Clinicopathologic Review of Ovarian Masses in Korean Premenarchal Girls

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Abstract

Objective: To review the clinicopathological characteristics of ovarian masses in Korean premenarchal girls.

Design: The data collected from hospital medical records were reviewed retrospectively regarding age, presentation, diagnosis, treatment, and outcome.

Participants: There were 65 premenarchal girls who underwent surgery at Seoul St. Mary’s Hospital between January 1990 and March 2012.

Results: The most common presenting symptom was abdominal pain (n=31, 47.7%), followed by palpable abdominal masses 16 (n=16, 24.6%), abdominal distension (n=8, 12.3%), vaginal bleeding (n=4, 6.2%), incidental finding (n=3, 4.6%), difficulty in urination or defecation (n=2, 3.1%), and prenatal sonographic findings (n=1, 1.5%). Of the patients with benign tumors, including non-neoplastic lesions and benign cysts, 26 (51%) underwent cystectomy, 6 (11.8%) underwent oophorectomy, 17 (33.3%) underwent unilateral salpingo-oophorectomy and none underwent bilateral salpingo-oophorectomy. Of the patients with malignant tumors, 2 (14.3%) underwent bilateral salpingo-oophorectomy, 7 (50%) underwent unilateral salpingo-oophorectomy and none underwent bilateral salpingo-oophorectomy. Of the patients with malignant tumors, 2 (14.3%) underwent oophorectomy, and 2 (14.3%) underwent cystectomy.

Conclusion: Abdominal pain was the most common symptom. However, the incidence of abdominal distension was higher in patients with malignant tumors than in those with benign tumors. We assessed clinical features, operative outcomes, and histological classifications of Korean premenarchal girls with ovarian masses. Further studies with a larger number of subjects are needed to confirm our results.

Key words: premenarchal girl, ovarian mass, clinicopathological observation.

Introduction

In children, indications for gynecologic surgery are divided into 3 categories: congenital anomalies, genital traumas and pathological lesions of the genital tract. Adnexal lesions are the most common indication for gynecologic surgery in children. Although the frequency of adnexal surgery in children is low, it is important to save patients from death and preserve their fertility.

Ovarian cysts develop from mature follicles in hormonally active ovaries, so that they are often detected after puberty. Ovarian masses, whether cystic, solid or both, are generally considered rare in the
premenarchal age group [1]. The actual incidence of pediatric ovarian lesions is unknown. However, ovarian neoplasms are estimated to occur at a rate of approximately 2.6 in 100,000 girls per year and malignant tumors make up about 1% of all childhood malignant tumors [1-3]. Ovarian masses receive surgical interventions for various causes. Most patients have nonspecific symptoms. Patients may present with acute abdominal pain and signs of peritonitis which are difficult to distinguish from acute appendicitis. Patients with ovarian lesions which secrete hormones may present with pseudoprecocity, masculinization or the signs of endocrine disturbances. Some patients may have huge masses, which can cause ureteral obstructions, hydronephrosis, urinary frequency or difficulty, and bowel obstruction [1, 4-6].

There have been many studies on the clinical characteristics of patients who have developed adnexal masses in the pediatric population. However, age criteria vary among the different studies. Therefore, more information about the clinical characteristics of children with adnexal masses is necessary to establish procedures for early diagnoses and to adequately manage such lesions in children. This study analyzed the clinicopathological characteristics of 65 Korean premenarchal girls who underwent surgery at Seoul St. Mary’s Hospital due to adnexal masses between January 1990 and March 2012.

Materials and Methods
This study included 65 premenarchal patients who underwent surgery due to adnexal masses at our hospital between January 1990 and March 2012. Clinicopathological data was retrospectively collected from the patients’ hospital medical records. The records were analyzed in terms of demographic characteristics, initial symptoms, complications, surgical methods and histological diagnoses. The study was approved by the Institutional Review Board (KC12RISI0881). All statistical analyses were performed using Student’s t test and the Fischer exact test. A p value of <0.05 was considered statistically significant.

Results
In our study, 65 premenarchal girls underwent ovarian tumor surgeries. The mean age of the patients at presentation was 9.6±3.3 years (range, 0.08-15 years). The most common presenting symptom was abdominal pain (n=31, 47.7%), followed by palpable abdominal masses (n=16, 24.6%), abdominal distension (n=8, 12.3%), vaginal bleeding (n=4, 6.2%), incidental finding (n=3, 4.6%), difficulty in urination or defecation (n=2, 3.1%), and prenatal ultrasonographic findings (n=1, 1.5%) (Table 1). Three patients’ masses were discovered incidentally during appendectomy. These patients were diagnosed with appendicitis or panperitonitis at the Department of Surgery and underwent operations, revealed ovarian masses. There are 2 patients that complained of atypical symptom, such as difficulty in urination or defecation. One patient complained of difficulty in urination, which prompted her visit to our clinic. We checked trans-abdominal ultrasonography (TAS), which revealed that there was a huge mass in her pelvic cavity and that the bladder was not visible. Computed tomography (CT) showed that there was a huge mass in the pelvic cavity containing fat and calcified material, which was accompanied by right hydronephrosis. The other patient complained of chronic constipation. CT showed a huge pelvic mass containing fat, calcified material, and hair.

One patient’s ovarian cyst was discovered via a prenatal sonography when she was at the gestational age of 25 weeks. Ultrasoundography performed at the gestational age of 25 weeks revealed ovarian cyst. Ultrasoundography performed immediately after birth revealed an ovarian cyst measuring 3.0 cm. Follow-up sonography performed 1 month after the birth revealed a 3.0X3.5-cm ovarian cyst, which was surgically removed. Of the 31 patients who complained of abdominal pain, 24 (77.4%) had ovarian torsion, 1 (3.2%) had ovarian cyst rupture, and 6(19.4%) had inflammation or adhered to the adjacent organs (Table 1). This pain was induced by the adhesions to the adjacent organs due to the traction by the masses.

| Table 1. Initial symptoms and signs at primary initial presentation. |
|---------------------------------------------------------------|
| Initial symptom and sign                  | No. of patients(%) |
|-------------------------------------------|--------------------|
| Abdominal pain                             | 31(47.7)           |
| Abdominal palpable mass                    | 16(24.6)           |
| Abdominal distension                       | 8(12.3)            |
| Vaginal bleeding                           | 4(6.2)             |
| Incidental finding                         | 3(4.6)             |
| Difficulty in urination/ defecation         | 2(3.1)             |
| Prenatal ultrasonographic finding          | 1(1.5)             |
| Causes of pain                             | No. of patients(%) |
| Torsion                                    | 24(77.4)           |
| Rupture                                    | 1(3.2)             |
| Others                                     | 6(19.4)            |

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Comparisons between the patients with torsion (group 1) and those without torsion (group 2) are shown in Table 2. The mean ages were 9.3±2.2 years (range, 4-13 years) in group 1 and 9.8±3.8 years (range, 0.08-15 years) in group 2. There were no significant differences in age (p=0.5136). The chief complaint was abdominal pain (n=24, 100%) in group 1, while the chief complaints were palpable abdominal masses (n=16, 39%), abdominal pain (n=7, 17.1%), prenatal ultrasonographic findings (n=1, 2.4%) and others (n=17, 41.5%)(p<0.0001) in group 2. The mean size of the ovarian cysts was 6.7±2.7 cm (range, 2-15 cm) in group 1 and 10.6±6.0 cm (range, 0.7-22 cm) in group 2. Tumor size was slightly larger in group 2 than in group 1 (p=0.0009). In group 1, 5 patients (20.8%) had non-neoplastic lesions, 18 patients (75%) had benign tumors, and 1 patient (4.2%) had a malignant tumor. In group 2, 2 patients (4.9%) had non-neoplastic lesions, 26 patients (63.4%) had benign tumors, and 13 patients (31.7%) had malignant tumors (p=0.0063). Table 3 shows the histologic types of the ovarian tumors in our series. A small cyst measuring 2.0 cm was found in a group 1 patient. It was incidentally found by the general surgery team during an operation. The patient complained of nonspecific abdominal pain and was diagnosed with panperitonitis. In the surgical field, the appendix was found to be normal but the right ovary had a 2-cm cyst containing fat and hair. It was twisted 270° and caused pain. Two non-neoplastic patients were included in group 2. One patient had a 0.7-cm follicular cyst and complained of acute abdominal pain; her TAS showed considerable hypoechoic fluid in the pelvic cavity suggestive of hemoperitoneum. Her pathologic diagnosis was follicular cyst. The other patient was also diagnosed with follicular cyst and complained of acute abdominal pain; her TAS showed a 7-cm within homogenous isoechoic cyst. In the surgical field, the hemorrhage was noted within the cyst.

The majority of ovarian masses were benign (67.7%), 7 ovarian masses (10.8%) were non-neoplastic, and 14 ovarian masses (21.5%) were malignant (Table 3). The most common type of benign ovarian tumors were dermoid cysts (64.6% of all ovarian masses) and the most common type of malignant ovarian tumors were immature teratomas (7.7% of all ovarian masses). In our study, there was 1 patient with a corpus luteum. An 11-year-old premenarchal girl visited our clinic due to acute abdominal pain. Transrectal ultrasonography showed a right ovarian cyst and color Doppler revealed no blood flow. A twisted ovarian cyst was found during the operation. Her pathologic diagnosis was a corpus luteal cyst.

### Table 2. Comparisons between patients with torsion (group 1) and those without torsion (group 2).

| Pathological Diagnosis | Group 1 (n=24) | Group 2 (n=41) | P value* |
|------------------------|---------------|---------------|---------|
| Age (years), mean±SD (range) | 9.3±2.2 (4-13) | 9.8±3.8 (0.08-15) | 0.5136 |
| Chief complaint, n (%) | | | <0.0001 |
| Mass | 0 | 16 (39.0) | |
| Pain | 24 (100.0) | 7 (17.1) | |
| Prenatal finding | 0 | 1 (2.4) | |
| Others | 0 | 17 (41.5) | |
| Size (cm), mean±SD (range) | 6.7±2.7 (2-15) | 10.6±6.0 (0.7-22) | 0.0009 |
| Oophorectomy, n (%) | 14 (58.3) | 23 (56.1) | 0.8606 |
| Pathology, n (%) | | | 0.0063 |
| Nonneoplastic lesion | 5 (20.8) | 2 (4.9) | |
| Benign | 18 (75.0) | 26 (63.4) | |
| Malignant | 1 (4.2) | 13 (31.7) | |
| Location, n (%) | | | 0.1445 |
| Right | 13 (54.2) | 19 (46.3) | |
| Left | 11 (45.8) | 16 (39.0) | |
| Both | 0 | 6 (14.6) | |

*P value of <0.05 was considered significant.

### Table 3. Pathological diagnoses of the ovarian tumors.

| Pathology | No. of patients (%) |
|-----------|--------------------|
| Nonneoplastic lesion | 7 (10.8) |
| Corpus luteum | 1 (1.5) |
| Simple/follicular cyst | 6 (9.2) |
| Benign | |
| Dermoid cyst | 42 (64.6) |
| Serous cystadenoma | 2 (3.1) |
| Malignant | |
| Dysgerminoma | 3 (4.6) |
| Juvenile granulose cell tumor | 2 (3.1) |
| Yolk sac tumor | 2 (3.1) |
| Mucinous cystadenocarcinoma | 1 (1.5) |
| Mixed germ cell tumor | 1 (1.5) |
| Immature teratoma | 5 (7.7) |

Ovarian surgeries included cystectomy, oophorectomy, unilateral salpingo-oophorectomy, unilateral salpingo-oophorectomy with contralateral ovarian biopsy, and bilateral salpingo-oophorectomy via laparotomy. Table 4 shows the surgical methods. Of the 51 patients with benign tumors, including non-neoplastic lesions and benign cysts, 26 (51%) underwent cystectomy, 6 (11.8%) underwent oophorectomy, and 17 (33.3%) underwent unilateral salpingo-oophorectomy, and none underwent bilateral salpingo-oophorectomy. Of the 14 patients with ma-
lignant tumors, 2 (14.3%) underwent bilateral salpingo-oophorectomy, 7 (50%) underwent unilateral salpingo-oophorectomy, 2 (14.3%) underwent oophorectomy, and 2 (14.3%) underwent cystectomy. Half of the patients with benign tumors underwent conservative operations otherwise, half of the patients with malignant tumor underwent unilateral salpingo-oophorectomy ($p=0.0135$). Table 5 shows the differences in initial symptoms between the patients with benign tumors and those with malignant tumors ($p=0.0077$). In all patients with benign tumors, abdominal pain was the most common symptom (56.9%), followed by palpable abdominal masses (21.6%). However, in all patients with malignant tumors, abdominal palpable mass (35.7%) and abdominal distension (35.7%) were most common symptoms. The frequency of abdominal pain was higher in patients with malignant tumors than in those with benign tumors (14.3% versus 56.9%).

Fourteen patients had malignant tumors in our series (Table 6). Of the 14 patients 4 had a palpable abdominal mass, 7 complained of abdominal distension, 2 complained of abdominal pain, and 1 patient had a vaginal bleeding. The tumor stages ranged between Ia and IIc. Two patients with yolk sac tumors and 1 patient with an immature tertoma had elevated αFP. Five patients (1 patient with a granulosa cell tumor, 2 patients with immature teratomas, 1 patient with a yolk sac tumor, and 1 patient with a mucinous cystadenocarcinoma) had nonspecifically elevated CA125. Nine patients underwent unilateral salpingo-oophorectomy and received combination chemotherapy. Of the 4 patients, 3 received chemotherapy using cisplatin and etoposide, and 1 received chemotherapy using vincristine, adriamycin D, and cyclophosphamide, and 1 patient (case 13) received no adjuvant therapy. All of them have been free of disease. Of the 3 patients with dysgerminomas, 2 underwent unilateral salpingo-oophorectomy and 1 underwent ovarian cystectomy. They received no adjuvant therapy, and were free of disease. Oophorectomy was a common operative procedure as compared with ovarian cystectomy (n=28, 43.1%). All patients underwent laparotomy.

Table 4. Types of surgery in the patients with benign tumors, including non-neoplastic lesions, and those with malignant tumors.

| Type of surgery | Benign | Malignant | Total |
|-----------------|--------|-----------|-------|
| Cystectomy      | 26 (51.0) | 6 (11.8) | 32 (54.6) |
| Oophorectomy    | 6 (11.8) | 2 (14.3) | 8 (12.3) |
| Unilateral salpingo-oophorectomy | 17 (33.3) | 7 (50.0) | 24 (36.9) |
| Unilateral salpingo-oophorectomy + Contralateral ovari biopsy | 2 (3.9) | 1 (7.1) | 3 (4.6) |
| Bilateral salpingo-oophorectomy | 0 | 2 (14.3) | 2 (3.1) |

P=0.0135 by Fisher’s exact test
Table 5. Comparison of initial symptoms between the patients with benign tumors and those with malignant tumors.

| Symptom and Sign                        | Benign No. of patients (%) | Malignant No. of patients (%) | p-value* |
|-----------------------------------------|-----------------------------|-------------------------------|----------|
| Abdominal pain                          | 29 (56.9)                   | 2 (14.3)                      | 0.0061   |
| Abdominal mass                          | 11 (21.6)                   | 5 (35.7)                      | 0.2764   |
| Incidental finding                      | 3 (5.9)                     | 0                             | 1.0000   |
| Prenatal sono finding                   | 1 (2.0)                     | 0                             | 1.0000   |
| Vaginal bleeding                        | 3 (5.9)                     | 1 (7.1)                       | 1.0000   |
| Urination/defecation difficulty         | 1 (2.0)                     | 1 (7.1)                       | 0.3870   |
| Abdominal distension                    | 3 (5.9)                     | 5 (35.7)                      | 0.0091   |
| Total                                   | 51 (100)                    | 14 (100)                      |          |

*p-values are obtained by Fisher’s exact test.

Table 6. Summary of the patients with malignant tumors.

| Patients | Age at diagnosis | Symptom               | Histologic type                        | Operation                      | Stage | Adjuvant treatment | Outcome | Tumor marker |
|----------|------------------|-----------------------|----------------------------------------|-------------------------------|-------|-------------------|---------|--------------|
| 1.       | 9yrs             | Vaginal bleeding      | Juvenile granulosa cell tumor          | Lt ovary cystectomy           | Ia    | None              | NED     | β-hcg<2      |
| 2.       | 12yrs            | Abdominal distension  | Immature teratoma                      | Lt Ad                         | Ic    | Carboplatin-V16   | NED     | β-hcg<2, CA-125:58, αFP:21, CA125:258, B-hcg<2, |
| 3.       | 14yrs            | Abdominal distension  | Juvenile granulosa cell tumor          | Rt Ad, PLND, partial omentectomy | Ia    | Carboplatin-V16   | NED     |              |
| 4.       | 14yrs            | Abdominal distension  | Yolk sac tumor                         | T/H, both Ad, PLND            | Ic    | None              | Recur at lung, 2yrs later, EOD | CA125:244, αFP: 2125, CA125:102, CA19-9:19, CA125:69, CA19-9:185, αFP: 83 |
| 5.       | 14yrs            | Abdominal distension  | Mucinous cyst adenoca                  | Lt Ad                         | Ic    | None              | NED     |              |
| 6.       | 8yrs             | Abdominal distension  | Immature teratoma                      | Rt oophorectomy, partial omentectomy | Ic    | Cisplatin-V16     | NED     |              |
| 7.       | 10yrs            | Abdominal distension  | Immature teratoma                      | Lt Ad                         | Ia    | Cisplatin- V16    | NED     |              |
| 8.       | 13yrs            | Abdominal pain, torsion | Dysgerminoma                           | Rt Ad                         | Ia    | None              | NED     | -            |
| 9.       | 8yrs             | Palpable mass         | Dysgerminoma                           | Lt Ad                         | Ia    | None              | NED     | -            |
| 10.      | 11yrs            | Palpable mass         | Dysgerminoma                           | Lt ovary cystectomy           | Ia    | None              | NED     | -            |
| 11.      | 12yrs            | Constipation, abdominal distension | Endodermal sinus tumor | Rt Ad, partial omentectomy, rectal serosal biopsy, Lt ovary biopsy | Iic   | Cisplatin-V16     | EOD     | αFP: 2450    |
| 12.      | 12yrs            | Palpable mass         | Immature teratoma                      | Lt Ad, omental biopsy, Lt peritoneal biopsy | Ic    | None              | NED     | -            |
| 13.      | 7yrs             | Abdominal pain         | Immature teratoma                      | Lt Ad                         | Ia    | None              | NED     | CA125:28, CA19-9:7.2 |
| 14.      | 13yrs            | Palpable mass         | Mixed germ cell tumor(Yolk sac tumor + dysgerminoma) | T/H, both Ad, PLND, omentectomy | IIIc  | carboplatin-V16, Cisplatin-Cytoxan, carboplatin-taxol | EOD     | -            |

*NED: negative evidence of disease. EOD: Expire of disease. Rt= right, Lt= left, Ad=adnexectomy, T/H=total hysterectomy, PLND=Pelvic lymph node dissection, VP16=etoposide, ERT=external radiation therapy.
Discussion

Menarche starts with changes in hormones of the hypothalamus, pituitary gland, and ovaries. The mechanisms of these changes are as follows: during puberty, estradiol inhibition decreases in response to tonic luteinizing hormone (LH) secretion. Tonic LH secretion is stimulated independently of ovarian steroid control and then the inhibitory feedback response to estradiol on tonic LH secretion decreases [7]. Therefore, it is important to evaluate the characteristics of ovarian masses which develop during puberty. Menarche occurs at the age between 9.1 and 17.7 years of age and at a mean age of 13 years [8]. In our study, patients ranged in age from 1 month to 14 years, with a mean age of 9.6±3.3 years at the time of surgery.

Adnexal masses in premenarchal patients can manifest in many ways. Patients with adnexal masses may have acute abdominal pain which is difficult to differentiate from that of acute appendicitis. Such patients can also be referred with large palpable masses or abdominal distension. In this study, patients had abdominal pain (47.7%), palpable abdominal masses (24.6%), incidental ultrasonographic findings (4.6%), and precocious puberty (6.2%). Our results were similar to those of previous studies which reported that primary initial symptoms requiring surgeries were abdominal pain (48%-56%), abdominal palpable mass (9%-22%), incidental ultrasonographic findings (5%-15%) and precocious puberty (2%-7%) [1, 4]. It is thought that abdominal pain is the most common symptom of premenarchal ovarian masses. In our study, the indications for abdominal pain were ovarian torsion (77.4%), ovarian cyst rupture (3.2%) and others (19.4%). Approximately 37% of our patients who required operations had ovarian torsion.

In pediatric patients, a longer infundibulopelvic ligament leads to ovarian torsion more frequently [6]. Ovarian torsion is more common on the right side and thus many patients with ovarian torsion can be misdiagnosed with acute appendicitis before surgery [9, 10]. It is still controversial whether the sigmoid colon helps prevent left adnexal torsion or whether left lesions are more commonly missed and managed nonsurgically [1, 11]. Treatment methods for ovarian torsion have changed; in the past, salpingo-oophorectomy was the treatment of choice. Historically, detorsion of the ovary has been avoided to prevent the theoretical risk of pulmonary embolisms from the infundibulopelvic vein [12]. More recently, investigators have conducted detorsion and preservation of the ovary even when the ovary appears markedly necrotic [13, 14]. Ovarian function has been shown to recover after detorsion in adults [11]. Cohen et al. [13] reported that, as assessed by ultrasonography, 93% of the necrotic or bluish ovaries were restored to normal ovarian function 3 months after detorsion and preservation of the ovaries. In our study, 42% of the patients with ovarian torsion underwent detorsion and more conservative surgeries. Preservation of future fertility is important in the surgical treatment of premenarchal girls. Surgical management of benign masses and functional cysts should be conservative and mainly includes ovarian cystectomy or simple excision of the lesions.

Although technically difficult, treatment methods minimize the risk of subsequent infertility and help conserve as much normal ovarian tissue as possible in order to maintain reproductive and endocrine functions. In our study, in the treatment of benign tumors and functional cysts, the ovaries were completely removed in 25 patients (49%). Of these 25 patients, 19 underwent unilateral resection of the fallopian tube (37%) and 2 (3.9%) only underwent biopsy. All of our patients were treated by laparotomy.

Breen et al. [15] reported that 64% of the ovarian masses found in children and adolescents were neoplastic, while 36% were non-neoplastic. Van Winter et al. [16] stated that in children and adolescents under the age of 20 years, 64.3% of the ovarian masses were non-neoplasics, 27.6% were benign, and 8.1% were malignant. In our study, we found that 10.8% of the ovarian masses were non-neoplastic, 67.7% were benign, and 21.5% were malignant. Germ cell tumors were the most common among the pediatric ovarian neoplasms. In children, the frequency of germ cell tumors varies from 67% to 85% [1, 12, 17-19]. Among them, teratoma is the most common form and constitutes 60%-73% of all ovarian tumors, of which 15% are malignant [5, 12, 20]. In our study, 79.2% of the germ cell tumors were mature teratomas, 9.4% were immature teratomas, and 5.7% were dysgerminomas. Sex cord tumors, which are derived from ovarian stroma, can also develop in premenarchal girls. They constitute 10%-25% of all pediatric ovarian neoplasms. Granulosa cell tumors are the most common form and are associated with hyperestrogenism or precocious puberty [5, 12, 20]. In our study, 3.4% of all ovarian masses and 14.3% of all ovarian malignant tumors were granulosa cell tumors. Among the patients with granulosa cell tumors, 1 patient (50%) showed pseudoprecocity, but the other did not. Unlike in adult patients, the incidence of ovarian tumors is lower in children than in adults. In adults, 80% of the ovarian tumors are of epithelial origin. In children, 7%-20% of the ovarian tumors are of epithelial origin [1, 5, 20, 21]. In our study, 1.7% of the ovarian masses
and 7.1% of the malignant ovarian tumors were of epithelial origin. If malignancies were suspected by frozen section examination, the patients underwent staging operations and were treated with combination chemotherapy. Fourteen patients were diagnosed with malignant tumors. Four patients underwent staging operations and 7 patients were treated with chemotherapy. Three patients died, but the remaining patients have had uneventful courses so far.

Conclusion

In conclusion, premenarchal girls with ovarian masses may have variable symptoms. Abdominal pain is the most common symptom. The incidences of abdominal distension and palpable masses were higher in patients with malignant tumors than in those with benign tumors (5.9% versus 35.7%, 21.6% versus 35.7%). Unlike in adults, ovarian tumors of premenarchal girls generally originate from the germ cell line and treatments have relatively good prognoses even when the tumors are malignant. Epithelial and sex cord-stromal tumors are relatively uncommon in children and their clinical behaviors are unpredictable.

This is a regional study, so it may not be applicable to the general population. We assessed clinical features, operative outcomes, and histological classification in Korean premenarchal girl with ovarian masses. Further studies with a larger number of subjects are needed to confirm our results.

Competing Interests

The authors have declared that no competing interest exists.

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