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Case report

Omega-3 fatty acids for the treatment of hypertriglyceridemia during the second trimester

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ABSTRACT

Objective: Serum triacylglycerol (TG) levels increase during pregnancy. High serum TG levels may elicit acute pancreatitis; therefore, it is important that pregnant women are managed well to abrogate the rapid rise of TG levels in pregnancy. The aim of this study was to report on the effect of eicosapentaenoic acid administration on pregnant women with hypertriacylglycerolemia in the second trimester.

Method: We report on four patients who presented to Kumamoto University Hospital from January 2005 to March 2013.

Findings: All four patients delivered neonates at term without complicating acute pancreatitis. Additionally, in three cases of multipara, the maximum serum TG levels were decreased to 10% to 49% of their preceding pregnancy.

Conclusion: Oral eicosapentaenoic acid administration might be a safe and useful treatment for hypertriacylglycerolemia during pregnancy and may prevent the development of acute pancreatitis.

Introduction

Maternal serum triacylglycerol (TG) levels increase two to four times during normal pregnancy [1], but levels rarely exceed 300 mg/dL [2]. Unlike common complications of chronic hypertriacylglycerolemia (HTG), such as arterial sclerosis or coronary artery disease [3], acute HTG may cause acute pancreatitis [4]. Pancreatitis can develop in pregnant women and is a life-threatening complication that can be prevented by controlling serum TG levels [5]. However, most medications for treating HTG are not safe for use during pregnancy; therefore, dietary intervention often is the only option in such cases. Following our first case of a pregnant woman who was administered eicosapentaenoic acid (EPA) for HTG [6], we experienced three more cases and are now convinced of the efficacy and safety of EPA. Here, we report four cases of HTG in pregnant women treated with eicosapentaenoic acid (EPA) during pregnancy, along with a review of the current literature.

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Case reports

Case 1

We have previously reported on a 27 y old (gravida 2, para 1) woman as the first woman who was administered EPA for HTG during pregnancy in Kumamoto University Hospital (see previous report [6] and Table 1).

Case 2

A 37 y old (gravida 2, para 1) woman had undergone laparoscopic surgery for an ovarian tumor when she was 33 y old. A close relative had past history of HTG and acute pancreatitis. Although her serum TG level was increased (417 mg/dL), her total cholesterol and Apo protein levels were normal at her preoperative examination. Therefore, she was diagnosed as type I or V HTG and received dietary intervention (Table 1, Fig. 1). One y later, she had her first pregnancy and received prenatal care at our hospital. Her serum TG levels had gradually increased and reached 4020 mg/dL; she was started on dietary intervention at 34 wk and 3 d of gestation. She had a spontaneous delivery at

41 wk and 1 d of gestation. Her serum TG levels rapidly decreased to normal levels (217 mg/dL) 4 wk after parturition. Three years later, she had her second pregnancy. As gestation progressed, her serum TG levels increased. At 18 wk and 4 d of gestation, oral EPA was initiated at 1800 mg/d and its dosage was increased to 4500 mg/d. Peak serum TG levels during her second pregnancy reached 1979 mg/dL at 40 wk of gestation, and decreased to 49% of her preceding pregnancy. She gave birth naturally at 41 wk and 1 d of gestation.

Case 3

A 30 y old (gravid 2, para 0) woman (Table 1) had been managed in the hospital's Department of Gastroenterology for Crohn's disease. Although her physician was aware that the patient's serum TG levels were at the upper limit of normal, she did not order further examination of HTG because serial blood examination did not reveal any additional increases in the TG level. The patient became pregnant after intracytoplasmic sperm injection at a local clinic. At 12 wk and 6 d of gestation, she was referred to our hospital for the management of her pregnancy. Her serum TG levels were 182 mg/dL at her first visit but increased to 620 mg/dL at 23 wk and 6 d of gestation. Despite dietary restrictions for 3 wk, TG levels remained abnormally high. Oral EPA administration (600 mg/d) was therefore started at 26 wk of gestation. Her serum TG levels remained at ~300 mg/dL without any complications. She delivered her baby after an oxytocin-induced labor at 38 wk and 5 d of gestation.

Case 4

A 31 y old (gravida 2, para 1) woman was transferred to our hospital for treatment of acute pancreatitis at 26 wk and 6 d of her first pregnancy (Table 1, Fig. 1). Her serum TG (11 480 mg/dL), total cholesterol (1172 mg/dL), and pancreatic amylase (416 U/L) levels were increased significantly on admission. Additionally, her father had past history of HTG and acute pancreatitis and she was diagnosed as type V HTG. Her symptoms improved after medication and she underwent a cesarean delivery at 30 wk and 1 d of gestation to prevent possible secondary infection of a pancreatic cyst. She had a favorable postoperative course and was discharged on postoperative day 28. Details of her first pregnancy were reported in a study published by her gastroenterologists [7]. Her serum TG levels were maintained in the normal range by dietary intervention. Two years later, she had a second pregnancy. Her serum TG levels gradually increased to 342 mg/dL; therefore, EPA administration at 600 mg/d was initiated from 15 wk and 6 d of gestation and its dosage was titrated up to 2700 mg/d according to her serum TG levels. The maximum serum TG levels of her second pregnancy reached 1163 mg/dL at 27 wk and 6 d of gestation, but there were no findings of acute pancreatitis. She had a vaginal birth after cesarean delivery at 39 wk and 3 d of gestation. Her serum TG levels were normal 1 mo after delivery and she no longer required any medication.

Discussion

Cases 1, 2, and 4 were multiparous and had been diagnosed with HTG during antecedent pregnancies. Cases 1 and 4 had been complicated with acute pancreatitis in the previous pregnancies. Case 3 was a primigravida who had a high serum TG level detected before pregnancy. For cases 1 and 4, the

Table 1
Clinical features of each case

	Age	G-P		Acute pancreatitis	Serum TG level in first trimester (Mg/dL)	Serum TG level at EPA start (Mg/dL)	Maximum of serum TG level (Mg/dL)	Delivered gestational week	Type of delivery	Birth weight (g)	Intrapartum hemorrhage (g)	Postpartum serum TG level (Mg/dL)	Diagnosis of HTG	Familial history
Case 1	27	2-1	Preceding pregnancy (2 y ago) EPA (−)	Yes (35 wk 2 d)	-	Unused	12,020 (35 wk 2 d)	38 wk 5 d	NTVD	2,895	540	165	Type V	Father:HL (no treatment, detail is unknown)
			Present	No	72	782 (27 wk 2 d)	1,691 (33 wk 2 d)	37 wk 0 d	NTVD	2,635	230	383		
Case 2	37	2-1	Preceding pregnancy (3 y ago) EPA (−)	No	319	Unused	4,020 (34 wk 3 d)	41 wk 1 d	NTVD	3,715	650	217	Type I or V	Close relative:HTG, acute pancreatitis
			Present	No	131	531 (18 wk 4 d)	1,979 (40 wk 0 d)	41 wk 1 d	NTVD	3,950	580	358		
Case 3	30	2-0	Present	No	182	336 (26 wk 6 d)	620 (23 wk 6 d)	38 wk 5 d	Oxytocin	3,365	490	113	Un-known	-
Case 4	31	2-1	Preceding pregnancy (2 y ago) EPA (−)	Yes (26 wk 6 d)	-	Unused	11,480 (26 wk 6 d)	30 wk 1 d	C/S	1,679	627	222	Type V	Father:HTG, acute pancreatitis
			Present	No	408	342 (15 wk 6 d)	1,163 (27 wk 6 d)	39 wk 3 d	NTVD (VBAC)	3,575	500	48		

C/S, cesarean section; EPA, eicosapentaenoic acid; G-P, gravida-para; HL, hyperlipidemia; HTG, hypertriacylglycerolemia; NTVD, normal transvaginal delivery; TG, triacylglycerol; VBAC, vaginal birth after cesarean

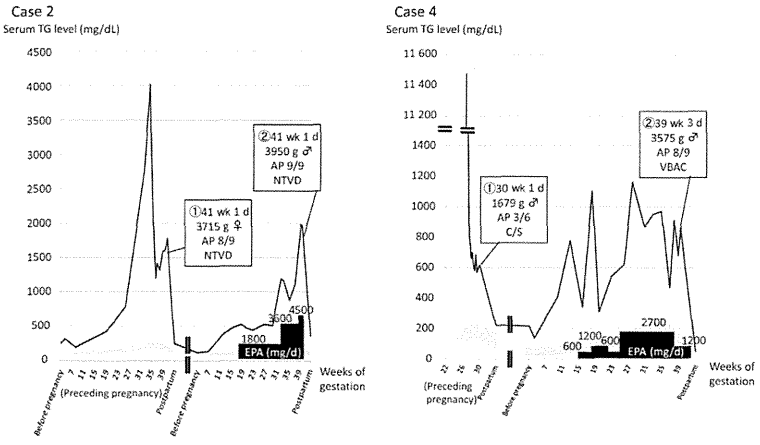

Fig. 1. Clinical presentation of hypertriacylglycerolemia during pregnancy for cases 2 and 4. The gray shaded area indicates the normal range of serum triacylglycerol. AP, Apgar score; NTVD, normal transvaginal delivery; C/S, cesarean section; TG, triacylglycerol; VBAC, vaginal birth after cesarean.

periodic checkup of their serum TG levels was not done until they developed acute pancreatitis.

The gestational times at which EPA administration was started ranged from 15 to 27 wk of gestation. The serum TG levels at this time were 782 mg/dL (case 1), 531 mg/dL (case 2), 336 mg/dL (case 3), and 342 mg/dL (case 4). The initial EPA dosage varied from 600 to 1800 mg/d and the administrated dose was increased when the serum TG levels reached beyond 600 to 1000 mg/dL, up to 4500 mg/d (case 2). None of the patients developed acute pancreatitis during EPA administration. Additionally, all patients delivered babies at term. For the multiparas (cases 1, 2, and 4), the maximum serum TG levels during pregnancy with EPA administration were lower than that during their previous pregnancy (without EPA administration), with their levels decreasing to 14%, 49%, and 10%, respectively. None of the patients showed any side effects, such as diarrhea or increasing intrapartum hemorrhage, and none of infants had complicated respiratory distress syndrome.

The normal serum TG level in nonpregnant women is < 150 mg/dL. TG levels increase during pregnancy [1], but rarely exceed 300 mg/dL. Furthermore, although TG is an essential lipid in the human diet, it is well known that an acute increase of serum TG levels could induce acute pancreatitis [4].

Acute pancreatitis is an uncommon complication of pregnancy with an incidence of 0.03% pregnancies and HTG accounts for 4.4% of pancreatitis cases [8]. Recent reports revealed that maternal and perinatal mortality rates of complicated acute pancreatitis have improved by 3.6% [9], but the management of acute pancreatitis during pregnancy, including fibrates, statins, and plasma exchange, remains a challenge [10,11].

A treatment gaining attention is the use of ω-3 fatty acids, including docosahexaenoic acid (DHA) and EPA, to improve HTG during pregnancy. The reported mortality rates from cardiovascular disease of Inuit populations of Greenland who ate raw seal were one-seventh of white populations [12]. The blood EPA levels are high in the Greenland Inuit and the low mortality rate could not be derived from genetic factors. Instead their environment, and especially their diet plays a critical role. EPA is found in seal meat, edible seaweed, and fish oils (e.g., mackerel, cod liver, herring, salmon, and sardine).

EPA has pharmacologic effects, including inhibition of arteriosclerosis and impairment of platelet aggregation without major complications. The combination of EPA and DHA has proven effective in improving HTG. High intakes of ω-3 fatty acids significantly lower rates of coronary disease in Japan [14]. The mechanism by which EPA improves HTG is not fully elucidated, but one possibility is the inhibition of a liver-activating enzyme that synthesizes lipoprotein. In pregnant women with preeclampsia, administration of ω-3 fatty acids can improve placental circulation; however, the effects remain controversial. Although some investigators have reported infants with complicated respiratory distress syndrome following prenatal administration of EPA [13], our four cases gave birth at term and the babies did not develop any respiratory complications. On the basis of our case series, we suggest that EPA is safe and effective to abrogate the rapid rise of serum TG levels and prevent acute pancreatitis during pregnancy.

Although prophylactic EPA administration to pregnant women who may develop HTG should be considered, this calls for further investigation. First, screening of HTG during prenatal checkup is not common. We pay little attention to the serum TG level during pregnancy if the patient does not have a history of acute pancreatitis or HTG. For cases 1 and 4, serum TG levels were not evaluated in the first trimester of their first pregnancy. In Japan, the energy intake of fat, especially animal fat, has increased fourfold in the past 40 y. It seems rational to consider screening serum TG levels in all pregnancy cases in Japan.

Another issue is how pregnant women ingest EPA. As we have mentioned, EPA is found in fish, including mackerel. The consumption of fish in Japanese people is ~500 g/wk per person, corresponding to 128 mg/d of EPA, which is two-thirds of the daily recommended EPA intake. However, the FDA has recommended that pregnant women eat ≤340 g/wk of seafood to prevent the excessive intake of methyl mercury in 2004. The administration of a high-purity EPA product or supplement

seems the most effective way to ingest EPA without an excessive intake of seafood.

Conclusion

Taking into consideration the recent literature and our small case series, we suggest that EPA administration improves HTG during pregnancy and prevents the onset of acute pancreatitis. However, further study is required to establish the significance, indication, and dosage of EPA to control serum TG levels during pregnancy.

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ORIGINAL PAPER

Clinicopathological heterogeneity in ovarian clear cell adenocarcinoma: a study on individual therapy practice

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Abstract Ovarian clear cell adenocarcinoma (CCA) has been believed to be a lethal histological subtype of an epithelial ovarian adenocarcinoma (EOA); its precursor has been assumed to be endometriosis. However, it has been reported that CCAs occasionally exhibit different clinical behaviors, suggesting that CCAs might not belong to a single category. We focused on CCAs combined with other histological types of EOAs; we re-evaluated the pathology of 46 CCAs and divided them into two subgroups: 35 CCAs alone (pure-type CCAs); and 11 CCAs with other histological types, endometrioid adenocarcinomas (EAs) or/and serous adenocarcinomas (SAs) (mixed-type CCAs). Immunohistochemical analysis for expression of ARID1A, p53, PTEN, Annexin 4, hepatocyte nuclear factor-1β (HNF-1β), and WT-1 was employed. We identified that patients with endometriosis were younger than those without endometriosis in pure-type CCAs ($P < 0.005$). In mixed-type CCAs, the immunohistochemical-staining patterns revealed internal transition of each histological component. In pure-type CCAs, expressions of ARID1A and p53 were mutually altered, and altered expression of p53 was associated with worse prognosis than that of ARID1A ($P < 0.001$). Our results provide evidence that CCAs would have clinicopathological heterogeneity, determining the patient's prognosis. Furthermore, immunohistochemical analysis may shed light on the selection of appropriate treatment, including chemotherapy.

Keywords Ovarian clear cell adenocarcinoma · Molecular pathology · Endometriosis · Heterogeneity · Prognosis · Therapeutic strategy

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Introduction

Ovarian clear cell adenocarcinomas (CCAs) were initially reported in 1899 by Peham [1]. They closely resembled renal cell carcinomas and were thought to be mesonephric in origin; therefore, in 1939, Schiller stated that ovarian tumors comprised clear and hobnail cells as mesonephromas [2]. Subsequently, Scully et al. [3] described a frequent association of CCAs with endometriosis in 1967 and suggested that CCAs originated from a Müllerian duct, similar to other major histological types of epithelial ovarian adenocarcinomas (EOAs). Consequently, in 1973, the World Health Organization (WHO) recognized CCAs as a distinct histological entity in the classification of EOAs [4].

Although comprising fewer than 3.7 % of EOAs worldwide [5], the prevalence of CCAs is 25 % in Japan [6]. CCAs have received much attention owing to their poor prognosis. Combination chemotherapy, with platinum plus paclitaxel, has been adopted as the standard regimen for front-line treatment of CCAs; this treatment is similar to that for serous adenocarcinomas (SAs) and endometrioid adenocarcinomas (EAs). In several retrospective studies [5, 7–10], the response rate (RR) to first-

line therapy with this regimen ranged between 22 and 56 %, compared with RR >70 % for patients with SAs. Recio et al. [11] showed that platinum-based chemotherapy did not improve 5-year overall survival. However, the mechanism underlying the chemoresistance of CCAs to platinum-based therapy is not well understood.

Morphologic studies over the past four decades showed an association of CCAs with endometriosis, and recent studies suggested that endometriosis was considered to be a precursor lesion of CCAs [12–14]. However, it has been reported that CCAs occasionally exhibit different clinical behaviors with better or worse prognoses [15, 16]. Because of these distinctive clinical and pathological features, the correct classification of CCAs is of critical importance. However, there is a difficulty of classification due to an occasional histological mixture of EOAs in CCAs. The WHO limits their classification to those mixed carcinomas in which one or more component other than the predominant component account for at least 10 % of the tumor on histopathological examination. Kurman and Craig reported that CCAs were found in association with other types of EOAs, although the most common mixtures were EAs and SAs [17]. In contrast, mixed carcinomas composed of CCAs and mucinous adenocarcinomas (MAs) were rare. Among mixed carcinomas of EOAs, mixed carcinomas composed of SAs and CCAs were indistinguishable from SAs with respect to clinical features. Some investigators suggested that they represented a variant of SAs and were not related to CCAs [18]. In regard to mixed carcinomas composed of CCAs and EAs, Köbel et al. [19] reported that CCAs and EAs tended to be observed together. Although mixed carcinomas have not been discussed in detail until the present, it is important to comprehensively investigate the clinical characteristics of CCAs.

We conducted a pathological re-evaluation of CCAs treated at Kumamoto University Hospital from 1990 to 2008, and investigated the association with endometriosis and patient age to determine the clinicopathological features. We performed immunohistochemical study for expression of ARID1A, p53, and PTEN as tumor suppressor genes for CCAs, SAs, and EAs, respectively. It was reported that alternative expression of ARID1A, p53, and PTEN corresponded to the mutation and loss of heterozygosity in each gene [20–27]. We included the immunohistochemical study for expression of Annexin 4, hepatocyte nuclear factor-1β (HNF-1β) as characterized genes for CCAs, and WT-1 as a distinctive gene for SAs [28–30]. Furthermore, we examined the heterogeneity of CCAs, including mixed carcinoma composed of CCAs and other histological types of EOAs.

Materials and methods

Human samples

Samples of CCAs were collected from 55 patients who underwent complete surgery at Kumamoto University Hospital from 1990 through 2008. Tissue blocks were prepared at a rate of one sample/cm according to the size of the tumor. They were routinely fixed in 10 % neutral buffered formalin and then embedded in paraffin blocks. These blocks were sectioned at 3 μm and stained by hematoxylin and eosin. Two independent gynecological pathologists determined the tumor type according to the WHO histological classification of ovarian tumors [4], and 46 cases were consistent with the original diagnosis. Furthermore, we divided 46 cases into two subgroups consisting of either CCA component alone (pure-type CCAs) or CCAs together with endometrioid, serous, or mucinous components (mixed type CCAs); in this study, mixed-type CCAs were defined when CCAs coexisted with other histological types of EOAs even to just a small degree. We investigated age and clinical stage according to the International Federation of Obstetrics and Gynecology (FIGO) system. Follow-up data were available for all 46 patients to evaluate the prognosis. We obtained consent from all the patients in this study.

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissue specimens were cut into 3-μm sections and mounted on silanized glass slides. These slides were deparaffinized in xylene, rehydrated through serial dilutions of alcohol, and washed in Tris-buffered saline (0.05 M Trizma base, 0.9 % NaCl, pH 7.4), according to the supplier's recommended protocol. These sections underwent antigen retrieval in a citrate buffer (0.01 M; pH 6.0) by microwave, or proteinase K and warm bath. They were stained with antibodies: p53, ARID1A, PTEN, HNF-1β, Annexin 4, and WT-1. Details of antibodies used and staining conditions were presented in Table 1. They were counterstained with hematoxylin. The immunoreaction was visualized using diaminobenzidine.

Evaluation of immunostaining

Immunostained slides were evaluated. The p53 staining was given an immunoreactive score obtained by multiplication based on the intensity of nuclear staining and quantity of cells stained according to the previously reported grading system [25]. The staining intensity was

Table 1 Details of antibodies used in this study

Antigen	Clone	Product code	Supplier	Dilution	Antigen retrieval
p53	Mouse monoclonal DO-7	N1581	Dako cytomation	1:1	Microwave
ARID1A	Mouse monoclonal	sc-32761	Santa cruz biotechnology	1:200	Microwave
PTEN	Rabbit monoclonal, 138G6	#9559	Cell signaling	1:200	Microwave
HNF-1β	Goat polyclonal	sc-7411	Santa cruz biotechnology	1:400	Microwave
Annexin4	Rabbit polyclonal	ab33009	Abcam	1:400	Microwave
WT-1	Mouse monoclonal, 6F-H2	M3561	Dako cytomation	1:25	Proteinase K, warm bath

Table 2 Clinical data of the 46 patients

Age (y)		
Median (range)	51.1 ± 1.8 years (range 28–91 years)	
Subgroup, *n* (%)		
Pure-type CCAs	35 (76.1%)	
Mixed-type CCAs	11 (23.9%)	
Histological pattern of mixed-type CCAs, *n* (%)		
CCAs and EAs	2 (18.2%)	
CCAs and SAs	8 (72.7%)	
CCAs and EAs and SAs	1 (9.1%)	
FIGO stage, *n* (%)		
I	33 (71.7%)	
II	4 (8.7%)	
III	7 (15.2%)	
IV	2 (4.3%)	
Endometriosis, *n* (%), median age (y)		
with endometriosis	34 (73.9%), 49.4 ± 1.7 years	*
without endometriosis	12 (26.1%), 55.5 ± 4.7 years	*

CCAs clear cell adenocarcinomas, *EAs* endometrioid adenocarcinomas, *SAs* serous adenocarcinomas

* $P < 0.012$

divided into four categories: 0, negative; 1, weakly positive; 2, moderately positive; 3, strongly positive. The most intensely staining slides were deemed to be the upper limit. The quantity of cells stained was scored as follows: 0, no staining; 1, 1–10 %; 2, 11–50 %; 3, 51–80 %; 4 >80 % of tumor nuclei stained. With p53 staining, the multiplied immunoreactive score of 8–12 was considered strong immunoreactivity, 4–6 was moderate, 1–3 was weak, and 0 was negative. It was previously indicated that all carcinomas with strong immunoreactivity (score 8–12) showed p53 missense mutations, although some negative carcinomas (score 0) also revealed p53 frameshift mutations [25]. For ARID1A and PTEN, we defined loss of function as negative nuclear staining by the multiplied immunoreactive score, inversely with p53, because previous studies showed that negative staining of ARID1A and PTEN demonstrated gene mutation [20–24, 26, 27]. HNF-1β, Annexin 4, and WT-1 were binarized as negative or positive, and immunostaining in >50 % of tumor cells was positive [28–30].

Statistical analysis

The χ^2 test and Student's t test for unpaired data were used for statistical analysis. Patient survival distribution was calculated using the Kaplan–Meier method. The significance of the survival distribution in each group was tested by the log rank test. $P < 0.05$ was considered to be statistically significant. All values were given as the mean ± SD.

Results

Patient characteristics

The characteristics of the study population are presented in Table 2. Median age at diagnosis was 51.1 ± 1.8 years (range 28–91 years). Of the 46 tumors, 71.7 % were stage I, 8.7 % were stage II, 15.2 % were stage III, and 4.3 % were stage IV (FIGO classification). Endometriosis was histologically observed in 34/46 (73.9 %) of the cases. Patients with endometriosis were aged 49.4 ± 1.7 years, whereas those without endometriosis were aged 55.5 ± 4.7 years, showing that those with endometriosis were significantly younger ($P < 0.012$).

We identified 35 pure-type CCAs and 11 mixed-type CCAs coexisting with serous or/and endometrioid elements. Mixed-type CCAs coexisting with mucinous

Table 3 Characteristics of pure-type and mixed-type CCAs

Pure-type CCAs (total number: 35)		
FIGO stage, *n* (%)		Recurrence and/or dead, n
I	29 (82.9%)	3
II	3 (8.6%)	0
III	2 (5.7%)	1
IV	1 (2.9%)	0
Existence of endometriosis, *n* (%), median age (y)		
with endometriosis	29 (82.9%), 49.1±1.8 years	4
without endometriosis	6 (17.1%), 63.4±6.2 years **	0
Mixed-type CCAs (total number: 11)		
FIGO stage, *n* (%)		
I	4 (36.4%)	0
II	1 (9.1%)	0
III	5 (45.5%)	3
IV	1 (9.1%)	0
Existence of endometriosis, *n* (%), median age (y)		
with endometriosis	5 (45.5%), 51.0±4.2 years	2
without endometriosis	6 (54.5%), 46.3±5.4 years	1

CCAs clear cell adenocarcinomas, *EAs* endometrioid adenocarcinomas, *SAs* serous adenocarcinomas

** $P < 0.005$

adenocarcinoma were not observed in our cases. The characteristics of pure-type and mixed-type CCAs are presented in Table 3. In pure-type CCAs, patients with endometriosis were younger than those without endometriosis ($P < 0.005$). In contrast, in mixed-type CCAs, there was no correlation between age and endometriosis.

Immunohistochemical findings

In 46 cases, negative staining (score 0) of ARID1A, strong positive staining (score 8–12) of p53, and negative staining (score 0) of PTEN were observed in 28 (60.9 %), 10 (21.7 %), and 2 (4.3 %) cases, respectively.

Positive staining of Annexin 4, HNF-1β, and WT-1 was found in 38 (82.6 %), 36 (78.3 %), and 3 (6.5 %) of 46 cases.

In pure-type CCAs, the immunohistochemical results are shown in Table 4. The negative staining of ARID1A, strong positive staining of p53, and negative staining of PTEN were observed in 23 (65.7 %), 5(14.3 %) and 0 (0.0 %) of 35 cases, respectively. Positive expressions of Annexin 4, HNF-1β, and WT-1 were observed in 35 (100.0 %), 34 (97.1 %), and 0(0.0 %) 35 cases, respectively. Endometriosis was observed in 18 (78.3 %) of 23 cases with negative staining of ARID1A, and in 4 (80.0 %) of five cases with strong positive staining of p53.

The immunohistochemistry results of mixed-type CCAs are demonstrated in Figs. 1 and 2. Two cases composed of CCAs and EAs had negative staining of PTEN in both areas of the CCAs and EAs (Fig. 2a–d). Eight cases composed of CCAs and SAs had various expression patterns. Negative staining of ARID1A in areas of the CCAs

Table 4 Number of cases observed with altered expression by immunohistochemical analysis in pure-type CCAs

Total number of pure-type CCAs 35				
Markers	Altered expression, *n* (%)	Endometriosis, *n* (%) in cases of altered expression	Recurrences and/or deaths, *n* (%) in cases of altered expression	Altered expression, *n* (%) in 4 recurrence and/or dead cases
ARID1A	23 (65.7 %)	18/23 (78.3 %)	0/23 (0.0 %)	0/4 (0.0 %)
p53	5 (14.3 %)	4/5 (80.0 %)	3/5 (60.0 %)	3/4 (75.0 %)
PTEN	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0/4 (0.0 %)
ARID1A and p53	1 (2.9 %)	0/1 (0.0 %)	0/1 (0.0 %)	0/4 (0.0 %)
Annexin4	35 (100.0 %)	29/35 (82.9 %)	4/35 (11.4 %)	4/4 (100.0 %)
HNF-1β	34 (97.1 %)	29/34 (85.3 %)	4/34 (11.8 %)	4/4 (100.0 %)
WT-1	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0/4(0.0 %)

CCAs clear cell adenocarcinomas

Case	Age (years)	Hystological type	PTEN		ARID1A (Baf250a)		Annexin4		HNF-1β		p53		WT-1		En
			CCA	EA	CCA	EA	CCA	EA	CCA	EA	CCA	EA	CCA	EA	
1	59	CCA+EA	-	-	+	+	-	-	-	-	-	-	-	-	+
2*	60	CCA+EA	-	-	+	+	-	-	-	-	-	-	-	-	+
			CCA	SA	CCA	SA	CCA	SA	CCA	SA	CCA	SA	CCA	SA	
3*	51	CCA+SA	+	+	+	+	+	+	-	-	-	-	-	-	+
4	48	CCA+SA	+	+	-	-	-	-	-	-	-	-	-	-	+
5	37	CCA+SA	+	+	-	-	-	-	+	-	-	-	-	-	+
6	32	CCA+SA	+	+	-	-	+	-	+	-	-	-	-	-	-
7	56	CCA+SA	+	+	-	-	-	-	-	-	-	+	-	-	-
8	49	CCA+SA	+	+	+	+	-	-	-	-	-	+	-	+	-
9*	28	CCA+SA	+	+	+	+	-	-	-	-	-	+	+	+	-
10	61	CCA+SA	+	+	-	+	-	-	-	-	+	+	+	+	-
			CCA	EA	SA	CCA	EA	SA	CCA	EA	SA	CCA	EA	SA	CCA	EA	SA	CCA	EA	SA	
11	52	CCA+EA+SA	+	+	+	+	+	+	+	-	+	-	-	-	-	-	+	-	-	-	-

Fig. 1 Immunohistochemical characteristics in mixed-type CCAs. Positive immunostaining is demonstrated as *plus*, and negative as *minus*. Abnormal expression is surrounded by red. *EA* endometrioid adenocarcinoma, *SA* serous adenocarcinoma, respectively. *En* endometriosis; *plus* surrounded with *yellow* indicates that endometriosis was observed; *minus* surrounded with *green* indicates that endometriosis was not found. *Asterisk* recurrence and/or death

was observed in 5/8 (62.5 %), and in one case among these five cases positive staining of ARID1A was found in SAs (Fig. 2e–h). Strong positive staining of p53 in areas of the SAs was found in 4 (50.0 %) of eight cases, and in one case among of these four cases positive staining was observed in both areas of the CCAs and SAs (Fig. 2i–l). The negative staining of PTEN was not observed in all eight cases. One case composed of CCA, EA, and SA had altered expression of Annexin 4 and p53 without endometriosis.

Overall, in pure-type CCAs, the frequency of endometriosis was high. There was no significant difference between the involvement of endometriosis and expression of each protein. However, in mixed-type CCAs, endometriosis was not found in any of the p53 strong positive staining cases.

Patient prognosis

Of 35 patients with pure-type CCAs, 29 (82.9 %) had stage I disease, 3 (8.5 %) had stage II, 2 (5.7 %) had stage III, and 1 (2.9 %) had stage IV. In pure-type CCAs, the median survival time was 65.5 months (range 31–194 months), and recurrence and/or death occurred in 4/35 (11.4 %). In these 4 cases, negative staining of ARID1A was not found; however, strong positive staining of p53 was observed 3/4 (75.0 %) (Table 4). This indicated that altered expression of ARID1A and p53 showed contrary prognosis ($P < 0.001$).

Of 11 patients with mixed-type CCAs, 4 (36.4 %) had stage I disease, 1 (9.1 %) had stage II, 5 (45.4 %) had stage III, and 1 (9.1 %) had stage IV. In mixed-type CCAs, the median survival time was 60.4 months (range 1–160 months) and recurrence and/or death occurred in 3/11 (27.3 %). In patients with recurrence and/or death, a significant difference between prognosis and expression of p53 and ARID1A was not found.

Discussion

Recent pathological and molecular evidences have suggested that endometriosis serves as a precursor of CCAs [21, 31, 32]. In approximately 60 % of endometriosis-associated EOAs including CCAs, the carcinomas are adjacent to endometriosis or arise directly from atypical endometriosis, suggesting that malignant transformation

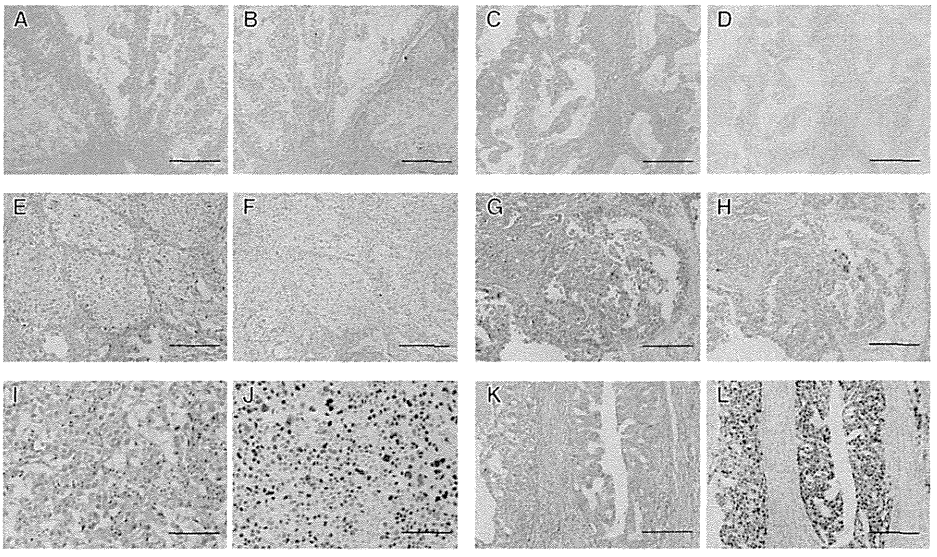

Fig. 2 Immunohistochemistry of mixed-type CCAs. **a–d** Case 1 of Fig. 2 is presented. **a**, **b** In the area of CCAs, PTEN was negative in the nucleus or cytoplasm of glandular cells. **c**, **d** In the area of EAs, PTEN was negative in the nucleus or cytoplasm of glandular cells. **e–l** Case 10 of Fig. 2 is presented. **e**, **f** In the area of CCAs, ARID1A was negative in the nucleus of glandular cells. **g**, **h** In the area of SAs, ARID1A was positive in the nucleus of glandular cells. **i**, **j** In the area of CCAs, p53 was positive in the nucleus of glandular cells. **k**, **l** In the area of SAs, p53 was positive in the nucleus of glandular cells. **a–l** ×100. *Scale bar* 200 μm

occurs in a subset of patients with ovarian endometriosis. In our current study, endometriosis was also histopathologically observed in 73.9 % of CCAs. Among pure-type CCAs selected in this study, endometriosis was observed in 82.9 % of cases, and patients with endometriosis were significantly younger than those without endometriosis. These results show that endometriosis represents an important site of the origin of pure-type CCAs. However, endometriosis was observed in 45.5 % of mixed-type CCAs, and there was no significant difference between the ages of patients with and without endometriosis. Furthermore, as for the prognosis, the ratio of cases of recurrences and/or deaths of mixed-type CCAs was higher than pure-type CCAs. This demonstrated that difference of characteristic between pure-type and mixed-type CCAs.

The p53 gene is the most frequently altered gene in human cancer [25]. Recently, it was reported that mutation of p53 was an important determinant of aggressive biological behavior, resulting in poor outcome of patients with several types of cancer [33]. In contrast, changes in chromatin can influence the epigenetic regulation of many genes, inducing transcription, DNA replication, and DNA damage repair in cancer. Chromatin remodeling is essential for all nuclear activities, and ARID1A is a chromatin remodeling factor [24, 25]. ARID1A is recognized as a tumor suppressor gene and also provides a potential approach to determine which of the numerous epigenetic changes in cancer confer a selective growth advantage. It was previously reported that the AIRD1A mutation is an early event in neoplastic transformation as well as p53 [22, 23, 34]. Importantly, the regulation of p53-related genes by ARID1A raises the possibility that ARID1A molecularly cooperates with p53 to inhibit tumor growth. Therefore, it is possible that in non-transformed cells, ARID1A and p53 collaborate as a pair of gatekeepers that prevent carcinogenesis by transcriptional activation of tumor-inhibiting downstream genes. Furthermore, it is thought that concurrent mutations in ARID1A and p53 are not required for carcinogenesis; in other words, both genes are mutually exclusive in tumor [22].

With the exception of one case, our immunohistochemical analysis demonstrated that in pure-type CCAs, tumors with mutated ARID1A contain wild-type p53 and tumors with mutated p53 harbor wild-type ARID1A. Both ARID1A and p53 appear to be essential for tumor suppression of pure-type CCAs, and concurrent mutations in

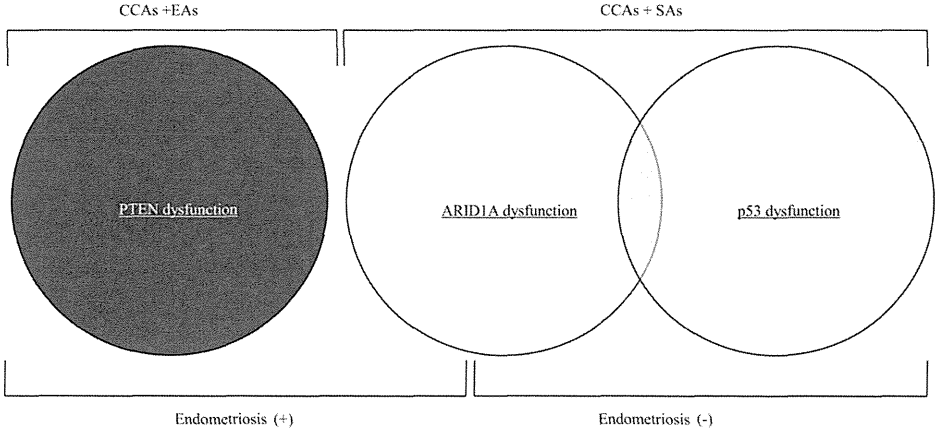

Fig. 3 Association between dysfunction of tumor suppressor genes and endometriosis in mixed-type CCAs. This figure presents a concise summary of mixed-type CCAs with altered expression of PTEN, p53, and/or ARID1A. Mixed-type CCAs lacking PTEN and ARID1A function were associated with endometriosis. Mixed-type CCAs lacking p53 function were not associated with endometriosis

both genes are not required for their carcinogenesis. Our results also suggest that ARID1A and p53 genes were mutually exclusive in CCAs [22]. Interestingly, we found altered expression of p53 and normal expression of ARID1A in pure-type CCAs with recurrence or death. Conversely, we found altered expression of ARID1A and normal expression of p53 in pure-type with good prognosis. This result indicated that pure-type CCAs may be divided into two subgroups with mutation in either ARID1A or p53; these subgroups correlate with prognosis. Henceforward, recognition of this subgroup of CCAs may play an indispensable role in determining patient prognosis and selecting the appropriate chemotherapy.

In this study, 11 cases were categorized by the present pathological review into mixed-type CCAs. Immunohistochemical results indicated that mixed-type CCAs could be divided into at least four groups accompanied with dysfunction of tumor suppressor genes, PTEN, ARID1A and/or p53, correlated with endometriosis: (1) In mixed-type CCAs coexisting with EAs lacking PTEN function, endometriosis was observed. This type of CCAs may have the characteristics of EAs, indicating intratumoral heterogeneity of CCAs. (2) In mixed-type CCAs coexisting with SAs lacking ARID1A function, endometriosis was observed. Altered expression of Annexin 4 or HNF-1β was found. These characteristics of CCAs were observed in the area of SAs, indicating intratumoral heterogeneity. (3) In mixed-type CCAs coexisting with SAs lacking p53 function, endometriosis was not observed. In these CCAs, ARID1A function was preserved. Altered expression of WT-1 was found in the area of CCAs. These results implied the intratumoral heterogeneity of CCAs showing the characteristics of SAs. (4) In mixed-type CCAs coexisting with SAs lacking both ARID1A and p53 function, endometriosis was not observed. These findings indicated intratumoral heterogeneity of CCAs that exhibited the characteristics of both CCAs and SAs (Fig. 3). These immunohistochemical results demonstrated that mixed-type CCAs containing SAs with altered expression of p53 exhibited different carcinogenesis from other mixed-type CCAs, independent of endometriosis. Furthermore, the rates of altered expression of Annexin 4 and HNF-1β were lower, and expression of WT-1 was higher than in pure-type CCAs. These immunostaining results also led us to infer that mixed-type CCAs have different characteristics from pure-type CCAs.

Conclusion

We revealed that CCAs should be first divided into two subgroups: pure type and mixed type. Furthermore, in clinical practice, we must consider that it is necessary to examine the molecular characteristics of individual CCAs. CCAs must be categorized into three subtypes: pure-type CCAs, CCAs with serous characteristics, and CCAs with other histological characteristics. We must deal with CCAs according to intratumoral heterogeneity, and these findings may be applied to clinical treatment, including chemotherapy. Moreover, we should consider intratumoral

heterogeneity because the difference in expression between ARID1A and p53 can influence the prognosis in pure-type CCAs. A new chemotherapy strategy is necessary, based on the expression of ARID1A and p53, including molecular targeting therapy. Detailed pathological review is important to acknowledge the existence of other histological types of EOAs. To the best of our knowledge, this is the first study to show the immunohistochemical differences between pure-type and mixed-type CCAs, and the first to mention the need for classification of CCAs to apply to appropriate chemotherapy according to clinicopathological heterogeneity.

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Conflict of interest The authors have no conflict of interest.

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Embolization for post-partum rupture of ovarian artery aneurysm: Case report and review

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Abstract

Spontaneous rupture of an ovarian artery aneurysm most commonly presents with abdominal pain in a multiparous woman in the early post-partum period. Aneurysms of the ovarian artery have been reported in the published work very infrequently. In our case, a 31-year-old multiparous woman experienced sudden left lower quadrant abdominal pain on the second post-partum day. Angiography showed rupture of a left ovarian artery aneurysm, which was successfully embolized using gelatin sponge particles. The patient resumed menstruation 3 months after the embolization and concurrently conceived, ultimately giving birth at term without complications. Interventional radiology appears to be a highly safe and effective technique for diagnosis and management of a ruptured ovarian artery aneurysm with minimal risk of impairing subsequent fertility.

Key words: angiography, early post-partum period, fertility, ovarian artery aneurysm, transcatheter arterial embolization.

Introduction

Spontaneous rupture of an ovarian artery aneurysm is extremely rare; 21 cases have been reported in the English-language published work during the past 5 decades. The episodes tend to occur during pregnancy or in the early post-partum period. Here we report a case of spontaneous rupture of the left ovarian artery in the early post-partum period and also review the published reports about this condition. Written consent was obtained from the patient.

Case

A 31-year-old woman, gravida 6, para 4, with a history of cesarean delivery 13 years ago, was transferred to our University Hospital complaining of severe lower abdominal pain 2 days after a vaginal delivery at 39 weeks of gestation. Her pregnancy course had been uneventful. In late pregnancy, her blood count showed a white blood cell count of 11 700/mm^3, hemoglobin 10.7 g/dL, and platelets 270 000/mm^3. She gave birth to a healthy male infant weighing 2958 g in a local clinic, and the placenta was delivered spontaneously. Examination following delivery revealed a minimal tear of the cervix, which was repaired properly. The estimated blood loss was 378 g.

On the second post-partum day, the patient experienced sudden onset of abdominal pain in the left lower quadrant, and she was immediately transferred to our hospital by ambulance. On arrival, she was alert but appeared uncomfortable due to the pain. At the initial physical examination, her vital signs indicated body temperature, 37.1°C; heart rate, 92 b.p.m.; respiratory rate, 16 breaths/min; and blood pressure, 96/56 mmHg. Abdominal examination showed left

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lower quadrant tenderness without rebound. Initial laboratory test results indicated an elevated white blood cell count of 16 700/mm^3 and a decreased hemoglobin level of 7.9 g/dL. Ultrasonographic examination of the abdomen and pelvis demonstrated no intraperitoneal effusion, but an 8-cm-diameter hematoma was visualized inferior to the left kidney. Enhanced computed tomography (CT) of the abdomen and pelvis confirmed a massive hematoma inferior to the left kidney that extended to the left retroperitoneal region (Fig. 1). Tortuous vascular structures were observed beside the hematoma and there was no extravasation.

A reformatted CT scan indicated that the tortuous vessel was the left ovarian artery with an aneurysm formation (Fig. 2). There was no indication of rupture or dehiscence of the uterine scar from the previous cesarean delivery on magnetic resonance imaging (MRI) or ultrasonography, and there were no foci of actual bleeding. Therefore, we selected expectant management.

Six days after delivery, the patient's hemoglobin level decreased to 6.4 g/dL, and she received a blood transfusion. Emergent angiography was undertaken to locate the origin of bleeding. She underwent catheterization through a right femoral approach with 4-Fr catheters (Pig-tail, MIK, Duck head; Medikit) and a 2.5-Fr microcatheter (Renegerd-18; TARGET, Boston Scientific). Angiography revealed the tortuous left ovarian artery with segmental dilatations, suggesting that the ovarian artery aneurysms remained. Although there was no extravasation of the contrast medium, selective embolization of the left ovarian artery using gelatin sponge particles was performed. After embolization, an angiogram showed complete exclusion of the aneurysm, and the patient's anemia stopped progressing. Follow-up study by ultrasound examination revealed a shrinking left retroperitoneal hematoma.

The patient had an uneventful postoperative course and was discharged 14 days after the embolization. Enhanced CT 2 months after embolization demonstrated a small hematoma in the retroperitoneal space, and did not show any other aneurysm or vascular malformation. She resumed menstruation 3 months after the embolization, and she concurrently became pregnant. Twelve months after the embolization, an elective cesarean delivery was performed at 39 weeks of gestation in the local clinic. The post-partum period was uneventful.

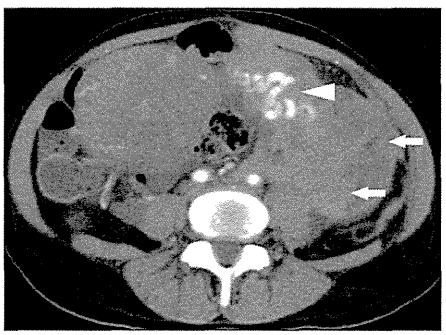

Figure 1 Abdominal computed tomography scan after injection of iodine contrast media on admission at 2 days after delivery. Transverse plane showed a large left retroperitoneal hematoma (arrows). Tortuous vascular structures on the left side of uterus (arrow head) suggested vascular aneurysm. No extravasation was found in this image.

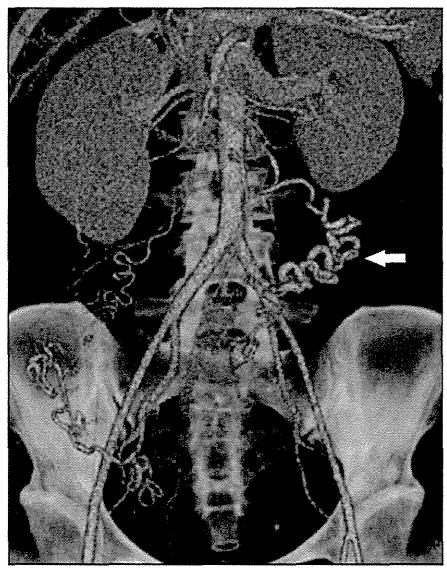

Figure 2 Reformatted computed tomography scan on admission at 2 days after delivery revealed the left torturous ovarian artery with aneurysm formation (arrow).

Discussion

The spontaneous rupture of an ovarian artery aneurysm is extremely rare. Our review of the published work revealed 21 cases of ovarian artery aneurysm dating back to 1963, as shown in Table 1.[1–21] The age of the subjects ranged from 23 to 69 years, and 14 of the 21 cases were associated with pregnancy. In 10 of those 14, the rupture of the ovarian artery aneurysm occurred within the first 5 days after delivery. In our case, the patient had sudden onset of abdominal pain 2 days after delivery, which is consistent with previous reports.

The physiology and anatomy of the cardiovascular system dramatically change during pregnancy. The enlarging uterus induces local fluctuations in blood pressure in the aorta and ovarian arteries, and dilation of the pelvic arteries occurs in conjunction with the increased uterine blood flow. It is widely accepted that involution of the uterus and the return of other genital organs to their prepregnant state in the post-partum period takes 3–4 weeks. Two mechanisms may be involved with the formation of an ovarian artery aneurysm during this time. First, failure of puerperal involution of a segment of ovarian artery after the pregnancy may induce subsequent aneurysm formation.[4] Second, there may be pregnancy-related changes in the vascular walls.[1,3,4] In a combination of animal and human studies, arterial changes were correlated with high levels of circulating steroid hormones during pregnancy.[22] Moreover, all of the 14 previously reported cases that were associated with pregnancy occurred in multiparous or grand multiparous women, just as in our case, suggesting that repeated pregnancy is a risk factor for the development of an ovarian artery aneurysm.

In the present case, the ovarian artery aneurysm during the post-partum period occurred on the left side. However, it is notable that in previous reports, 11 of the 14 cases of aneurysm associated with pregnancy were located in the right ovarian artery. Although the cause of ovarian artery aneurysms during the post-partum period remains poorly understood, there may be a connection to the physiological rotation of the gravid uterus. Post-partum uterine involution induces a dextrorotated uterus to return to the normal prepregnant position, and the associated physiological changes may be involved in the formation of ovarian artery aneurysms on the right side.

The rupture of an ovarian artery aneurysm should be considered in cases of flank pain or abdominal pain in the early post-partum period, and the diagnosis can be confirmed by a reformatted CT scan. Selective angiography is a useful tool both to identify the bleeding from the aneurysm and to manage the bleeding via transcatheter arterial embolization (TAE). In eight of the 21 cases, TAE of ovarian arteries was performed, as shown in Table 1. In two of those eight, the emergent laparotomy was performed following TAE and the ovarian artery was ligated, because embolization was unsuccessful. In our case, the patient was discharged 14 days after TAE with an uneventful recovery. Over the past 3 decades, the role of TAE has evolved from a novel treatment to a major option in the management of obstetric hemorrhage.[23] Interventional radiology has modified this attitude further by offering a minimally invasive alternative of controlling active hemorrhage.

The impact of TAE on fecundity has not been studied in great detail. Several studies have reported cases with uneventful pregnancy outcomes,[24–29] and the fertility of patients who underwent embolization of uterine arteries does not appear to be statistically different from that of the general population.[30] In our case, the patient conceived 3 months after the embolization, and uneventfully gave birth at term. There are no studies evaluating the effect of ovarian artery embolization on ovarian function. However, a follow-up study of nontarget ovarian artery embolization following uterine artery embolization suggests that most patients do not develop ovarian failure.[31] To the best of our knowledge, the present case is the first report of a successful pregnancy following unilateral ovarian artery embolization of a ruptured ovarian artery aneurysm. Larger prospective studies are required to understand fecundity following unilateral or bilateral ovarian artery embolization.

In conclusion, the rupture of an ovarian artery aneurysm should be suspected in a multiparous patient presenting with flank pain or abdominal pain in the early post-partum period. Interventional radiology is an effective technique for both diagnosis and management of a ruptured ovarian artery aneurysm, and it may be the best option for patients who wish to preserve their fecundity.

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Disclosure

The authors have no conflicts of interest related to this article.

Table 1 Reported cases of ruptured ovarian artery aneurysm

	Reference	Year	Age (years)	Obstetric status	Onset day of pregnancy-related case	Side	Treatment	Treatment outcome
1	Caillouette and Owen[1]	1963	29	4G4P	Post-partum day 2	Left	Laparotomy	Success
2	Tsoutsoplides[2]	1967	35	6G3P	Post-partum day 4	Left	Laparotomy	Success
3	Riley[3]	1975	38	6G6P	During delivery	Right	Laparotomy	Success
4	Burnett and Carfrae[4]	1976	32	2G2P	Post-partum day 4	Right	Laparotomy	Success
5	Jafari and Saleh[5]	1977	26	5G4P	Post-partum day 1	Right	Laparotomy	Success
6	Mojab and Rodriguez[6]	1977	23	—	Post-partum month 1	Right	Laparotomy	Success
7	Siu *et al.*[7]	1986	45	6G5P	—	Left	Laparotomy	Success
8	Høgdall *et al.*[8]	1989	31	4G3P	39w of gestation	Right	Laparotomy	Success
9	King[9]	1990	36	5G5P	Post-partum day 4	Right	Embolization	Success
10	Belfort *et al.*[10]	1993	38	3G2P	Post-partum day 0	Right	Laparotomy	Success
11	Guillem *et al.*[11]	1999	38	3G2P	Post-partum day 4	Right	Embolization	Success
12	Blachar *et al.*[12]	2000	38	12G11P	Post-partum day 3	Right	Laparotomy	Success
13	Panoskaltsis *et al.*[13]	2000	37	4P	39w of gestation	Left	Laparotomy	Success
14	Manabe *et al.*[14]	2002	53	—	—	Left	Laparotomy	Success
15	Nakajo *et al.*[15]	2005	55	2G2P	—	Right	Embolization	Success
16	Kale *et al.*[16]	2005	30	5G5P	—	Left	Laparotomy	Success
17	Rathod *et al.*[17]	2005	40	—	Post-partum day 0	Right	Embolization	Success
18	Poilblanc *et al.*[18]	2008	39	5G4P	Post-partum day 5	Right	Embolization	Success
19	Kirk *et al.*[19]	2009	69	3G3P	—	Left	Embolization	Success
20	Tsai and Lien[20]	2009	48	2G2P	—	Left	Embolization and laparotomy	Embolization was unsuccessful
21	Chao and Chen[21]	2009	46	3G2P	—	Left	Embolization and laparotomy	Embolization was unsuccessful
22	Our case	2014	31	6G4P	Post-partum day 2	Left	Embolization	Success

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Radiofrequency thermal treatment with chemoradiotherapy for advanced rectal cancer

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Abstract. We previously reported that patients with a clinical complete response (CR) following radiofrequency thermal treatment exhibit significantly increased body temperature compared with other groups, whereas patients with a clinical partial response or stable disease depended on the absence or presence of output limiting symptoms. The aim of this study was to evaluate the correlation among treatment response, Hidaka radiofrequency (RF) output classification (HROC: termed by us) and changes in body temperature. From December 2011 to January 2014, 51 consecutive rectal cancer cases were included in this study. All patients underwent 5 RF thermal treatments with concurrent chemoradiation. Patients were classified into three groups based on HROC: with ≤9, 10-16, and ≥17 points, calculated as the sum total points of five treatments. Thirty-three patients received surgery 8 weeks after treatment, and among them, 32 resected specimens were evaluated for histological response. Eighteen patients did not undergo surgery, five because of progressive disease (PD) and 13 refused because of permanent colostomy. We demonstrated that good local control (ypCR + CR + CRPD) was observed in 32.7% of cases in this study. Pathological complete response (ypCR) was observed in 15.7% of the total 51 patients and in 24.2% of the 33 patients who underwent surgery. All ypCR cases had ≥10 points in the HROC, but there were no patients with ypCR among those with ≤9 points in the HROC. Standardization of RF thermal treatment was performed safely, and two types of patients were identified: those without or with increased temperatures, who consequently showed no or some benefit, respectively, for similar RF output thermal treatment. We propose that the HROC is beneficial for evaluating the efficacy of RF thermal treatment with chemoradiation for rectal cancer, and the thermoregulation control mechanism in individual patients may be pivotal in predicting the response to RF thermal treatment.

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Key words: rectal cancer, radiofrequency thermal treatment, chemoradiation, Hidaka RF output classification, thermoregulation

Introduction

Hyperthermia has a long history and is widely used in various medical fields (1). Radiofrequency (RF) hyperthermia (HT) has been performed in Japan and is associated with two major issues: i) this modality has not been approved as a standardized treatment in oncology, and ii) there is a risk of a fatal complication, the hot spot phenomenon, which is induced by RF thermal therapy itself (2,3). Many randomized trials of HT have demonstrated a significant improvement in clinical outcome for several tumor types (4-6). However, due to the lack of standardization parameters, and absence of a reference point for this therapy, clinical studies have had contradictory outcomes, thereby raising doubts about efficacy.

Conversely, rectal cancer shows higher local recurrence rates than colon cancer after surgery (7-9). Since the National Comprehensive Cancer Network Practice Guidelines for treatment of primary rectal cancer were specified in 2009, neoadjuvant chemoradiation (NACR) has been accepted as the standard therapy worldwide, except in Japan. Many studies have demonstrated that NACR increases local control but exerts no influence on overall survival (10-12). New strategies that incorporate neoadjuvant therapy are required for rectal cancer.

We reported that hyperthermo-chemoradiotherapy (HCRT) for rectal cancer is performed safely (13). The main endpoint of this study was the evaluation of the pathological and clinical responses after HCRT using the Hidaka RF output classification (HROC: termed by us).

Materials and methods

Between December 2011 and January 2014, 51 consecutive patients with primary rectal cancers were included in this

study. Patients received pre-treatment and post-treatment diagnostic examinations, including computed tomography (CT), positron emission tomography/CT (PET/CT), and magnetic resonance imaging (MRI), at Hidaka Hospital. The extent and location of the tumor were classified according to the tumor-node-metastasis TNM staging (14). Patients underwent HCRT at Hidaka Hospital. Operations were performed at the Department of General Surgical Science, Gunma University, or at the Division of Surgery, Hidaka Hospital. Each resected specimen was evaluated histologically at the Department of Pathology, Gunma University. The study was approved by the Ethics Committees of the Hidaka Hospital and Gunma University. Each patient gave written informed consent prior to enrollment in the study.

Chemoradiotherapy. Intensity-modulated radiotherapy was administered conventionally once daily 5 times/week using TomoTherapy® (Hi-Art® treatment system; Accuray). Neoadjuvant radiotherapy (NART) consisted of 50 Gy delivered to the posterior pelvis in 25 fractions of 2 Gy each. Concurrent neoadjuvant chemotherapy was delivered in 5-day courses during the first to fifth weeks of NART. Capecitabine was administered orally at a dose of 1,700 mg/m^2/day.

Hyperthermia. RF thermal treatment was performed using the Thermotron-RF 8 (Yamamoto Vinita Co., Ltd., Japan) and administered once a week for 5 weeks with a 50-min irradiation. From December 2011 to November 2012, 19 patients underwent abdominal hyperthermia treatment and the RF output was retrospectively evaluated and from November 2012 to January 2014, 32 patients prospectively received a standardized increasing output method (which we termed neothermia) based on retrospective data. Details of the method for increasing output have been reported previously (15). Briefly, group A included patients with a thickness of fat of the abdominal wall <16 mm, visceral fat area <100 cm^2 and total fat area <190 cm^2, and group B included patients with either one of the aforementioned factors. For patients in group A, the output was increased to 50 W/min, whereas patients in group B received 25 W/min. The operator started the output from 200 W and increased to 1,200 W until output limiting symptoms occurred and then decreased the output by 100 W. Most patients did not complain and continued the first RF thermal treatment. Subtracting 100 W output was judged as the optimal energy output dose without output limiting symptoms. From the second to fifth RF thermal treatment, this output was applied for 50 min. These principles were maintained in patients with neothermia in this prospective study.

Thermal output. A sensor catheter with 4 temperature points was placed in the rectum of 12 patients while it was attached to the skin on the lateral abdominal side, as well as in 39 patients who received neothermia and in 7 who did not. The accumulated thermal output was calculated from the estimated internal temperature of patients during the 50-min duration of each irradiation. An increased thermometric scale of the skin and the rectum was added to the pretreatment axillary temperature of the patients to obtain a hypothetical internal body temperature. Temperature and output curves were recorded at 1-min intervals from treatment initiation to completion (50 min).

Table I. Patient characteristics.

Characteristics	Data
Total no. of patients	51
Age (years)	
Median	62
Range	33-89
Gender, n (%)	
Female	13 (25.5)
Male	38 (74.5)
Stoma, n (%)	
(-)	41 (83.7)
(+)	8 (16.3)
Tumor location, n (%)	
Ra	5 (9.8)
Rb	30 (58.8)
RbP	15 (29.4)
P	1 (2.0)
Primary tumor, n (%)	
T2	9 (17.6)
T3	36 (70.6)
T4	6 (11.8)
Regional lymph node status, n (%)	
N(-)	30 (58.8)
N(+)	21 (41.2)
Distant metastasis, n (%)	
M0	46 (90.2)
M1	5 (9.8)
TNM stage[a], n (%)	
Stage 1	7 (13.7)
Stage 2	21 (41.2)
Stage 3	18 (35.3)
Stage 4	5 (9.8)
Tumor differentiation, n (%)	
Well differentiated	27 (52.9)
Moderately different	21 (41.2)
Poorly differentiated	3 (5.9)
A-V distance (cm)	
Median	3.0
Average (± SE)	2.70 (0.33)

[a]Pretreatment tumor staging was clinical, if available, by CT and MR.

RF output. Details of the HROC have been reported previously (15). Briefly, the total accumulated irradiation output (W/min) was classified into four groups: ≤26,000, 260,001-32,600, 32,601-39,500, and ≥39,501, as 1 point, 2 points, 3 points, and 4 points, respectively. The HROC was further classified into three groups: ≤9, 10-16, and ≥17 points, which were the sum of the five treatments.

Evaluation of objective response. All patients were evaluated according to the Response Evaluation Criteria in Solid