RESEARCH ARTICLE

Prognosis of Eight Chinese Cases of Primary Vaginal Yolk Sac Tumor with a Review of the Literature

Qiong-Lan Tang[1&*], Xue-Feng Jiang[2&], Xiao-Ping Yuan[3], Yong Liu[4], Lin Zhang[5], Xiao-Feng Tang[6], Jia-Jia Zhou[7], Hai-Gang Li[1], Jian-Pei Fang[4], Lin Xue[8]

Abstract

Background: Primary vaginal yolk sac tumor is a rare malignancy in the pediatric population, and a diagnostic challenge and appropriate initial treatment remains unsolved. The aim of this study was to investigate the clinicopathologic features, treatment and prognosis of this tumor. Materials and Methods: Eight cases of primary vaginal yolk sac tumor were reported with a literature review. Results: There were 4 pure yolk sac tumor cases and four mixed germ cell tumors containing yolk sac tumor element, including two cases with embryonal carcinoma and two cases with embryonal carcinoma and dysgerminoma. Partial vaginectomy was performed in four cases and all patients received chemotherapy. 85 cases in literatures were reviewed and 9 cases were misdiagnosed. Follow-up data was available in 77 cases and 5-year overall survival rate was 87.6%. 5-year survival rate of biopsy with chemotherapy, conservative surgery with chemotherapy and radical surgery with chemotherapy was 91.1%, 100% and 28.6%, respectively ($p<0.001$). Compared to cases without relapse or metastasis after initial treatment, patients with relapse or metastasis had a shorter overall survival (35.6% *vs* 96.6%, $p<0.001$). Conclusions: Mixed germ cell tumor containing yolk sac tumor element was not uncommon and partial vaginectomy may be a good choice for primary vaginal mixed yolk sac tumor type to eradicate local tumor cells and provide complete information for pathological diagnosis and postoperative adjuvant therapy.

Keywords: Yolk sac tumor - vagina - clinicopathology - treatment - chemotherapy - prognosis

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Introduction

Malignant Germ Cell Tumors (GCTs) arising primarily from the vagina are extremely rare, comprising from 3 to 8% of all GCTs (Rescorla et al., 2003). Of the different histological subtypes, yolk sac tumor is the most common in the pediatric population (Davidoff et al., 1996; Terenziani et al., 2007). Primary vaginal yolk sac tumor is a rare entity, and is the most common germ cell tumor that occurs primarily in infants. The first case of primary vaginal yolk sac tumor was reported by Allyn DL (1971). About one hundred and twenty reported cases in the international medical literature. Although PEB (cisplatin, etoposide and bleomycin) chemotherapy without surgery have achieved in complete remission (CR) in some early cases (Tao et al., 2012), there are still some children's adverse outcomes due to erroneous histological diagnosis and inadvertent interventions (Goyal et al., 2014). A diagnostic challenge and appropriate initial treatment remains unsolved owing to the rarity of the malignancy. Herein, eight cases of primary vaginal yolk sac tumor were reported including 4 pure yolk sac tumor and four mixed germ cell tumors containing yolk sac tumor element, respectively, along with a literature review, summarizing the clinicopathologic features, diagnosis, differential diagnosis, treatment and outcome of the tumor to investigate the differential diagnosis, treatment and prognosis of primary vaginal yolk sac tumor.

Materials and Methods

Patient selection

One hundred and ninety-eight cases of vaginal malignant tumors and 124 cases of female yolk sac tumor were collected from the Department of Pathology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University and Sun Yat-sen University cancer center, Guangzhou, China, between January 1995 and June 2014. Thirty of them were excluded due to insufficient experimental materials. The remaining cases were histologically and immunophenotypically reviewed, and the diagnosis was based on the World Health Organization (WHO) classification for tumors of female genital organs (2014) (Kurman et al., 2014). In total, eight cases of primary

*[1]Department of Pathology, [3]Department of Radiology, [4]Department of Hematological Pediatrics, [7]Department of surgical Pediatrics, Sun Yat-sen Memorial Hospital, [6]Department of Ultrasonography, [8]Department of Pathology, the First Affiliated Hospital, Sun Yat-sen University, [5]Department of Laboratory, Sun Yat-sen University Cancer Center, [2]Department of Obstetrics and Gynecology, the First Affiliated Hospital, Jinan University, Guangzhou, China [&]Equal contributors *For correspondence: qionglantang@126.com*

vaginal yolk sac tumor were identified, including 2 patients described in the previous study (Liu et al., 2013), 2 archival and four consultative cases. The clinical and laboratory data of theses patients were collected and tumor staging evaluated according to the TNM classification (Kurman et al., 2014).

Hematoxylin and eosin (HE) and immunohistochemical staining

Four micrometer-thick sections from formalin-fixed paraffin-embedded blocks were cut for routine hematoxylin and eosin staining.

According to the manufacturer's recommendations, hyaline globules were stained by Periodic-acid Schiff (PAS).

The EnVision method was used for immunostaining with diaminobenzidine (DAB) as a substrate. A broad panel of antibodies included: cytokeratin (CK; AE1/AE3), CK8&18 (Zym5.2), CK20 (EP23), epithelial membrane antigen (EMA; GP1.4), Alpha fetoprotein (AFP; EP209), carcinoembryonic antigen (CEA; COL-1), human chorionic gonadotropin (HCG; ZSH17), CD30 (EP154), Oct-3/4 (N1NK), placental alkaline phosphatase (PLAP; EP194), CD117 (2E4), neuron specific enolase (NSE; E27), synaptophysin (Syn; UMAB112), chromoganin A (CgA, LK2H10), vimentin (V9), CD10 (56C6), myogenin (F5D), MyoD1 (EP212), estrogen receptor (ER; 6F11), progesterone receptor (PR; EP2), CDX2 (EP25), CD99 (PCB1), S-100 (15E2E2+4C4.9) and Ki-67 nuclear antigen (7B11). CgA was purchased in Dako Technologies Company (Glostrup, Denmark). All other antibodies were purchased in Beijing Zhongshan Biotechnology Co (Beijing; China).

The slides were treated by pressure-cooking in citric acid buffer (10mM, Ph 7.4) for 3 min before staining for CK, CK8&18, CK20, EMA, AFP, CEA, HCG, PLAP, CD117, NSE, Syn, CgA, vimentin, CD99 and S-100, and in ethylenediaminetetraacetic acid (EDTA; 1 mM, Ph 9.0) for 8 min before staining for CD30, Oct-3/4, CD10, myogenin, MyoD1, ER, PR, CDX2, and Ki-67.

HE, PAS and immunostaining staining was evaluated by two independent observers who were blinded to clinical data. All of the experiments were repeated three times. Differences were discussed to reach consensus.

Review of literature and statistical analysis

Articles from 1995 to 2014 that contain the keywords "vagina" and "yolk sac tumor" in the PubMed and MEDLINE databases were reviewed.

All statistical analyses were performed using the SPSS WIN program package 13.0 (SPSS, Inc., Chicago, IL, USA). Survival time was measured from primary diagnosis to death. Survival analysis was carried out using the log-rank test in association with Kaplan-Meier analysis. Differences were statistically significant when P-value<0.05.

Ethical approval

Each institution obtained approval to participate in the study as required by the local district research ethics committee. Informed consent was obtained from each patient and/or his or her legal guardian.

Results

Clinical features

The clinical characteristics of the eight patients with primary vaginal yolk sac tumor are listed in Table 1. The mean age of patients at diagnosis was 12.1 months (range 7-20 months). The only initial manifestation for all patients was persistently or discontinuously vaginal bleeding and the average clinical history was 35.8 days (range 10-60 days). The external genitalia of all cases were entirely normal, but digital rectal examination under general anesthesia revealed a firm mass in the posterior vaginal wall of all patients. Vaginal masses were also founded by magnetic resonance imaging (MRI) study. Three patients involved the cervix uteri. There are 5 regional lymph nodal metastasis. The average serum AFP level was 7996.1 ng/ml (153.8-23650 ng/ml, normal value less than 25 ng/ml). The serum NSE level of the first case was 22.3 ng/ml (normal value less than 16 ng/ml). The serum NSE, CEA, CA199 and HCG levels of the third case were 19.7 ng/ml, 8.3 ng/ml(normal value less than 5 ng/ml), 8.8 ng/ml (normal value less than 3.3 ng/ml) and 0 IU/L (normal value less than 0 ng/ml), respectively.

Pathological features

Two patients were histologically diagnosed based on

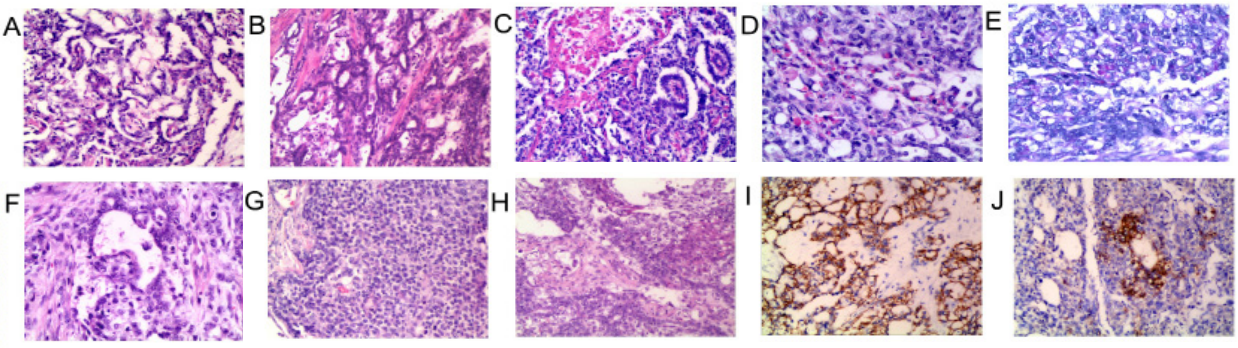

Figure 1. Histologic Features and Immunohistochemical Staining of Primary Vaginal Yolk Sac Tumor. A) Reticular pattern*; **B)** Glandular structures*; **C)** Schill-Duval bodies (right)*; **D)** Intra- and extracellular hyaline globules (red) (HE, original magnificent × 200); **E)** Hyaline globules positive for PAS (purple) (PAS, original magnificent ×200); **F)** Polyvesicular vitelline pattern*; **G)** Solid sheet-like growth pattern*; **H)** Hepatoid component (right)*; **I)** Tumor cells positive for CK (SP, original magnificent ×100); **J)** Tumor cells focal positive for AFP (SP, original magnificent ×100). *hematoxylin-eosin staining (HE), original magnificent × 100

a discharged tumor fragment falling out from the vagina, two based on vaginal biopsy under anesthesia and 4 based on exploratory laparotomy and partial vaginectomy. Grossly, there was a variegated cut surface, solid and cystic mass with sizes ranging from 1.0cm×0.6cm×0.5cm to 5.0cm×4.5cm×3.5cm and five with extensive necrosis and hemorrhage.

The histology and immunophenotype of the eight patients with primary vaginal yolk sac tumor are listed in Table 2. Histologically, the tumor was composed of large pleomorphic cells growing in several major patterns. The predominant growth pattern was one of irregular tubulo-acinar formations lined by large cells with clear cytoplasm, which occasionally projected into the lumina in "hobnail" fashion. These structures were closely apposed, with little intervening stroma, in many foci, often being arranged in a reticular pattern (Figure 1A), but in other regions were separated by dense fibrous connective tissue or by pools of mucin. Glandular structures were also prominent and presented as isolated glands, tubules and papillae (Figure 1B). Characteristic Schill-Duval bodies (rounded papillae containing a single central vessel and lined by columnar

Table 1. Clinical Features of Cases of Primary Vaginal Yolk Sac Tumor in this Series

No.	Age (months)	Initial symptom (lasting time) (days)	Physical examination	AFP and other tumor marker level before treatment	MRI features	CT features	Size of tumor	Stage
1	14	Discontinuously vaginal bleeding, 60	A 3cm×3cm mass with less clear boundaries and less smooth surface	AFP, 153.8ng/ml; NSE, 22.3ng/ml	A solid and cystic 2.2cm×1.9cm×2cm tumoral mass in the posterior wall of the vagina	No enlargement of pelvic and inguinal lymph nodes	2.5cm×2cm ×2cm	I
2	13	Persistently vaginal bleeding, 10	A huge mass with unclear boundaries	AFP, 8127ng/ml	A solid and cystic 3.4cm×3.0cm×3.0cm tumoral mass in the posterior wall of the vagina and cervix	Enlargement of many mesenteric lymph nodes (≥ 1.5cm in short axis) in the right lower abdomen	5cm×4cm ×2.5cm	III
3	20	Discontinuously vaginal bleeding,20; discharged tumor fragment falling out from the vagina	A 2cm×1.5cm mass	AFP, 576ng/ml NSE, 19.7ng/ml CEA, 8.3ng/ml CA199,8.8ng/ml HCG, 0IU/L	A solid and cystic 1.3cm×1.2cm×0.6cm tumoral mass in the posterior wall of the vagina	Enlargement of many pelvic lymph nodes in bilateral pelvic cavitie	1.8cm×0.8cm ×0.5cm	III
4	12	Persistent vaginal bleeding, 10	A 2cm×1.6cm mass in the vagina	AFP, 2255ng/ml	A solid and cystic 2.0cm×1.6cm×1.5cm tumoral mass in the posterior wall of the vagina and rectum	No enlargement of pelvic and inguinal lymph nodes	2cm×1.6cm ×1.5cm	III
5	7	Persistently vaginal bleeding, 60	A polypoid mass in the vagina	AFP, 23650ng/ml	A 4.5cm×2.6cm×2.5cm mass in the posterior wall of the vagina and rectum	Enlargement of pelvic lymph nodes and inguinal lymph nodes	4.5cm×2.6cm ×2.5cm	III
6	7	Persistently vaginal bleeding, 30	A polypoid mass in the vagina	AFP, 20592.3ng/ml	A 4.0cm×2.5cm×2.0cm mass in the posterior wall of the vagina and cervix uteri	No enlargement of pelvic and inguinal lymph nodes	4cm×2.5cm ×2cm	II
7	13	Persistently vaginal bleeding, 36	A polypoid mass in the vagina	AFP, 8030ng/ml	A solid and cystic 3.8cm×4.8cm×4.0cm mass in the posterior wall of the vagina, , cervix, rectum and bladder	Enlargement of many pelvic lymph nodes in bilateral pelvic cavities	2cm×2cm× 2cm and 3cm× 2cm×1cm	III
8	11	Discontinuously vaginal bleeding, 60	A polypoid mass in the vagina	AFP, 585ng/ml	A solid and cystic 5.0cm×4.5cm×3.5cm mass in the posterior wall of the vagina,cervix, rectum and bladder	Enlargement of many pelvic lymph nodes (≥ 1.5cm in short axis) in bilateral pelvic cavities and lung metastasis	5.0cm×4.5cm ×3.5cm	IV

AFP, a-fetoprotein; HCG, human chorionic gonadatropin; NSE, neuron specific enolase; CEA, carcinoembryonic antigen; CT, computerized tomographic imaging; MRI: magnetic resonance imaging

tumor cells) were also seen (Figure 1C). Occasional fields also revealed intra- and extracellular hyaline globules (Figure 1D), which positive for PAS (Figure 1E). Polyvesicular vitelline pattern was a rare variant and was composed of numerous cyctic spaces lined by a mesothelial-like epithelium that merged with columnar, tall vacuolated cells (Figure 1F). Foci characterized by solid sheet-like growth of clear cells were also observed (Figure 1G). Hepatoid component was seen in the tumor of case 2 and was composed of masses, nests, and broad bands of large polyhedral cells with occasional glandular formations and numerous hyaline bodies (Figure 1H). Cytologically, the tumor cells were cuboidal to cplumnar with clear vacuolated cytoplasm and hyperchromatic nuclei. Immunohistochemical analysis revealed that the tumor cells of all eight cases were positive for CK (Figure 1I), CK8/18, AFP (Figure 1J) and vimentin, and negative for NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER and PR. Focal EMA, CK20 and CEA expression was seen the tumor cells in case 1, 2, 5, 7 and 8. Case 1, 3, 4, 5, 7 and 8 were positive for HCG, respectively. CD30 expression was showed in case 1, 3, 7

Table 2. Pathological Findings and Immunophenotype of Primary Vaginal Yolk Sac Tumor in this Series

No.	Specimen origination	Gross observation	Histology of yolk sac tumor	Immunophenotype	Pathological diagnosis
1	Complete resection	A variegated cut surface, solid and cystic mass, 2.5cm×2cm ×2cm	Reticular form, Schill-Duval bodies, hyaline droplets, glandular structures, polyvesicular vitelline pattern, solid areas	CK, CK8/18, EMA(focal), AFP, CK20 (focal), CEA (focal), HCG, CD30, Oct-3/4, vimentin - positive CDX2, PLAP, CD117, NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER, PR - negative Ki-67, 30% (positive)	Mixed germ cell tumors, yolk sac tumor (75%) embryonal carcinoma (25%)
2	Complete resection	A partially cystic mass, containing large foci of hemorrhage and necrosis, 5.0cm×4.0cm ×2.5cm	Reticular form, Schill-Duval bodies, hyaline droplets, glandular structures, polyvesicular vitelline pattern, solid areas, hepatoid component	CK, CK8/18, EMA (focal), AFP, CK20 (focal), CEA(focal), vimentin – positive CDX2, HCG, CD30, Oct-3/4, PLAP, CD117, NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER, PR - negative Ki-67, 10% (positive)	Yolk sac tumor
3	Complete resection	A solid and cystic mass, 1.8cm×0.8cm ×0.5cm	Reticular form, Schill-Duval bodies, hyaline droplets, glandular structures, polyvesicular vitelline pattern, solid areas	CK, CK8/18, AFP, HCG, CD30, Oct-3/4, vimentin – positive EMA, CK20, CEA, CDX2, PLAP, CD117, NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER, PR – negative Ki-67, 15% (positive)	Mixed germ cell tumors yolk sac tumor (30%) embryonal carcinoma (70%)
4	Discharged tumor fragment	A partially cystic mass with hemorrhage and necrosis, 1.0cm×0.6cm ×0.5cm	Reticular form, Schill-Duval bodies, hyaline droplets, glandular structures	CK, CK8/18, AFP, HCG, CD117, vimentin – positive EMA, CK20, CEA, CDX2, CD30, Oct-3/4, PLAP, NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER, PR – negative Ki-67, 90% (positive)	Yolk sac tumor
5	Simple excisional biopsy	A polypoid mass, 1.5cm×1.0cm ×0.5cm	Reticular form, Schill-Duval bodies, hyaline droplets, glandular structures	CK, CK8/18, EMA(focal), AFP, CK20(focal), CEA(focal), CDX2(focal), HCG, vimentin – positive CD30, Oct-3/4, PLAP, CD117, NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER, PR – negative Ki-67, 50% (positive)	Yolk sac tumor
6	Discharged tumor fragment	A polypoid mass with hemorrhage and necrosis, 2.5cm×1.5cm ×1cm	Reticular form, Schill-Duval bodies, hyaline droplets,	CK, CK8/18, AFP, vimentin - positive EMA, CK20, CEA, CDX2, HCG, CD30, Oct-3/4, PLAP, CD117, NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER, PR – negative Ki-67, 40% (positive)	Yolk sac tumor
7	Simple excisional biopsy	A partially cystic mass, containing large foci of hemorrhage and necrosis, 1.0cm×0.8cm ×0.8cm	Reticular form, Schill-Duval bodies, hyaline droplets, glandular structures	CK, CK8/18, EMA(focal), AFP, CK20(focal), CEA(focal), HCG,CD30, Oct-3/4, PLAP, CD117, vimentin – positive CDX2,NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER, PR – negative Ki-67, 45% (positive)	Mixed germ cell tumors yolk sac tumor (70%) embryonal carcinoma (20%) dysgerminoma (10%)
8	Complete resection	A partially cystic mass, containing large foci of hemorrhage and necrosis, 5.0cm×4.5cm ×3.5cm	Reticular form, Schill-Duval bodies, glandular structures, solid areas	CK, CK8/18, EMA(focal), AFP, CK20 (focal), CEA (focal), HCG, CD30, Oct-3/4, PLAP, CD117, vimentin-positive, CDX2, NSE, Syn, CgA, CD10, myogenin, MyoD1, CD99, S-100, ER,PR-negative, Ki-67, 55% (positive)	Mixed germ cell tumors yolk sac tumor (15%) embryonal carcinoma (50%) dysgerminoma (35%)

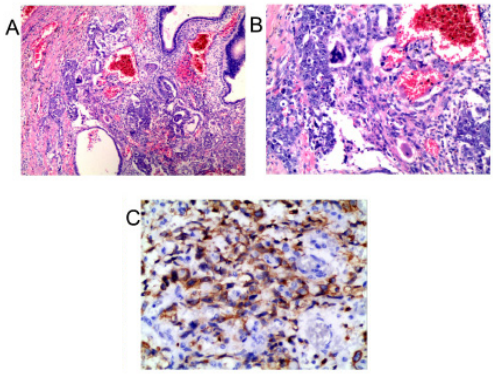

Figure 2. Histologic Features and Immunohistochemical Staining of Yolk Sac Tumor with Embryonal Carcinoma. A) glandular structures of yolk sac tumor and embryonal carcinoma with giant cell (HE, original magnificent×25); **B)** embryonal carcinoma with giant cell (HE, original magnificent×100); **C)** tumor cells of embryonal carcinoma positive for CD30 (SP, original magnificent ×100)

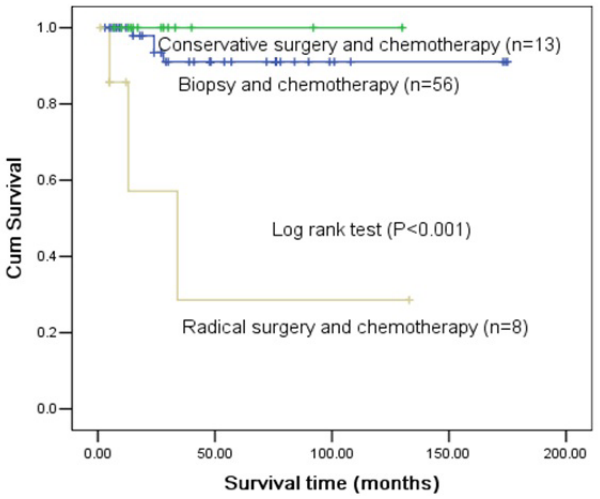

Figure 4. Kaplan-Meier Analysis Of Overall Survival of Patients with Different Initial Treatment

and 8. Case 4, 7, 8 were positive for CD117, respectively. Only two cases were positive for PLAP.

Four pure yolk sac tumors and four mixed germ cell tumors were diagnosed. Yolk sac tumor was a component of the mixed germ cell tumor in the first, 3rd, 7th and 8th cases. Embryonal carcinoma elements were also seen in these four cases. Some tumor cells in solid and undifferentiated form had a carcinomatous appearance, exhibited prominent variation in size and shape with numerous mitoses (often atypical) and were reactive for CK, CD30 and OCT3/4 (Figure 2). Dysgerminoma element was seen in the specimen of the seventh and eighth cases on exploratory laparotomy during chemotherapy and on partial vaginectomy before chemotherapy, respectively. These tumor cells grouped themselves in well-defined nests separated by fibrous strands and had large 'squared-off' nuclei, one or more prominent elongated nucleoli and positive for PLAP and CD117 (Figure 3).

Treatment and tumor response evaluation after chemotherapy and other treatment

The treatment and outcome of 8 cases are summarized in Table 3. Four cases underwent exploratory laparotomy and partial vaginectomy before chemotherapy and the postoperative serum AFP level was decreased dramatically. Exploratory laparotomy during chemotherapy was performed in the other four cases. All patients underwent chemotherapy. Follow-up data were available for all patients. The average cycle of AFP level returned to normal was 3.1 (1-10) cycles. Clinical and pathological complete remission (CR) was obtained after 5-12 courses of chemotherapy in the second, third, forth, fifth and eighth case. 7 patients survival and one died of lung metastasis, the mean survival time is 28.6 months (10-57months).

These four cases with partial vaginectomy were managed with primary PEB chemotherapy (cisplatin, etoposide and bleomycin; cisplatin 20mg/m^2/day from days 1-5, etoposide 100mg/m^2/day from days 1-5, bleomycin 20mg/m^2/day on days 2, 9 and 16; 50% of the dosage in the first and second cycle, 75% of the dosage in the third and fourth cycle and 100% of the dosage in the remaining cycles), and there was disappearance of primary lesion, no new lesion and normal serum AFP level after chemotherapy. However, the average elevated serum NSE, CEA and CA199 levels of the first case were 25.8 ng/ml (20.9-30.8 ng/ml), 7.6 ng/ml (6.7-8.6 ng/ml) and 4.5 ng/ml (3.8-5.3 ng/ml), respectively. The second, third and eighth case achieved in CR, with disappearance of primary lesion, no new lesion, lymph node reduced in short axis to <1 cm and tumor marker level returned to normal 27, 15, and 10 months after primary diagnosis, respectively.

The fifth patient underwent vaginal tumor excision and the left external iliac lymph node sampling after the fifth cycle of PEB and there was negative for malignancy of the suspected residual diseases. However, at the third month interval after six cycles of PEB, the serum AFP level was elevated to 307.3 ng/ml and tumor cells were seen in the discharged tumor fragment falling out from the vagina.

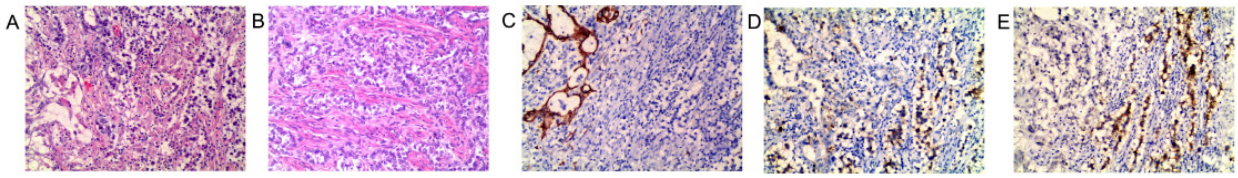

Figure 3. Histologic Features and Immunohistochemical Staining of Mixed Germ Cell Tumors, Including Yolk Sac Tumor, Embryonal Carcinoma and Dysgerminoma. A) yolk sac tumor (left) and dysgerminoma (right)*; **B)** embryonal carcinoma*; C) CK**; D) PLAP***; E) CD117***. *HE, original magnificent ×100;**positive expression in yolk sac tumor (left) while negative in dysgerminoma (right) (SP, original magnificent×100); ***negative expression in yolk sac tumor (left) while positive in dysgerminoma (right) (SP, original magnificent ×100)

Table 3. Treatment and Outcome of Primary Vaginal Yolk Sac Tumor in this Series

No.	Treatment before chemo-therapy and tumor marker level after operation	Chemotherapy (regimens*cycles) and other treatment during chemo-therapy	Total course	Cycles of AFP level returned to normal	AFP and other tumor marker level after chemotherapy and other treatment	The latest AFP and other tumor marker level	Tumor response evalution after chemotherapy and other treatment	Follow-up, (months and status)
1	Partialvagi-nectomy: AFP,65.1ng/ml; NSE, 30.8ng/ml	PEB*6	6	1	AFP, within the nor-mal range after the first PEB cycle, NSE CEA and CA199, remained slightly elevated level	AFP, 1.1ng/ml NSE, 24.6ng/ml CEA, 6.7ng/ml CA199, 3.8ng/ml	Disappearance of primary lesion,no new lesion, main-tenance elevated tumor marker level	40, alive; PR
2	Partial vaginectomy; AFP,822.9ng/ml; HCG,6.2IU/L	PEB*8	8	2	AFP, within the normal range after the second PEB cycle, HCG, within the normal range after the eighth PEB cycle	AFP, 3.6ng/ml HCG, 0IU/L	Disappearance of primary lesion, no new lesion, mesen-teric lymph node in short axis to <1cm, tumor marker level returned to normal	27, alive; CR
3	Partial vaginectomy; AFP,58.8ng/ml	PEB*6	6	1	AFP, within the normal range after the first PEB cycle	AFP, 5.0ng/ml	Disappearance of primary lesion,no new lesion, pelvic lymph nodes in short axis to <1cm,tumor marker level returned to normal	15, alive; CR
4	ND	BIP*1;IP*2; ex-ploratory laparotomy biopsy and negative for malignancy of the suspected residual diseases and the serum AFP level within the normal range; IP*2	5	2	AFP, within the normal range after the first IP cycle	AFP, 1.4ng/ml	Disappearance of primary lesion, no new lesion, tumor marker level re-turned to normal	57, alive; CR
5	Vaginal biopsy under anes-thesia; AFP, 43057ng/ml	PEB*5, exploratory laparotomy radical surgery and negative for malignancy of the suspected residual diseases and and the serum AFP level within the normal range; PEB*1; VIP*6	12	4	AFP, within the normal range after the sixth VIP cycle	AFP, 9.4ng/ml	Decurrence at the third month after 6 cycles of PEB and appear-ance new lesion and tumor marker level above the normal limits, negative for malignancy of the suspected residual diseases and tumor marker level returned to normal after 6 cycles of VIP	27, alive; AWR, CR
6	ND	JEB+A*6; tumor marker level above the normal limits after 3 months and negative for malignancy of the suspected residual diseases, PEB*2, PET/CT, a mass in retroperitoneal space and positive for tumor cells by biopsy and AFP remained slightly elevated level after further 4 cycles of PEB	12	3	AFP, remained slightly elevated level	AFP, 56.0ng/ml	Mestasis at the sixth month after 6 cycles of JEB+A, and AFP remained slightly elevated level after 6 cycles of PEB	29, alive; CR, PD,AWM, AWD,SD,PR

7	Vaginal biopsy under anesthesia; AFP, 7136.13ng/ml	CE*3, AFP, 239.7ng/ml; PE*1 and ETP*2, AFP, 91.9ng/ml; VIP*1, AFP, 416.9ng/ml; TC*1, AFP, 3171ng/ml,NVI*2, AFP, 15.9ng/ml; exploratory laparotomy and positive for malignancy of the suspected residual diseases (mixed germ cell tumors, embryonal carcinoma with dysgerminoma); AFP, 14.1ng/ml; NVI*3, AFP, 25.6ng/ml,GEMOX*1, AFP, 78ng/ml; CVB*1, AFP, 46.1ng/ml; IV*2, AFP, 221.3ng/ml; DC*1, AFP, 459.1ng/ml	18	10	AFP, within the normal range after the second NVI cycle and remained elevated level after the fifth NVI cycle	AFP, 3841ng/ml	Mestasis was confirmed by the increase in the enlargement of pelvic lymph nodes in bilateral pelvic cavities and lung metastasis at the nineteenth month after primary diagnosis, maintenance of tumor marker level above the normal limits.	24, DOD; AWM, AWD, PD
8	Partial vaginectomy; AFP,221ng/ml	PEB*6	6	2	AFP, within the normal range after the second PEB cycle	AFP, 5.0ng/ml	Disappearance of primary lesion, no new lesion	10, alive; CR

*ND, not done; NC, not clear; cm, centimeter; PEB, cisplatin, etoposide and bleomycin; PIB, cisplatin, ifosamide and bleomycin; PI, cisplatin and ifosamide; JEB+A carboplatin, etoposide, bleomycin and tetrahydropyranyl adriamycin; VIP, ifosfamide, cisplatin and etoposide; CE, etoposide and carboplatin; PE, etoposide and cisplatin; ETP, etoposide, cisplatin and pirarubicin; TC, nab-paclitaxel and Carboplatin; NVI, ifosfamide, nedaplatin and vinblastine; GEMOX, gemcitabine and oxaliplatin; CVB, Irinotecan, vindesine and bleomycin; IV, ifosfamide and vinblastine; DC, decotaxel and cyclophosphamide; PR, partial response; CR, complete remission; PD, progressive disease; SD, stable disease; AWM, alive with metastasis; AWD, alive with disease; AWR, alive with relapse, DOD, died of disease

Finally, the case after six cycles of VIP (ifosfamide, cisplatin and etoposide) achieved in CR 27 months after primary diagnosis.

The sixth and the seventh patients were transferred from another hospital. The sixth patient received JEB+A (carboplatin, etoposide bleomycin and tetrahydropyranyl adriamycin) chemotherapy and the serum AFP level dropped to the normal level after three cycles. Then the case underwent three more cycles of JEB+A chemotherapy. Three months later, AFP level was elevated to 800 ng/ml, but no lesion was found by exploratory laparotomy and local tumor resection of vagina. The patients received two cycles PEB in Sun Yat-sen University cancer center. However, two months later, a retroperitoneal mass was founded in PET-CT and metastasis was confirmed by punch biopsy from the mass. Then, this patient received further 4 cycles of PEB, and AFP level remained slightly elevated 29 months after primary diagnosis.

The seventh case was initially misdiagnosed as pure vaginal yolk sac tumor and underwent CE (etoposide and carboplatin, 3 cycles), PE (etoposide and cisplatin, 1 cycle), ETP (etoposide, cisplatin and pirarubicin, 2 cycles) and VIP (1 cycle) chemotherapy in another hospital. However, the size of vaginal mass was increased and the serum AFP level was persistent elevated. A mixed germ cell tumor was confirmed in Sun Yat-sen University cancer center, and AFP level returned to normal after two cycles of NVI (ifosfamide, nedaplatin and vinblastine). Dysgerminoma element was seen in the specimen of exploratory laparotomy and local tumor resection of vagina, and further three NVI cycles were used. Unfortunately, AFP level (25.6 ng/ml) elevated again. In spite of rescuing chemotherapy, the patient's serum AFP level still continued to elevate, the number of enlargement of pelvic lymph nodes (≥1.5cm in short axis) in bilateral pelvic cavities increased and tumor cells metastasized to lung. The girl died within 24 months after primary diagnosis.

The clinicopathologic characterization, treatment and prognosis of previously published primary vaginal yolk sac tumor

85 cases (including our own) of primary vaginal yolk sac tumor from 1995 to 2014 were summarized. The mean age was 14.0 months (range from 3.5 to 120 months). 37 cases (43.5%) were distributed in Asian (including 14 cases in China, 11 cases in India, 6 cases in Japan). 27 cases (31.8%) in America and 21 (24.7%) in Europe. Vaginal bleeding was still initial manifestation for all patients and the average clinical history was 37.7 days (range 2-120 days). The average of AFP at diagnosis was 11225.5ng/ml (rang from 19.4 to 104340 ng/ml).

9 cases were misdiagnosed in the 85 cases. One patient was misdiagnosed as vaginitis by clinical doctor (Watanabe et al., 2010). Rhabdomyosarcoma was diagnosed on initial frozen section in two cases (Handel et al., 2002), clear cell carcinoma in two cases (Lopes et al., 1999) as well as rhabdomyosarcoma in one case in the routine pathological diagnosis(Mauz-Körholz et al., 2000). Unfortunately, rhabdomyosarcoma on imprint smear cytology, and then as clear cell adenocarcinoma on punch biopsy, at last yolk sac tumor on radical hysterectomy were reported in one case (Goyal et al., 2014). 2 cases of mixed germ cell tumors were also misdiagnosed as pure vaginal yolk sac tumor, one is the seventh case of our own, which the embryonal carcinoma and dysgerminoma compositions were found

by complete tumor resection during treatment, and the other one reported by Rescorla (Rescorla et al., 2003), which mature teratoma was found by excisional biopsy after BEP*4 chemotherapy. There were 5 cases (5/85) with mixed germ cell tumor including our four cases. Only 25 cases had stage information: 10 in stage I, 6 in stage II, 8 in stage III, and 1 in stage IV.

Follow-up data was available in 77 cases. 7 died of neoplasms and 5-year overall survival rate was 87.6% with the longest survival time of 175 month. The initial treatment was divided into three groups: 56 patients received biopsy and chemotherapy, 13 patents underwent conservative surgery and chemotherapy including 8 partial vaginectomy (complete resection of tumor) and 5 simple tumor excision (incomplete resection of tumor), and 8 cases underwent radical surgery (radical hysterectomy and partial vaginectomy) and chemotherapy. 5-year survival rate of the three groups were 91.1%, 100%, 28.6%, respectively (log rank test, $P<0.001$) (Figure 4). Compared to cases without relapse or metastasis after initial treatment, patients with relapse or metastasis had a shorter overall survival (35.6% vs 96.6%, $P<0.001$). 12 cases relapsed: 9 cases (9/52) relapsed in the group of biopsy with chemotherapy; 2 cases relapsed in 5 cases with simple tumor excision, on the contrary, no recurrence in 8 cases with partial vaginectomy, and 1 (1/12) in radical hysterectomy with chemotherapy. The average time of recurrence was 10 months (4-19m). 50 cases received PEB-based chemotherapy which achieved in 5-year survival rate 93.6%, compared to 80.2% in no PEB-based chemotherapy ($p=0.142$). The 5-year survival rate of 72 cases with pure yolk sac tumor was 88.8%, while that of cases with mixed germ tumor was 66.7% ($p=0.332$).

Discussion

Yolk sac tumor is a primitive germ cell tumor with a variety of distinctive patterns which may also exhibit differentiation into endodermal structures, ranging from the primitive gut and mesenchyme to the derivatives of extra-embryonal (secondary yolk sac and alantois) and embryonal somatic tissues (intestine, liver and mesenchyme) (Nogales et al., 2012). Vaginal yolk sac tumor is a rare and highly malignant tumor and primarily occurs in infants, which usually occurs in patients under 3 years of age. The mean age was 14.0 months in this series and reviewed literatures, and this 10-year-old patient was the oldest among the reported cases (Ishi et al., 1998). 43.5% (37/85) of cases originated in Asian, which achieved high prevalence compared to 31.8% (27/85) in America and 24.7% (21/85) in Europe.

The clinical presentation includes vaginal bleeding with bloody/blood-tinged vaginal discharge or a polypoidal friable mass. The average clinical history was 37.7 days (range 2-120 days). Unfortunately, the longest time to delay diagnosis reached 4 months. It is not easy to identify the cause of vaginal bleeding in pediatric patients, because of the difficulty in examining. Rectal examination should be done to assess the extent of the disease. Pediatric rhinoscopy, nasal speculum or vaginoscopy was used to visualize the vaginal tumor under general anesthesia before the treatment, and then biopsy of the tumor should be taken at the same time (Tao et al., 2012).

Histologically, there are five major patterns present in most yolk sac tumors. The two more-common and distinct are the reticular or microcystic pattern and the endodermal sinus pattern. The characteristic features of vaginal yolk sac tumor are clear cells with varied patterns with Schiller-Duval bodies, PAS-positive, diastase resistant hyaline globules with intracytoplasmic AFP immunopositivity. AFP is considered a reliable marker to evaluate the treatment response and remission status (Arafah et al., 2012). AFP levels in patients with mixed germ cell tumors also increase when the tumors contain yolk sac tumor elements (Arita et al., 1980).

The main entities in the differential diagnosis include embryonal rhabdomyosarcoma (RMS) on clinical examination and clear cell carcinoma on histopathological examination. Vaginal embryonal rhabdomyosarcoma is a common tumor of infancy and has a much wider age of presentation ranging from 0.1 to 12.5 years. Grossly, embryonal rhabdomyosarcoma shows soft and polypoid growth with a typical grape-like configuration. On microscopy it shows a small round blue cell tumor with skeletal muscle differentiation, and is histologically distinct from yolk sac tumor. Immunohistochemical expression of myogenin and myoD1 is highly sensitive and specific for the diagnosis of RMS (Sebire et al., 2003). Microscopically, due to clear cells morphology, yolk sac tumor is often misdiagnosed as clear cell carcinoma (Watanabe et al., 2010). Vaginal clear cell carcinoma usually occurs in adolescence and has not been reported under the age of 6 years. It shows a characteristic abundance of clear cells in masses, sheets, nests and papillary formations, and may be associated with adenosis (Sebire et al., 2003). The hyaline globules in clear cell carcinoma are PAS-positive, diastase-sensitive (glycogen) and AFP immunonegative (Lacy et al., 2006). Vaginal yolk sac tumor is CK7, and CD10 negative, in contrast to clear cell carcinomas (Zirker et al., 1989).

It's worth noting that several cases were histological misdiagnosis in this series. As reported here, it could have been averted with the use of special stains and with immunohistochemistry and detection serum tumor markers. In addition, more important is to provide complete information by complete resection of tumor especially in mixed yolk sac tumor for pathological diagnosis and subsequently primary treatment. The reason is that all components of mixed germ cell tumors can occur widely metastatic diffusion and then form metastases, but chemotherapy should be based on the highest degree of malignancy of ingredients (Robboy et al., 2008). Furthermore, dysgerminoma was more suitable for surgery and radiotherapy. Mixed germ cell tumors containing a yolk sac tumor element was not uncommon. About 40% (13/33) of yolk sac tumor of the ovary were of mixed yolk sac tumor type were reported by Kojimahara et al (Kojimahara et al., 2013). Moreover the prevalence of mixed yolk sac tumor type reached 50% (4/8) in our own cases. Thus, Germ cell tumors should be complete removed and avoid re-excision to obtain complete information on the pathological diagnosis after

the failure of primary treatment. The components present and proportion should be also specified in the diagnostic report (Smith et al., 2006).

As with other rare disorders, the ideal management of vaginal yolk sac tumor in infancy remains unclear. Untreated patients have died within 2 to 4 months of presentation (Andersen et al., 1985). In recent years, there has been marked improvement in prognosis with pre- and postoperative adjuvant chemotherapy (PEB) (Mauz-Körholz et al., 2000). PEB chemotherapy alone has resulted in complete remission in some early cases (Tao et al., 2012) and may be more suitable for pure yolk sac tumor. In malignant ovarian germ cell tumors, treatment with fertility sparing operations and adjuvant chemotherapy with a BEP regimen showed a good outcome (Bilici et al., 2013; ANeeyalavira et al., 2014). Correspondingly more conservative surgery maintaining sexual and reproductive function in vaginal yolk sac tumor has gradually replaced the radical surgery. The goal of conservative surgery is to remove local bulk disease and make subsequent chemotherapy more effective, and also provide complete information for pathological diagnosis and postoperative adjuvant therapy. The extent of conservative surgery should require at least partial vaginectomy, which was performed with a free resection margin (Hwang et al., 1996). It was recognized that a small biopsy or local simple tumor excision may have missed the other elements of mixed germ tumor and residual cells in the vaginal wall can result in local recurrence even with effective chemotherapy (Hwang et al., 1996).

In summary, we reported eight primary vaginal yolk sac tumor, including 4 cases of pure yolk sac tumor and four mixed germ tumor, and reviewed 85 cases in literature. In our experience, partial vaginectomy combined with PEB regimen chemotherapy could be a good choice for primary vaginal yolk tumor to eradicate local tumor cells and provide complete information for pathological diagnosis and postoperative adjuvant therapy. However, this concept needs more cases and long time follow-up to further verify.

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