8. Kinoshita T, Nakajima T, Ohashi Y; National Surgical Adjuvant Study Group for Gastric Cancer. Adjuvant chemotherapy with uracil-tegafur (UFT) for serosa negative advanced gastric cancer: results of a randomized trial by national surgical adjuvant study of gastric cancer. J Clin Oncol 2005;23(16 Suppl):313S.

9. Nakajima T, Kinoshita T, Nashimoto A, Sairenji M, Yamaguchi T, Sakamoto J, et al. Randomized controlled trial of adjuvant uracil-tegafur versus surgery alone for serosa-negative, locally advanced gastric cancer. Br J Surg 2007;94:1468-76.

10. Akasu T, Moriya Y, Ohashi Y, Yoshida S, Shirao K, Kodaira S, et al. Adjuvant chemotherapy with uracil-tegafur for pathological stage III rectal cancer after mesorectal excision with selective lateral pelvic lymphadenectomy: a multicenter randomized controlled trial. Jpn J Clin Oncol 2006;36:237-44.

11. Noguchi S, Koyama H, Uchino J, Abe R, Miura S, Sugimachi K, et al. Postoperative adjuvant therapy with tamoxifen, tegafur plus uracil, or both in women with node-negative breast cancer: a pooled analysis of six randomized controlled trials. J Clin Oncol 2005;23:2172-84.

12. Yamamoto K, Izumi R, Hasegawa K, Nakajima H, Ohashi K, Kudo R, et al. Adjuvant oral 5-fluorouracil for cervical cancer: Japanese Gynecologic Oncology Group report. Int J Oncol 2004;24:1175-9.

13. Ho DH, Pazdur R, Covington W, Brown N, Huo YY, Lassere Y, et al. Comparison of 5-fluorouracil pharmacokinetics in patients receiving continuous 5-fluorouracil infusion and oral uracil plus N1-(2'-tetrahydrofuryl)-5-fluorouracil. Clin Cancer Res 1998;4:2085-8.

14. Taguchi T, Ikeda Y, Mikami Y, Kawai S, Arai Y, Mochimatsu I, et al. Combined radiotherapy and chemotherapy with carboplatin and UFT for head and neck squamous cell carcinoma. Anticancer Res 2003;23(1B):713-7.

15. Ichinose Y, Yano T, Asoh H, Yokoyama H, Maruyama R, Ushijima C, et al. UFT plus cisplatin with concurrent radiotherapy for locally advanced non-small-cell lung cancer. Oncology (Williston Park) 1999;13(7 Suppl 3):98-101.

16. Cellier P, Leduc B, Martin L, Vie B, Chevelle C, Vendrely V, et al. Phase II study of preoperative radiation plus concurrent daily tegafur-uracil (UFT) with leucovorin for locally advanced rectal cancer. BMC Cancer 2011;11:98.

17. Lorvidhaya V, Chitapanarux I, Sangruchi S, Lertsanguansinchai P, Kongthanarat Y, Tangkaratt S, et al. Concurrent mitomycin C, 5-fluorouracil, and radiotherapy in the treatment of locally advanced carcinoma of the cervix: a randomized trial. Int J Radiat Oncol Biol Phys 2003;55:1226-32.

18. Duenas-Gonzalez A, Zarba JJ, Patel F, Alcedo JC, Beslija S, Casanova L, et al. Phase III, open-label, randomized study comparing concurrent gemcitabine plus cisplatin and radiation

followed by adjuvant gemcitabine and cisplatin versus concurrent cisplatin and radiation in patients with stage IIB to IVA carcinoma of the cervix. J Clin Oncol 2011;29:1678-85.

19. Tanaka F, Yanagihara K, Otake Y, Miyahara R, Wada H. Angiogenesis and the efficacy of postoperative administration of UFT in pathologic stage I non-small cell lung cancer. Cancer Sci 2004;95:371-6.

20. Tanaka F, Otake Y, Yanagihara K, Kawano Y, Miyahara R, Li M, et al. Correlation between apoptotic index and angiogenesis in non-small cell lung cancer: comparison between CD105 and CD34 as a marker of angiogenesis. Lung Cancer 2003;39:289-96.

21. Tanaka F, Otake Y, Yanagihara K, Yamada T, Miyahara R, Kawano Y, et al. Apoptosis and p53 status predict the efficacy of postoperative administration of UFT in non-small cell lung cancer. Br J Cancer 2001;84:263-9.

22. Nakagawa T, Tanaka F, Takata T, Matsuoka K, Miyahara R, Otake Y, et al. Predictive value of dihydropyrimidine dehydrogenase expression in tumor tissue, regarding the efficacy of postoperatively administered UFT (Tegafur + Uracil) in patients with p-stage I nonsmall-cell lung cancer. J Surg Oncol 2002;81:87-92.

23. Nakano J, Huang C, Liu D, Masuya D, Nakashima T, Yokomise H, et al. Evaluations of biomarkers associated with 5-FU sensitivity for non-small-cell lung cancer patients postoperatively treated with UFT. Br J Cancer 2006;95:607-15.

24. Nagai N, Mukai K, Hirata E, Jin HH, Komatsu M, Yunokawa M. UFT and its metabolite gamma-butyrolactone (GBL) inhibit angiogenesis induced by vascular endothelial growth factor in advanced cervical carcinoma. Med Oncol 2008;25:214-21.

Table 1. Summary of patient characteristics and treatment of cervical cancer

Characteristic	Control group (n=147)	UFT group (n=162)
Median age (yr)	62.0±14.1	61.0±14.1
FIGO stage		
I	58 (39.5)	64 (39.5)
II	37 (25.2)	42 (25.9)
III	40 (27.2)	39 (24.1)
IV	12 (8.1)	17 (10.5)
Histological type		
Squamous cell carcinoma	134 (91.2)	133 (82.1)
Adenocarcinoma	9 (6.1)	17 (10.5)
Adenosquamous carcinoma	4 (2.7)	6 (3.7)
Undifferentated carcinoma	-	1 (0.6)
Others	-	5 (3.1)
Primary treatment		
Radiotherapy alone	65 (44.2)	58 (35.8)
Surgery alone	41 (27.9)	43 (26.5)
Surgery/radiotherapy	29 (19.7)	26 (16.0)
Surgery/radiotherapy/chemotherapy	6 (4.1)	14 (8.6)
Radiotherapy/chemotherapy	4 (2.7)	11 (6.8)
Surgery/chemotherapy	2 (1.4)	10 (6.3)

Values are presented as mean±SD or number (%). Chemotherapy means cispatin based therapy, not included the oral administration of UFT. Chemotherapy regimens were given to patients before the oral administration of UFT.UFT, tegafur-uracil; FIGO, International Federation of Gynecology and Obstetrics.

Table 2. Efficacy of UFT administration in the patients with cervical cancer

Variable	Overall survival rate (%)		
	Control group	UFT group	p-value
All patients	60.8	73.8	0.049
FIGO stage			
I	88.9	91.5	0.665
II	46.7	71.3	0.644
III	34.9	62.1	0.012
IV	20.8	35.3	0.318
Histologic type			
Squamous cell carcinoma	60.7	74.1	0.062
Adenocarcinoma	85.7	80.6	0.764
Adenosquamous carcinoma	25.0	62.5	0.290
Primary treatment			
Radiotherapy alone	48.7	64.3	0.068
Surgery alone	94.7	92.7	0.746
Surgery/radiotherapy	53.5	82.7	0.193

The effect of UFT administration on overall survival rate was analyzed according to FIGO staining, histological type, and primary treatment. A p-value between patients with and without UFT administration.UFT, tegafur-uracil; FIGO, International Federation of Gynecology and Obstetrics.

Table 3. Adverse events in the UFT group

Toxicity	Grade				
	1	2	3	4	Unknown
Hematological adverse events					
Leukopenia/neutropenia	1	6	1	0	0
Thrombocytopenia	0	1	0	0	0
Anemia	0	1	0	0	0
Elevation of serum transaminases	2	2	0	0	3
Non-hematological adverse events					
Nausea/vomiting	12	2	2	0	1
Loss of appetite	7	3	4	0	0
Diarrhea	3	3	1	0	0
Abdominal discomfort	2	0	0	0	0
Abdominal pain	1	1	1	0	0
Rash	4	0	0	0	0
Skin/nail pigmentation	3	2	0	0	0
Stomatitis	2	0	0	0	1
Itching	1	0	0	0	0
Tremor	1	1	0	0	0
Dysgeusia	3	0	0	0	0
General fatigue	0	0	0	0	1
Bloody stool	0	0	0	0	1
Total	42	22	9	0	7

UFT, tegafur-uracil.

Fig.1A

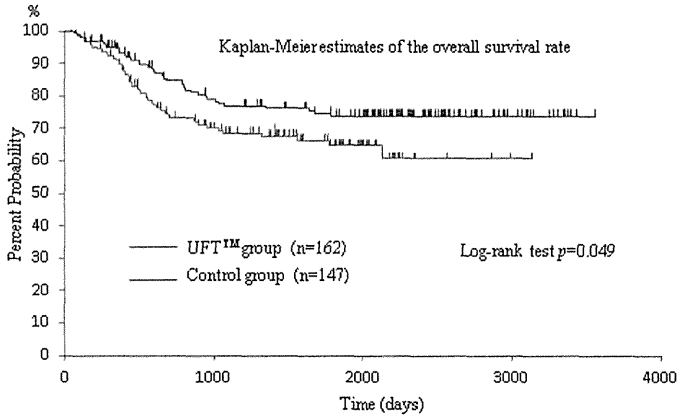


Fig.1B

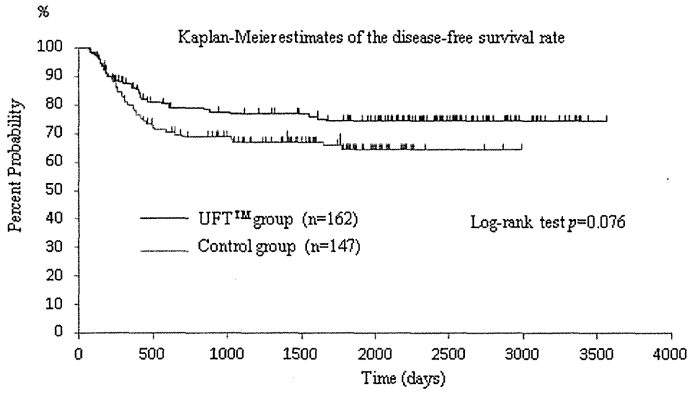


Fig. 1. Survival curve among 309 patients with uterine cervical cancer in the tegafur-uracil (UFT) and the control group. (A) Overall survival (p=0.049). (B) Disease-free survival (p=0.076).

Fig.2A

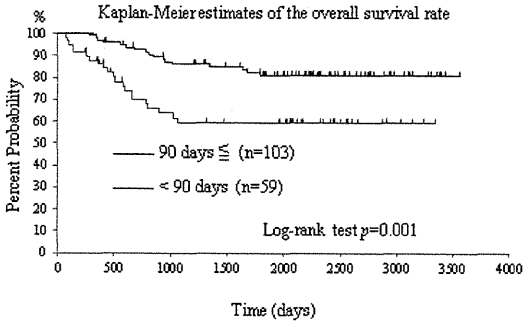


Fig.2B

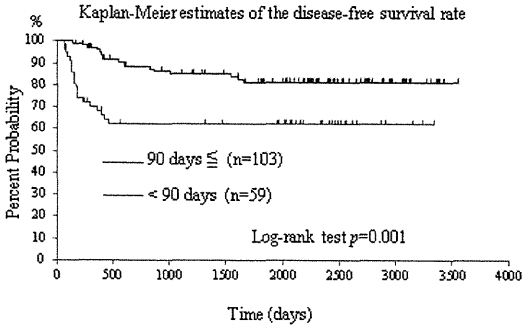


Fig. 2. Among the tegafur-uracil (UFT)-treated patients, those who received the drug for ≥90 days had significantly higher survival and disease-free rates than those received the drug for <90 days. (A) Overall survival (p=0.001). (B) Disease-free survival (p=0.001).

Supplementary Table 1. Efficacy of UFT administration in the patients with cervical cancer

Variable	Disease-free survival (%)		
	Control group	UFT group	p-value
All patients	59.8	68.5	0.076
FIGO stage			
I	89.2	91.6	0.661
II	61.6	71.6	0.855
III	38.3	62.5	0.026
IV	10.4	37.6	0.204
Histologic type			
Squamous cell carcinoma	64.6	75.2	0.083
Adenocarcinoma	85.7	80.8	0.694
Adenosquamous carcinoma	25.0	62.5	0.242
Primary treatment			
Radiotherapy alone	48.9	65.6	0.082
Surgery alone	94.9	92.7	0.701
Surgery/radiotherapy	59.5	83.2	0.093

The effect of UFT administration on disease free survival rate was analyzed according to FIGO staining, histological type, and primary treatment. A p-value between patients with and without UFT administration.UFT, tegafur-uracil; FIGO, International Federation of Gynecology and Obstetrics.

Supplementary Table 2. Efficacy of long-term oral administration of UFT in the patients with cervical cancer

Variable	Overall survival rate (%)		p-value
	Administration period <90 days	Administration period ≥90 days	
All patients	59.6	81.4	0.001
FIGO stage			
I	84.4	94.9	0.130
II	54.8	79.8	0.072
III	55.3	66.8	0.334
IV	17.1	57.1	0.001
Histologic type			
Squamous cell carcinoma	59.6	81	0.003
Adenocarcinoma	71.4	87.5	0.416
Adenosquamous carcinoma	66.7	50.0	0.081
Primary treatment			
Radiotherapy alone	44.7	74.9	0.010
Surgery alone	88.9	93.6	0.594
Surgery/radiotherapy	87.5	80.4	0.759

We compared the overall survival rate in the patients who received the drug for ≥90 days with those who received the drug for <90 days. A p-value between patients received the drug for 90 days or more, and for less than 90 days.

UFT, tegafur-uracil; FIGO, International Federation of Gynecology and Obstetrics.

Supplementary Table 3. Efficacy of long-term oral administration of UFT in the patients with cervical cancer

Variable	Disease-free survival (%)		p-value
	Administration period <90 days	Administration period ≥90 days	
All patients	62.2	81.4	0.001
FIGO stage			
I	84.4	94.9	0.134
II	58.7	79.3	0.063
III	61.1	64.8	0.467
IV	17.1	60	<0.001
Histologic type			
Squamous cell carcinoma	62.2	81	0.003
Adenocarcinoma	71.4	87.5	0.361
Adenosquamous carcinoma	66.7	66.7	0.715
Primary treatment			
Radiotherapy alone	49.1	75.3	0.006
Surgery alone	88.9	93.5	0.594
Surgery/radiotherapy	88.9	80.8	0.761

We compared the desease free survival rate in the patients who received the drug for ≥90 days with those who received the drug for <90 days. A p-value between patients received the drug for 90 days or more, and for less than 90 days.

UFT, tegafur-uracil; FIGO, International Federation of Gynecology and Obstetrics.

Fig.S1

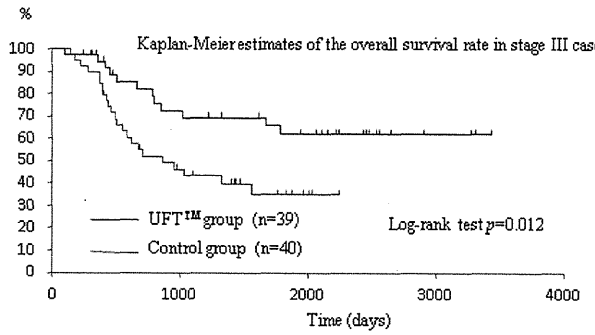

%
Kaplan-Meier estimates of the overall survival rate in stage III cases
100
90
80
70
60
50
40
30
20
10
0
UFT™ group (n=39)
Control group (n=40)
Log-rank test p=0.012
0
1000
2000
3000
4000
Time (days)

Fig.S2

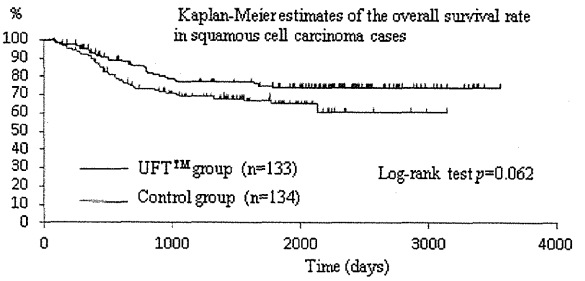
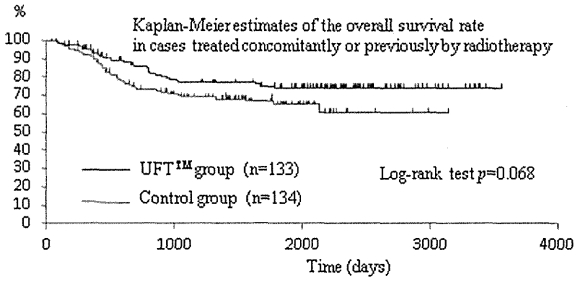

%
Kaplan-Meier estimates of the overall survival rate
in squamous cell carcinoma cases
100
90
80
70
60
50
40
30
20
10
0
UFT™ group (n=133)
Control group (n=134)
Log-rank test p=0.062
0
1000
2000
3000
4000
Time (days)

Fig.S3



Supplementary Fig. 1. Survival curve among 309 patients with uterine cervical cancer in the UFT and the control group. (A) The overall survival rate in stage III cases (p=0.012). (B) The overall survival rate in cases of squamous cell carcinoma (p=0.062). (C) The overall survival rate in cases that were treated concomitantly or previously by radiotherapy (p=0.068).

Nutrition 31 (2015) 409–412

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Case report

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

Junko Nakao M.D. *, Takashi Ohba M.D., Ph.D., Kiyomi Takaishi M.D., Ph.D., Hidetaka Katabuchi M.D., Ph.D.

Department of Obstetrics and Gynecology, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

ARTICLE INFO

Article history:
Received 4 July 2014
Accepted 3 September 2014

Keywords:
Pregnancy
Hypertriacylglycerolemia
Pancreatitis
EPA
Second trimester

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.

* Corresponding author. Tel.: +81 96 373 5269; fax: +81 96 363 5164 008-651-085-869-236.
E-mail address: amethyst_j_0229@yahoo.co.jp (J. Nakao).

http://dx.doi.org/10.1016/j.nut.2014.09.006
0899-9007/

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 w1 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

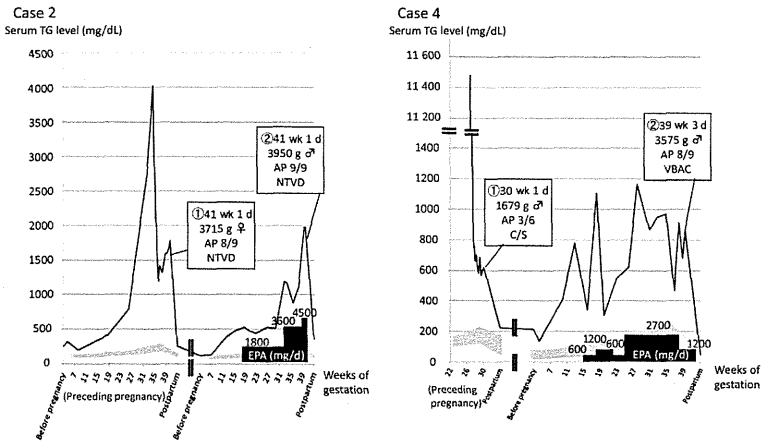


Fig. 1. Clinical presentation of hypertriacylglycerolemia during pregnancy for cases 2 and 4. The gray shaded area indicates the normal range of serum triacylglycerol. AP, Apgar score; NTVD, normal transvaginal delivery; C/S, cesarean section; TG, triacylglycerol; VBAC, vaginal birth after cesarean.

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.

References

[1] Fahraeus L, Larsson-Cohn U, Wallentin L. Plasma lipoproteins including high density lipoprotein subfractions during normal pregnancy. Obstet Gynecol 1985;66:468–72.
[2] Papadakis EP, Sarigianni M, Mikhailidis DP, Mamopoulos A, Karagiannis V. Acute pancreatitis in pregnancy: an overview. Eur J Obstet Gynecol Reprod Biol 2011;159:261–6.
[3] Kaplan NM. The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Arch Intern Med 1989;149:1514–20.
[4] Lloret Linares C, Pelletier AL, Czernichow S, Vergnaud AC, Bonnefont-Rousselot D, Levy P, et al. Acute pancreatitis in a cohort of 129 patients referred for severe hypertriglyceridemia. Pancreas 2008;37:13–8.
[5] Crisan LS, Steidl ET, Rivera-Alsina ME. Acute hyperlipidemic pancreatitis in pregnancy. Am J Obstet Gynecol 2008;198:e57–9.
[6] Takaishi K, Miyoshi J, Matsumura T, Honda R, Ohba T, Katabuchi H. Hypertriglyceridemic acute pancreatitis during pregnancy: prevention with diet therapy and omega-3 fatty acids in the following pregnancy. Nutrition 2009;25:1094–7.
[7] Ido Y, Gushima R, Ozaki T, Maki Y, Nonaka K, Kaku E, et al A case of severe acute pancreatitis with hyperlipidemia in a pregnant woman. Nihon Shokakibyo Gakkai Zasshi 2012;109:1236–42.
[8] Eddy JJ, Gideonsen MD, Song JY, Grobman WA, O'Halloran P. Pancreatitis in pregnancy. Obstet Gynecol 2008;112:1075–81.
[9] Eddy J, Gideonsen M. Pancreatitis in pregnancy: a 10 year retrospective of 15 midwest hospitals. NIH Public Access 2008;112:1075–81.
[10] Briggs GG, Freeman RK, Yaffe SJ, editors. FENOFIBRATE. Drugs in pregnancy and lactation. 9th ed. Philadelphia,PA: Wolters Kluwer; 2011.
[11] Sivakumaran P, Tabak SW, Gregory K, Pepkowitz SH, Klapper EB. Management of familial hypertriglyceridemia during pregnancy with plasma exchange. J Clin Apheresis 2009;24:42–6.
[12] Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet 1978;2:117–9.
[13] Sato S, Ohkuchi A, Kawano M, Iwanaga N, Furukawa Y, Matsumoto H. Effect of eicosapentaenoic acid agent on aggravated hypertriglyceridemia during pregnancy. J Obstet Gynaecol Res 2013;39:1541–4.
[14] Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, et al. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulat 2006;113:195–202.

Med Mol Morphol
DOI 10.1007/s00795-014-0090-z

ORIGINAL PAPER

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

Yuji Matsuo · Hironori Tashiro · Hiroyuki Yanai · Takuya Moriya · Hidetaka Katabuchi

Received: 25 August 2014 / Accepted: 15 October 2014

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

Y. Matsuo · H. Tashiro · H. Katabuchi (✉)
Department of Obstetrics and Gynecology, Faculty of Life Sciences, Kumamoto University, Honjo 1-1-1, Chuou-ku, Kumamoto 860-8556, Japan
e-mail: buchi@kumamoto-u.ac.jp

H. Yanai
Department of Pathology, Okayama University Hospital, Okayama, Japan

T. Moriya
Department of Pathology 2, Kawasaki Medical School, Kurashiki, Japan

Published online: 15 November 2014

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