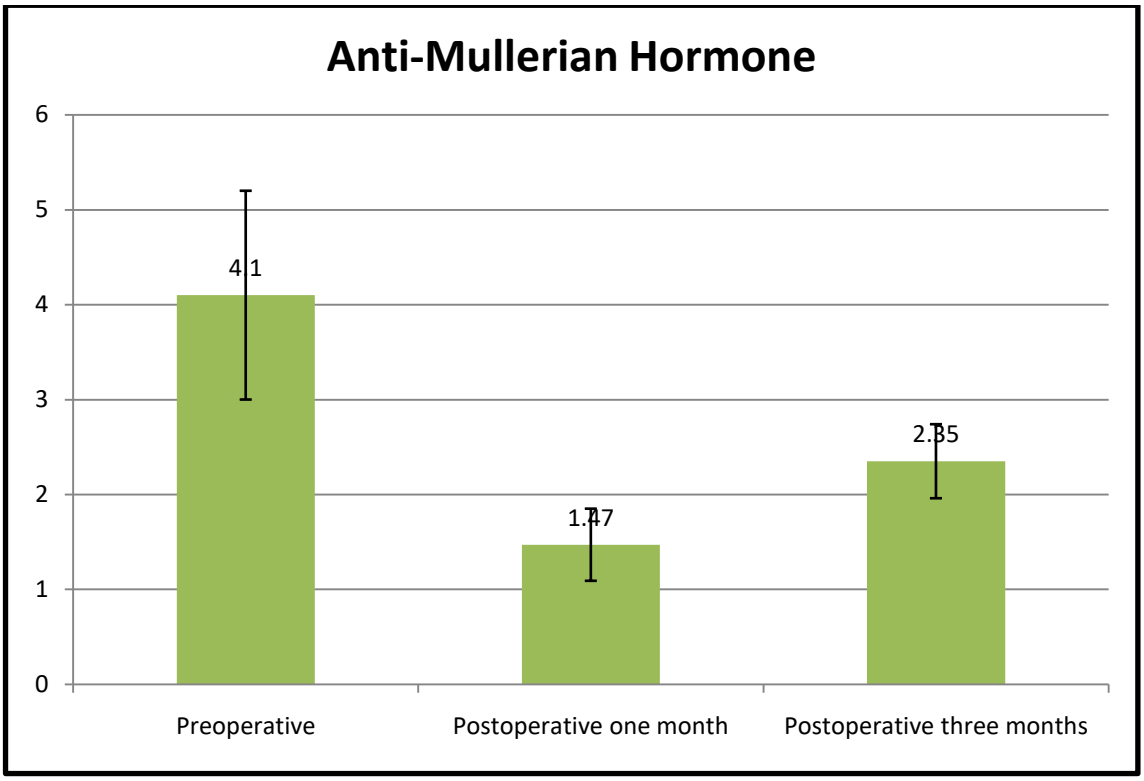

Fig (1); Bar chart for the pre and post-operative Anti-Mullerian Hormone (AMH) Level among the studied group

Table (2), showed that there was statistically significant higher reduction among younger than older age groups (49.7% versus 41.5%) with no statistically significant difference regarding parity and patients' complaint.

Table (2); Relation between reduction (AMH reduction) and patients' characteristics:

Variables	***Percent of AMH reduction***	***(range)***	**test**	**P-value**
Age				
<31 years	49.7%	(31.3%-66.2%)	2.4	**0.02***
≥31 years	41.5%	(26.7%-51.9%)		

Parity*:*				
Nulliparous	46.6%	(29.1%-66.2%)	0.8	0.4
Multiparous	43.6%	(26.7%-56.3%)		
Complaint				
Pain	44.7%	(26.8%-61.9%)	0.9	0.3
Infertile	50.3%	(31.3%-66.2%)		

Table (3), showed that there was statistically significant higher reduction among bigger than smaller cyst size (51.5% versus 39.2%) and also more reduction among dermoid than serous than mucinous cyst with no statistically significant difference regarding unilateral and bilateral cyst.

Table (3); Relation between reduction (AMH reduction) and ovarian cyst characteristics:

Variables	Percent of AMH reduction	(range)	test	P-value	
The ovarian cyst size:					
≥69mm	51.5%	(26.7%-66.2%)	3.9	**0.001****	
<69mm	39.2%	(26.9%-51.9%)			
The ovarian cyst side:					
Unilateral	43.4%	(26.7%-61.9%)	1.6	0.1	
Bilateral	49.9%	(31.3%-66.2%)			
The ovarian cyst type:					
Serous cystadenoma	44.1%	(29.1%-50.4%)		**0.001****	**0.003*(1)**
Dermoid	53.8%	(40.6%-66.2%)	19.8		**0.009*(2)**
Mucinous cystadenoma	34.8%	(26.7%-51.9%)			**0.001**(3)**

** Statistically highly significant difference ($P \leq 0.001$), * Statistically significant difference ($P \leq 0.05$),(1) Serous versus dermoid, (2) Serous versus Mucinous, (3) Dermoid versus Mucinous

Figure (2), showed that there was statistically significant higher reduction among patients with higher pre-operative Anti-Mullerian Hormone (≥ 5 ng/ml) than<5 ng/ml (50.6% versus 40.7%).

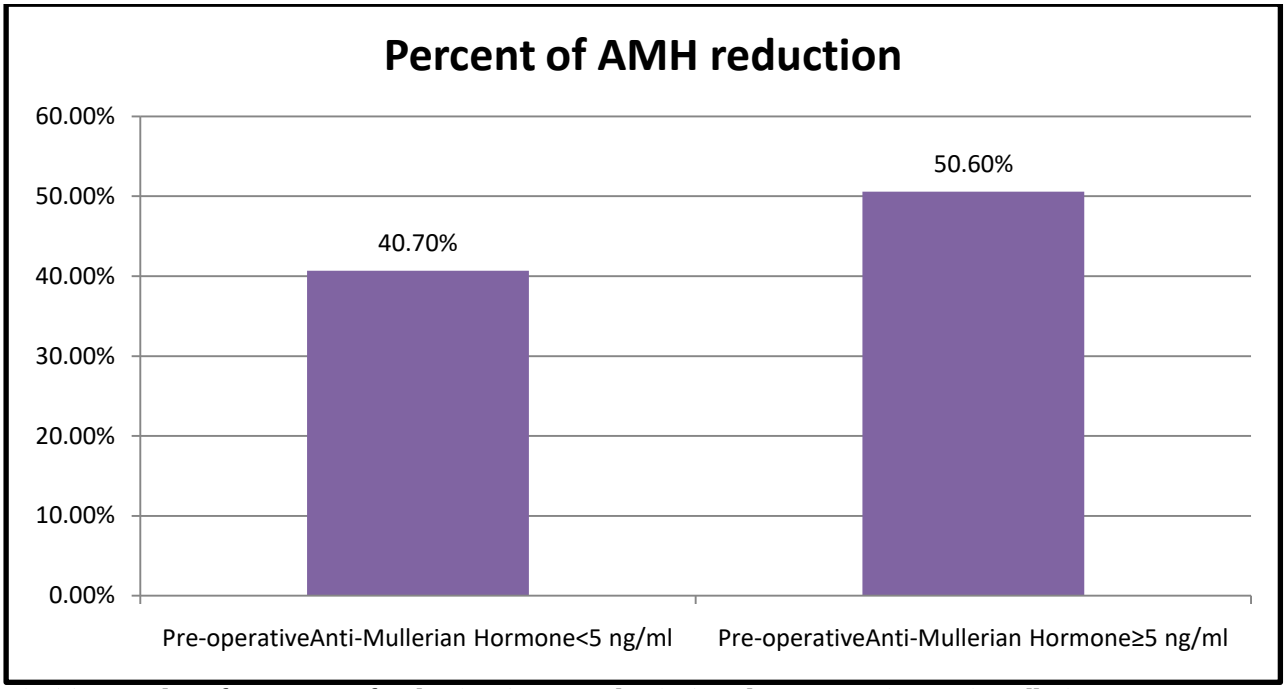

Fig (2): Bar chart for percent of reduction (AMH reduction) and Pre-operative Anti-Mullerian Hormone level among the studied group.

Table (4), showed that there was statistically significant positive correlation between percent of reduction and AMH levels (pre- & post-operatively). But regarding age and cyst size, there was no statistically significant correlation.

Table (4): Correlation between reduction (AMH reduction) and patients' characteristics

Variable	***percent of reduction***		
	r^	**p**	**SIG**
Age	-0.1	>0.05	NS
Ovarian cyst size	0.08	>0.05	NS
Preoperative AMH	**0.4**	**0.004***	**S**

Postoperative one month AMH	**0.5**	**0.003***	**S**
Postoperative three months AMH	**0.3**	**0.02***	**S**

IV. DISCUSSION

Management of benign ovarian cysts is mandated when there is chance for malignancy (cysts larger than 4 cm and abnormal serum CA-125 levels) and intolerable symptoms. Surgical removal of the cysts is required for persistent ones measuring 5-10 cm which have not shown any shrinkage after expectant management for several cycles should be considered**[1].**

Laparoscopic cystectomy is considered the first-line choice for benginoverian cyst and associated with better pain control and shorter hospital stay. Laparoscopic stripping is the standard laparoscopic method for these ovarian tumors **[6].**

However, the best and most efficient conservative laparoscopic technique remains a controversial issue in the literature. The debate is between excision and ablation of the capsule. The reported benefits in favors of surgery include a decrease in the recurrence of symptoms and signs and a reduction in the recurrence of the benginoverian cyst. There are also some reports that show an increase in responsiveness to ovarian stimulation and cumulative pregnancy rate (PR) in randomized controlled trials**[7]**.

Concern has been expressed over the risk of damaging ovarian reserve owing to excision of the capsule or to the use of electrocautery in surgically treating benginoverian cyst. Ablation may have a higher risk of recurrence, but excision may result more significant damage to ovarian follicular reserve, which could compromise future ovarian response during in-vitro fertilization**[8]**.

Many tests and markers are used to determine the ovarian reserve, such as serum levels of estradiol, follicle-stimulating hormone, anti-mullerian hormone (AMH), inhibin-B, and antral follicle count (AFC), of which AMH is the best marker. Compared with AMH, AFC may be a more accurate marker of post-surgery ovarian reserve due to the laterality of diseases), consequently, AMH and AFC are widely used**[9]**.

The anti-Mullerian hormone [AMH] belongs to the β-class of growth factor transforming and falls from primary granulosa cells to small antral follicles, and is associated with the small antral follicles. The anti-Mullerian hormone is the only menstrual cycle independent ovarian marker. The AMH acts as an ovarian reserve marker, provide many advantages over other markers; it is stable during the menstrual cycle, and is fairly autonomous from the use of hormone therapy. AMH level decreases after surgical cyst excision**[6]**.

Many studies reported increased risk of ovarian damage and decreased serum AMH levels after laparoscopic cystectomy in women with benginoverian cyst. Although some studies reported that laparoscopic surgery did not damage the ovaries, and laparoscopic cystectomy generally harmed the ovaries, resulting in decreased post-surgery ovarianreserve**[7]**.

The present study showed that the ovarian cyst size in the study group was (69±1.6) ranged from (48 to 103) mm, divided equally to two groups less and more than 69 mm, most cysts were unilateral (70.6%), 10 women (29.4%) had serous, 10 women (29.4%) had mucinous and 14 women (41.2%) of the studied group were dermoid cyst type. this was in agreement with **Amooee, et al., [1]**whose study included 60, women with average age of 25.8 years. Ovarian cysts included in their study consisted of benign cysts including dermoid cyst (n=23), mucinous cystadenoma (n=11), and serous cystadenoma (n=26). Ovarian cyst diameters measured from 4.6-13 cm with mean size of 7.6 cm.

Similarly, **Awad et al., [6]**found that there were 9 (18%) simple cysts with focal inflammation of the fibrous wall and sloughed lining, 11 (22%) were endometriotic cysts, 12 (24%) were benign serous cyst, 11 (22%) were hemorrhagic retention cyst with fibrolytic wall and 7 (14%)were cystic teratomata.

Our results found that there was highly statistically significant reduction in AMH pre and post-intervention 4.1±1.01 versus 2.35±0.39 respectively with 45.4% percent of reduction) this was in agreement with **Elsemary et al., [7]**whose study reported that the serum levels of AMH decreased significantly 3 months postoperatively (4.12±3.4 versus .62±2.7 P < 0.001) and**Awad et al., [6]**who reported that AMH decreased after laparoscopy when compared with values before it (1.56±0.14 ng/ml Vs 0.71±0.15 ng/ml).

Additionally, in another prospective research 40 women with benginoverian cyst (as study group) diagnosed as infertility, 36 women with infertility factor tubes (as the control group 1), and 22 women with other benign ovarian cysts (as the control group 2] were also reported. The impact on ovarian reserves afterlaparoscopic cystectomy was assessed by serum AMH levels**[8].**

As regard to serum AMH, current results were also in consistent with **Celik et al., [10]**that measured the serum AMH until 6 month after cystectomy and noticed it gradually declined.

Also **Chang et al., [11]**reported that the serum AMH declined gradually after cystectomy after one month and that 65% preoperative level recovered 3 months later.

In contrast to our results, **Lee et al., [12]**and **Biacchiardi et al., [13]**showed no statistically significant decline in serum AMH level 3 months after the surgery

The role of patients age, parity, complaint, cyst size, cyst type and bilaterally were compared with the changes of AMH level after laparoscopic cystectomy and the following result showed that there was statistically significant higher reduction among younger than older age groups (49.7% versus 41.5%), bigger than smaller cyst size (51.5% versus 39.2%), higher reduction among patients with higher pre-operative Anti-Mullerian Hormone (≥ 5 ng/ml) than<5 ng/ml (50.6% versus 40.7%) and also more reduction among dermoid than serous than mucinous cyst with no statistically significant difference regarding unilateral and bilateral cyst, parity and patients' complaint.

Elsemary et al., [7]found that Patients older than 38 years had significantly lower baseline serum level of AMH when compared with those younger than 38 years (1.56 ± 2.49 vs. 4.12 ± 3.48 ng/ml; $P = 0.003$). Both groups had significantly lower AMH levels ($P < 0.001$) 3 months after the operation. Women with large and small cysts (3 cm as a cut-off value) had nosignificant difference regarding the baseline level of AMH (4.01 ± 3.55 vs. 2.86 ± 2.58 ng/ml; $P = 0.149$). The decrease in AMH after the operation was similar between the two study groups. However, regarding AMH level between those with large and small cysts 3 months after the

operation (P = 0.366), there was no significant difference. There were 19 (31.7%) patients with bilateral and 41 (68.3%) patients with unilateralbenginoverian cyst. The baseline AMH level was comparable between these two groups (3.29 ± 3.10 vs. 4.21 ± 3.71 ng/ml; P = 0.074). The AMH level decreased significantly 3 months after the operation in those with unilateral (P < 0.001) and bilateral (P < 0.001) benginoverian cyst .Those with single unilateral cysts had significantly higher baseline levels of AMH when compared with those with single bilateral cysts (4.21 ± 3.82 vs. 2.59 ± 1.89 ng/ml; P = 0.006). Moreover, in those with multiple bilateral cysts, the baseline serum levels of AMH were significantly higher compared with those with single bilateral ones (4.49 ± 4.78 vs. 2.59 ± 1.98 ng/ml; P = 0.026). The trend of reduction of AMH levels after the operation was similar in all groups.

Similarly to our results, Statistical analysis of AMH levels in studied population at **Amooee, et al., [1]** study before the operation showed a mean value of 3.77 ng/mL which declined to 1.87 (ng/mL 0.67-3.07 ng/mL) 1 month after the operation. This declinewas significant with the p:<0.001. Measurement of AMH 3 months after the surgery revealed significant elevation to a mean level of 2.48 ng/mL (95% CI: 1.08-3.88 ng/mL) (p<0.001). Patients younger than 30 years old had greater decline in serum AMH level when compared to over 30 year-old patients. The difference of values was significant. Regarding the primary AMH level, they found that those with initial level of AMH greater than 5 ng/ml developed more decline in AMH levels than those with primary AMH less than 5 ng/ml after 3 months.

For evaluation of primary cyst size effect on AMH variation after cystectomy patients were categorized as follows: Group 1) cyst size 40-65 mm, Group 2) cyst size 65-90 mm, Groups 3) cyst size larger than 90 mm. analysis for AMH level variation after 3 months showed significantly higher decline in AMH level in group 3 compared to group 1 with higher percentage of AMH decline among group 3 (p=0.006).

Finally, regarding AMH level reduction within each type of ovarian cysts, variations of serum AMH levels were significant (p<0.001). Long term recovery of ovarian reserve after ovarian cystectomy did not vary significantly among serous and mucinous cystadenoma (p=0.48) but pairwise comparison of each type with dermoid cyst revealed significant difference in total recovery. Overall recovery was highest in mucinous cystadenoma (68%) and lowest in dermoid cysts (62%).

V. CONCLUSIONS

Decreased serum AMH may be contributed to decreased ovarian reserve after laparoscopic ovarian cystectomy. This can result from thermo-coagulation used for hemostasis during the operation. More multi-centric studies in the same direction are required involving large sample size of patients in order to explore the exact etiology of ovarian reserve reduction. Repeated further tests after 1 year post-operative to see the final effect on the biochemical markers.

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