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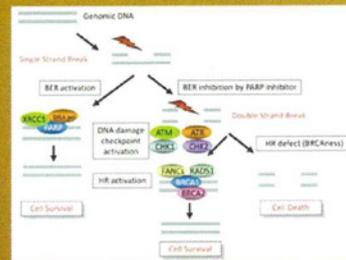
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Poly(ADP-ribose) polymerase (PARP) is required for base excision repair (BER). When homologous recombination (HR)-defective cells are treated with PARP inhibitors, the subsequent inhibition of both BER and HR leads to "synthetic lethality", resulting in the generation of unrepaired DNA single-strand breaks, accumulation of double-strand breaks, collapsed replication forks and eventual cell death. See page 424.

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Association between carotenoids and outcome of cervical intraepithelial neoplasia: a prospective cohort study

Takuma Fujii · Naoyoshi Takatsuka · Chisato Nagata · Koji Matsumoto · Akinori Oki · Reiko Furuta · Hiroo Maeda · Toshiharu Yasugi · Kei Kawana · Akira Mitsuhashi · Yasuo Hirai · Tsuyoshi Iwasaka · Nobuo Yaegashi · Yoh Watanabe · Yutaka Nagai · Tomoyuki Kitagawa · Hiroyuki Yoshikawa

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Abstract

Background It has been suggested that micronutrients such as alpha-tocopherol, retinol, lutein, cryptoxanthin, lycopene, and alpha- and beta-carotene may help in the prevention of cervical cancer. Our aim was to investigate whether serum concentrations and/or dietary intake of

micronutrients influence the regression or progression of low-grade cervical abnormalities.

Methods In a prospective cohort study of 391 patients with cervical intraepithelial neoplasia (CIN) grade 1–2 lesions, we measured serum micronutrient concentrations in addition to a self-administered questionnaire about dietary intake. We evaluated the hazard ratio (HR) adjusted for CIN grade, human papillomavirus genotype, total energy intake and smoking status.

The Japan HPV and Cervical Cancer (JHACC) Study Group.

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Results In non-smoking regression subjects, regression was significantly associated with serum levels of zeaxanthin/lutein (HR 1.25, 0.78–2.01, $p = 0.024$). This benefit was abolished in current smokers. Regression was inhibited by high serum levels of alpha-tocopherol in smokers ($p = 0.042$). In progression subjects, a significant protective effect against progression to CIN3 was observed in individuals with a medium level of serum beta-carotene [HR 0.28, 95 % confidence interval (CI) 0.11–0.71, $p = 0.007$], although any protective effect from a higher level of serum beta-carotene was weaker or abolished (HR 0.52, 95 % CI 0.24–1.13, $p = 0.098$). Increasing beta-carotene intake did not show a protective effect (HR 2.30, 95 % CI 0.97–5.42, $p = 0.058$).

Conclusions Measurements of serum levels of carotenoids suggest that regression is modulated by smoking status. Maintaining a medium serum level of beta-carotene has a protective effect for progression; however, carotene intake is not correlated with serum levels of carotenoids.

Keywords Human papillomavirus · Cervical intraepithelial neoplasia · Low-grade squamous intraepithelial lesion · Micronutrients · Carotenoids

Introduction

Persistent infection with human papillomavirus (HPV) may potentially lead to the development of cervical cancer. Most women are exposed to at least one type of genital HPV in their lifetime [1]. HPV infections often cause cervical intraepithelial neoplasia 1 (CIN1) [2]. Only a subset of individuals with CIN1 progress to CIN3 or invasive cervical cancer, suggesting that environmental cofactors are related to cervical carcinogenesis [3–5]. Numerous environmental candidates such as oral contraceptives, parity, smoking status, micronutrient status, nutrient intake, *Chlamydia trachomatis* infection and herpes simplex virus type 2 infection have been investigated as potential cofactors related to progression of CIN.

Much attention has been given to the role of dietary factors and serum micronutrients in the etiology of cervical cancer and CIN. Carotenoids and tocopherols are lipid-soluble micronutrients with potent antioxidant activities and modulatory effects on immunity. Recent publications have reported that the association of carotenoids and tocopherols with reduced risk has not been observed consistently [6–10]; however, these inconsistent results may be due to the study designs. Furthermore, the majority of case-control studies of the associations between micronutrients and outcome of CIN were conducted to assess either dietary intake or circulating micronutrients only [7–9, 11].

Foods are composites of several biologically active dietary components. Micronutrients in foods, as well as other possible anti-carcinogenic compounds such as detoxification enzymes, may have synergistic effects and interact with one another [11–13]. A recent multi-center cohort study reported an association between dietary intake of micronutrients and outcome of CIN. However, this study reported no information about circulating micronutrients [6]. Conversely, some prospective cohort studies reported an association between circulating micronutrient levels and outcome of CIN but no information about dietary intake [14, 15]. Both dietary intake and circulating serum concentrations of micronutrients are important in assessing the role of micronutrients in cervical carcinogenesis. We previously conducted a case-control study including 156 pairs of women with CIN1–3 and matched controls with normal cytology and found an inverse relationship between serum levels of alpha-carotene, lycopene and zeaxanthin/lutein and the risk of CIN development [16]. Because retrospective analysis of previous study findings provides only limited information, we report here the results of a prospective study that was conducted in an attempt to confirm these findings.

Materials and methods

Study design

We used follow-up data from the Japan HPV and Cervical Cancer Study, a prospective non-intervention cohort study conducted to identify determinants of low-grade squamous intraepithelial lesion (LSIL)/CIN regression and progression. Among a total of 570 study subjects with low-grade cervical abnormalities (cytological LSIL and histological CIN1/2) recruited from nine hospitals between 1998 and 2004, 391 women with data concerning serum micronutrients and complete entry questionnaires were enrolled in the present study. Details of the design, methods and primary results have been provided elsewhere [17, 18]. Participants entered the study only after voluntarily giving signed, informed consent. The subjects were routinely followed at 3- to 4-month intervals and received cytology and colposcopy examinations at each visit. To avoid interference of the biopsy procedure on the natural course of the disease, cervical biopsy was performed only when women had HSIL smears and major colposcopic changes that were suggestive of progression to CIN3 or worse. Progression was defined as histological CIN3 lesions or worse, diagnosed on central pathology review. We defined regression as at least two consecutive negative smears and normal colposcopy. Women were regarded as having persistent lesions when they did not have either regression or

progression over the period of follow-up. At enrollment, study subjects were tested for cervical HPV-DNA and circulating serum micronutrients. Information about smoking and dietary intake was obtained from a self-administered questionnaire. Participants were not obliged to answer the questionnaire and their participation was unrelated to their clinical evaluation, treatment or follow-up evaluation. The simplified diet history questionnaire used in the current study had been developed and validated previously [19]. Originally, a prototype diet history questionnaire including 169 traditional Japanese foods and dishes was developed. To alleviate the participants' burden, our simplified diet history questionnaire was developed to employ a stepwise regression method to select from the 169 diet history questionnaire items. This simplified questionnaire was composed of 14 categories: (1) dishes of meat and vegetables; (2) meat (without dishes including vegetables); (3) fish; (4) cereals; (5) eggs and soybean products; (6) vegetables; (7) seaweed; (8) juice; (9) fruits; (10) milk and dairy products; (11) desserts and snacks; (12) pickles; (13) seasoning; and (14) alcoholic beverages. Supplement use was not assessed in this study because of a lack of complete information regarding availability. Because it was impossible to distinguish between intake of alpha- and beta-carotene from the questionnaire, total carotene intake was described. Questions on smoking habits included status (never, former or current smoker) and intensity (number of cigarettes smoked per day).

Circulating micronutrients

Blood was collected in foil-wrapped glass tubes without heparin. Serum was separated by centrifugation at $1,000\times g$ for 10 min and stored in the dark at $-70\text{ }^{\circ}\text{C}$ prior to sample preparation. Serum levels of retinol, alpha-tocopherol and various carotenoids were determined by a high-pressure liquid chromatography method described previously [21].

Statistical analysis

The association between smoking status and nutrient intake was analyzed by one-way analysis of variance. The association between smoking status and serum micronutrients was analyzed by analysis of covariance. The data were adjusted for age, body mass index (BMI) and alcohol intake frequency. For regression or progression, time to event was measured from the date of the index visit to the date of the visit at which cytological transition to normal or CIN3 was first detected. To estimate the association between the CIN outcomes and circulating serum micronutrients, serum micronutrient tertiles were examined.

Hazard ratios (HRs) and 95 % confidence intervals (CIs) for each tertile with reference to the lowest tertile were calculated using a proportional hazard model. For nutrient intake, identical estimation was conducted. The Brinkman Index (BI) was calculated by multiplying the average number of cigarettes smoked per day by the smoking years. We detected HPV-DNA in exfoliated cervical cells by a PCR-based methodology described previously [20]. HPV DNA was amplified by PCR using consensus-primers (L1C1/L1C2 + L1C2M) for the HPV L1 region. HPV genotypes were identified by a restriction fragment-length polymorphism (RFLP) PCR method that has been shown to identify at least 26 genotypes of genital HPV [18]. HRs were adjusted for potential confounders, including CIN grade, HPV genotype, age, total energy intake and smoking status. Statistical analyses were performed using Stata statistical software, release 11.1 (Stata Corporation; College Station, TX, USA).

Results

Of the 570 women enrolled in the parent study, 391 met the eligibility requirements of the current study for tests of serum micronutrients and completion of entry questionnaires. Of these, 329 and 62 women were diagnosed as CIN1 and CIN2, respectively. The mean age of the women was 36.3 years (median 36.0, range 19–54). Of the 391 women, regression, persistence and progression occurred in 218, 135 and 38, respectively.

Influence of smoking status on circulating levels and intake of micronutrients

At enrollment, 190 women had never smoked, while 142 women were current smokers (BI >100). Data from three women were lost and the remaining 56 women were past smokers. We found a 22 and 10 % decrease in carotene and vitamin E intake in current smokers compared with non-smokers, respectively (Table 1). Among the three groups, there was a significant difference in the intake of fiber, calcium, carotenes, vitamin A, vitamin C and vitamin E. As shown in Table 2, current smokers had significantly lower serum levels of alpha-carotene, beta-carotene and cryptoxanthin compared with non-smokers. Smokers had marginally lower levels of lycopene. Retinol, zeaxanthin/lutein and alpha-tocopherol were not related to smoking status.

The effects of serum micronutrients and nutrient intake in regression subjects

Significantly more inhibition of regression was observed in women in the middle tertiles of serum alpha-tocopherol

Table 1 Relationship between estimated daily nutrient intake and tobacco smoking status

Nutrient intake per day	Non smokers (N = 190)		Past smokers (N = 56)		Current smokers (N = 142)		p value
	Mean	SD	Mean	SD	Mean	SD	
Total energy intake (kcal)	2,220.1	576.1	2,221.6	679.7	2,149.1	574.9	0.520
Protein intake (g)	85.2	26.2	85.2	31.0	79.4	27.3	0.127
Fat intake (g)	60.2	21.9	62.9	27.2	59.0	22.6	0.566
Carbohydrate intake (g)	329.5	78.3	325.2	85.6	315.2	74.6	0.255
Fiber intake (g)	5.3	1.9	5.2	2.0	4.6	1.8	0.004
Calcium intake (mg)	740.8	292.2	738.3	337.6	620.9	274.2	0.001
Retinol intake (µg)	284.6	219.1	302.4	176.9	331.2	624.7	0.597
Carotene intake (µg)	4,943.5	2,439.7	4,856.3	2,532.1	3,866.8	2,083.5	0.000
Vitamin A intake (IU)	3,430.6	1,587.5	3,424.3	1,546.9	2,954.2	2,197.4	0.049
Vitamin C intake (mg)	134.0	65.6	133.3	65.9	113.4	56.4	0.008
Vitamin D intake (IU)	76.4	48.8	69.3	40.6	66.9	53.7	0.213
Vitamin E intake (mg)	8.4	2.8	8.3	3.2	7.5	2.7	0.021
Salt intake (g)	13.5	4.1	13.7	4.8	12.8	4.5	0.291
Cholesterol intake (mg)	323.7	122.6	322.9	160.2	304.7	137.5	0.412

Analysis of variance was used to examine the differences in the mean values of factors among groups
SD standard deviation

Table 2 Relationship between serum micronutrients and tobacco smoking status

	Non-smoker (N = 190)		Past smoker (N = 56)		Current smoker (N = 142)		P value
	Adjusted mean	95 % CI	Adjusted mean	95 % CI	Adjusted mean	95 % CI	
Serum retinol (µg/dL)	59.23	56.42–62.04	59.70	54.59–64.81	60.88	57.24–64.51	0.695
Serum α-carotene (µg/dL)	9.70	8.58–10.82	7.47	5.43–9.51	7.23	5.78–8.68	0.003
Serum β-carotene (µg/dL)	58.05	50.77–65.33	46.61	33.36–59.85	41.02	31.60–50.44	0.003
Serum zeaxanthin/lutein (µg/dL)	54.93	50.77–59.09	54.06	46.50–61.62	49.88	44.50–55.26	0.205
Serum cryptoxanthin (µg/dL)	31.19	25.61–36.76	23.61	13.46–33.76	21.27	14.05–28.49	0.03
Serum lycopene (µg/dL)	30.00	26.76–33.22	34.68	28.80–40.55	27.23	23.04–31.41	0.06
Serum α-tocopherol (µg/dL)	881.68	817.51–945.84	953.15	836.40–1,069.91	873.56	790.50–956.63	0.414

Analysis of covariance was used to examine the differences in the mean concentrations of the serum levels of micronutrients that are related to the effect of the smoking status. The data were adjusted for age (20–29, 30–39, or 40–54 years), BMI and alcohol intake frequency (0, 1–6, 7/week)

(HR 0.68, 95 % CI 0.49–0.95) as compared with women in the lower tertiles, but the linear trend was not statistically significant ($p = 0.882$). From the questionnaire, high-load intake of retinol significantly inhibited the regression (adjusted model: HR 0.59, 95 % CI 0.40–0.89) but the linear trend was not significant (Table 3).

Because serum levels of most carotenoids were low and carotene intake was small in smokers, the regression group was sub-analyzed stratifying by smoking status (never or current smokers) as shown in Tables 4 and 5. In non-smokers (Table 4), regression was observed in women in the upper tertiles of serum zeaxanthin/lutein (HR 1.25, 95 % CI 0.78–2.01) as compared with women in the lower and middle tertiles, and the linear trend was statistically

significant ($p = 0.024$). In current smokers, this was statistically abolished as shown in Table 5. In current smokers, a significant inhibition of regression was observed in women in the middle tertiles for serum alpha-tocopherol (HR 0.53, 95 % CI 0.27–0.94) as compared with women in the lower tertiles, and the linear trend was significant ($p = 0.042$) in the adjusted model (Table 5).

Effect of serum micronutrients and nutrient intake in progression subjects

In Table 6, a significant inverse relationship was observed in subjects with a medium level of serum beta-carotene (HR 0.28, 95 % CI 0.11–0.71, $p = 0.007$), although these

Table 3 HR of regression from entire CIN1/2 according to the serum micronutrients and nutrient intake questionnaire

	n	Person-months	Events	Cumulative 2-year rate (95 % CI)	Hazard ratio for regression (95 % CI)			
					Unadjusted	p value	Adjusted model	p value
Serum retinol							<i>p</i> for trend	0.812
Low (<55.2)	128	1,715.6	74	62.5 (53.6–71.4)	1		1	
Medium (55.2–67.9)	132	1,689.8	77	63.2 (54.4–72.0)	1.06 (0.77–1.46)	0.709	1.19 (0.86–1.65)	0.301
High (>67.9)	131	1,763.5	67	57.8 (48.6–67.4)	0.87 (0.62–1.21)	0.399	0.87 (0.62–1.22)	0.423
Serum α -carotene							<i>p</i> for trend	0.472
Low (<5.1)	127	1,654.9	71	60.9 (51.9–70.0)	1.00		1.00	
Medium (5.1–9.7)	133	1,750.0	68	57.3 (48.2–66.8)	0.91 (0.65–1.27)	0.574	1.00 (0.71–1.41)	0.984
High (>9.7)	131	1,764.0	79	65.2 (56.5–73.9)	1.04 (0.75–1.43)	0.828	1.26 (0.89–1.80)	0.19
Serum β -carotene							<i>p</i> for trend	0.095
Low (<28.3)	129	1,679.7	66	56.7 (47.7–66.2)	1.00		1.00	
Medium (28.3–57.6)	131	1,755.9	75	62.7 (53.8–71.6)	1.10 (0.79–1.53)	0.581	1.17 (0.83–1.66)	0.364
High (>57.6)	131	1,733.3	77	64.0 (55.2–72.9)	1.12 (0.80–1.56)	0.511	1.34 (0.93–1.93)	0.115
Serum zeaxanthin/lutein							<i>p</i> for trend	0.235
Low (<42.9)	130	1,645.9	76	62.7 (53.8–71.6)	1.00		1.00	
Medium (42.9–57.3)	130	1,803.1	70	58.1 (49.2–67.2)	0.85 (0.62–1.18)	0.341	0.97 (0.69–1.36)	0.868
High (>57.3)	131	1,719.9	72	63.5 (54.2–72.7)	0.89 (0.65–1.23)	0.488	1.05 (0.75–1.48)	0.768
Serum cryptoxanthin							<i>p</i> for trend	0.215
Low (<11.2)	129	1,659.5	74	63.9 (54.8–73.0)	1.00		1.00	
Medium (11.2–22.1)	130	1,754.7	67	56.8 (47.8–66.2)	0.87 (0.62–1.21)	0.406	0.91 (0.65–1.28)	0.592
High (>22.1)	132	1,754.7	77	63.1 (54.3–71.9)	0.99 (0.72–1.37)	0.974	1.07 (0.76–1.51)	0.694
Serum lycopene							<i>p</i> for trend	0.638
Low (<19.8)	129	1,713.7	69	58.6 (49.7–67.9)	1.00		1.00	
Medium (19.8–35.8)	131	1,780.3	79	66.3 (57.4–75.0)	1.07 (0.78–1.48)	0.67	1.07 (0.76–1.49)	0.705
High (>35.8)	131	1,674.9	70	58.5 (49.4–67.8)	1.02 (0.73–1.42)	0.914	1.08 (0.77–1.52)	0.662
Serum α -tocopherol							<i>p</i> for trend	0.882
Low (<753.0)	128	1,535.8	82	67.3 (58.7–75.6)	1.00		1.00	
Medium (753.0–983.9)	132	1,896.8	66	54.7 (45.9–64.0)	0.66 (0.48–0.91)	0.011	0.68 (0.49–0.95)	0.025
High (>983.9)	131	1,736.3	70	62.8 (53.2–72.3)	0.74 (0.54–1.01)	0.062	0.78 (0.56–1.09)	0.142
Retinol intake							<i>p</i> for trend	0.322
Low (<190.2)	130	1,555.8	74	62.8 (53.6–72.0)	1.00		1.00	
Medium (190.2–313.1)	130	1,755.6	74	63.3 (54.0–72.0)	0.89 (0.65–1.23)	0.484	0.76 (0.54–1.07)	0.12
High (>313.1)	131	1,857.5	70	57.8 (49.0–66.9)	0.80 (0.57–1.10)	0.172	0.59 (0.40–0.89)	0.011
Carotene intake							<i>p</i> for trend	0.325
Low (<3,281.4)	130	1,639.3	70	59.8 (50.6–69.1)	1.00		1.00	
Medium (3,281.4–5,042.8)	131	1,812.8	72	61.6 (52.5–64.7)	0.92 (0.66–1.28)	0.637	0.90 (0.63–1.28)	0.557
High (>5,042.8)	130	1,716.8	76	62.2 (53.5–71.0)	1.03 (0.74–1.42)	0.869	0.97 (0.65–1.46)	0.89
Vitamin A intake							<i>p</i> for trend	0.546
Low (<2,398.8)	130	1,601.8	70	61.5 (62.5–74.6)	1.00		1.00	
Medium (2,398.8–3,466.7)	131	1,834.7	72	59.7 (51.7–64.7)	0.90 (0.65–1.25)	0.541	0.91 (0.64–1.29)	0.599
High (>3,466.7)	130	1,732.4	76	62.6 (53.9–71.4)	1.01 (0.73–1.40)	0.948	0.93 (0.61–1.42)	0.727
Vitamin E intake							<i>p</i> for trend	0.147
Low (<6.7)	130	1,610.2	68	57.4 (48.3–66.7)	1.00		1.00	
Medium (6.7–8.7)	130	1,897.1	71	59.4 (50.5–68.5)	0.90 (0.64–1.25)	0.521	0.95 (0.66–1.39)	0.807
High (>8.7)	131	1,661.6	79	65.9 (57.1–74.6)	1.11 (0.80–1.54)	0.519	0.88 (0.54–1.43)	0.601

Cox's proportional hazard model showing the hazard ratio for regression in a cumulative 24-month period. The adjusted model was calculated by CIN grade (initial biopsy results; CIN1 or CIN2), HPV genotypes (HPV16/18/31/33/35/42/52/59, other high-risk types, low-risk types, or HPV negative) [17, 18], age, total calorie intake and smoking status (Brinkman index >100). The units of micronutrients are expressed as $\mu\text{g/dL}$.

Table 4 HR of regression from non-smoking CIN1/2 according to the serum micronutrients and nutrient intake questionnaire

	n	Person-months	Events	Cumulative 2-year rate (95 % CI)	Hazard ratio for regression (95 % CI)			
					Unadjusted	p value	Adjusted model	p value
Serum retinol							<i>p</i> for trend	0.292
Low (<55.2)	62	809.8	39	67.0 (54.5–79.0)	1		1	
Medium (55.2–67.9)	70	922.3	41	62.8 (50.9–74.6)	0.93 (0.60–1.44)	0.75	1.03 (0.65–1.63)	0.908
High (>67.9)	58	743.4	39	71.4 (58.7–83.1)	1.08 (0.69–1.68)	0.742	1.21 (0.74–1.98)	0.448
Serum α -carotene							<i>p</i> for trend	0.883
Low (<5.1)	46	560.7	28	64.4 (50.1–78.5)	1.00		1.00	
Medium (5.1–9.7)	62	789.7	38	66.1 (53.3–78.4)	0.97 (0.60–1.59)	0.918	1.22 (0.73–2.05)	0.449
High (>9.7)	82	1,125.1	53	68.7 (57.9–79.0)	0.93 (0.59–1.47)	0.76	1.26 (0.75–2.11)	0.384
Serum β -carotene							<i>p</i> for trend	0.206
Low (<28.3)	45	583.9	26	60.1 (45.8–74.7)	1.00		1.00	
Medium (28.3–57.6)	61	780.1	41	75.7 (62.7–86.9)	1.16 (0.71–1.90)	0.557	1.20 (0.71–2.03)	0.488
High (>57.6)	84	1,111.5	52	65.5 (54.8–76.0)	1.03 (0.64–1.65)	0.91	1.23 (0.73–2.07)	0.439
Serum zeaxanthin/lutein							<i>p</i> for trend	0.024
Low (<42.9)	56	729.3	34	64.8 (51.4–77.8)	1.00		1.00	
Medium (42.9–57.3)	61	817.3	38	66.7 (54.2–78.9)	1.00 (0.63–1.59)	1	1.12 (0.69–1.84)	0.642
High (>57.3)	73	928.9	47	68.6 (57.1–79.5)	1.05 (0.68–1.64)	0.813	1.25 (0.78–2.01)	0.352
Serum cryptoxanthin							<i>p</i> for trend	0.129
Low (<11.2)	47	650.1	28	64.7 (50.0–79.1)	1.00		1.00	
Medium (11.2–22.1)	61	740.7	38	68.2 (55.3–80.4)	1.23 (0.75–2.00)	0.414	1.24 (0.74–2.08)	0.412
High (>22.1)	82	1,084.7	53	67.5 (56.8–77.8)	1.16 (0.73–1.83)	0.536	1.35 (0.82–2.22)	0.231
Serum lycopene							<i>p</i> for trend	0.269
Low (<19.8)	63	805.3	37	63.2 (50.7–75.7)	1.00		1.00	
Medium (19.8–35.8)	63	827.7	43	73.8 (61.5–84.8)	1.11 (0.71–1.72)	0.651	1.17 (0.73–1.87)	0.51
High (>35.8)	64	842.5	39	64.3 (52.0–76.4)	1.00 (0.63–1.55)	0.962	1.28 (0.79–2.07)	0.316
Serum α -tocopherol							<i>p</i> for trend	0.176
Low (<753.0)	60	731.7	39	67.1 (54.7–79.0)	1.00		1.00	
Medium (753.0–983.9)	63	829.9	40	67.5 (55.2–79.2)	0.91 (0.59–1.42)	0.676	0.96 (0.60–1.53)	0.866
High (>983.9)	67	913.9	40	66.5 (53.9–78.6)	0.81 (0.52–1.26)	0.344	0.96 (0.60–1.54)	0.859
Retinol intake							<i>p</i> for trend	0.892
Low (<190.2)	62	760.7	36	63.5 (50.5–76.4)	1.00		1.00	
Medium (190.2–313.1)	63	840.7	41	70.4 (57.9–82.0)	1.04 (0.67–1.63)	0.854	0.90 (0.53–1.54)	0.704
High (>313.1)	65	874.1	42	66.3 (54.5–77.7)	1.02 (0.65–1.59)	0.94	0.86 (0.48–1.53)	0.61
Carotene intake							<i>p</i> for trend	0.131
Low (<3,281.4)	47	606.4	29	67.7 (52.7–81.9)	1.00		1.00	
Medium (3,281.4–5,042.8)	71	959.6	40	62.1 (50.0–74.2)	0.88 (0.55–1.43)	0.615	0.89 (0.51–1.56)	0.676
High (>5,042.8)	72	909.5	50	70.8 (59.8–81.0)	1.16 (0.74–1.84)	0.515	1.08 (0.60–1.94)	0.804
Vitamin A intake							<i>p</i> for trend	0.134
Low (<2,398.8)	50	676.0	28	63.5 (48.8–78.2)	1.00		1.00	
Medium (2,398.8–3,466.7)	69	934.1	41	63.8 (51.7–75.8)	1.08 (0.67–1.75)	0.755	1.14 (0.65–1.99)	0.654
High (>3,466.7)	71	865.4	50	72.3 (61.3–82.4)	1.42 (0.89–2.25)	0.14	1.47 (0.79–2.73)	0.218
Vitamin E intake							<i>p</i> for trend	0.163
Low (<6.7)	51	631.5	29	61.3 (47.4–75.5)	1.00		1.00	
Medium (6.7–8.7)	62	884.3	39	66.0 (53.6–78.1)	0.98 (0.61–1.58)	0.932	1.38 (0.70–2.71)	0.354
High (>8.7)	77	959.7	51	70.3 (59.3–80.6)	1.16 (0.74–1.83)	0.519	1.44 (0.67–3.12)	0.352

Cox's proportional hazard model showing the hazard ratio for regression in a cumulative 24-month period in non-smokers. The adjusted model was identical to the model used in Table 3. The units of micronutrients are expressed as $\mu\text{g}/\text{dL}$.

Table 5 HR of regression from current smoking CIN1/2 according to the serum micronutrients and nutrient intake questionnaire

	n	Person-months	Events	Cumulative 2-year rate (95 % CI)	Hazard ratio for regression (95 % CI)			
					Unadjusted	p value	Adjusted model	p value
Serum retinol							<i>p</i> for trend	0.43
Low (<55.2)	47	614.0	27	64.0 (49.2–78.6)	1		1	
Medium (55.2–67.9)	38	417.6	24	70.5 (53.4–85.7)	1.29 (0.74–2.23)	0.369	1.54 (0.87–2.76)	0.141
High (>67.9)	57	780.5	21	42.9 (30.1–58.3)	0.60 (0.34–1.06)	0.08	0.54 (0.29–1.00)	0.05
Serum α -carotene							<i>p</i> for trend	0.898
Low (<5.1)	59	751.9	33	62.5 (49.2–75.8)	1.00		1.00	
Medium (5.1–9.7)	53	689.6	22	49.9 (35.3–66.7)	0.72 (0.42–1.24)	0.24	0.85 (0.48–1.53)	0.595
High (>9.7)	30	370.6	17	61.8 (43.6–80.2)	1.04 (0.58–1.87)	0.886	1.23 (0.63–2.39)	0.537
Serum β -carotene							<i>p</i> for trend	0.667
Low (<28.3)	63	788.0	31	58.1 (44.6–72.2)	1.00		1.00	
Medium (28.3–57.6)	53	700.2	27	54.5 (41.1–69.1)	1.02 (0.61–1.71)	0.94	1.07 (0.62–1.86)	0.808
High (>57.6)	26	323.9	14	66.6 (44.5–87.0)	1.06 (0.56–2.00)	0.854	1.04 (0.51–2.14)	0.915
Serum zeaxanthin/lutein							<i>p</i> for trend	0.373
Low (<42.9)	54	640.8	32	63.6 (50.0–77.0)	1.00		1.00	
Medium (42.9–57.3)	52	669.4	26	54.1 (40.4–69.0)	0.79 (0.47–1.33)	0.372	0.88 (0.51–1.52)	0.645
High (>57.3)	36	501.9	14	57.6 (37.9–78.8)	0.55 (0.29–1.02)	0.059	0.76 (0.37–1.53)	0.435
Serum cryptoxanthin							<i>p</i> for trend	0.866
Low (<11.2)	62	727.3	36	67.4 (53.9–80.2)	1.00		1.00	
Medium (11.2–22.1)	47	644.3	20	48.4 (33.9–65.2)	0.63 (0.36–1.09)	0.098	0.72 (0.39–1.31)	0.279
High (>22.1)	33	440.5	16	53.9 (36.6–73.1)	0.73 (0.40–1.31)	0.286	0.85 (0.44–1.64)	0.63
Serum lycopene							<i>p</i> for trend	0.517
Low (<19.8)	43	543.8	21	55.3 (39.9–71.9)	1.00		1.00	
Medium (19.8–35.8)	55	761.7	29	60.8 (46.7–75.1)	0.96 (0.55–1.69)	0.896	0.79 (0.42–1.48)	0.457
High (>35.8)	44	506.6	22	54.4 (39.2–70.9)	1.08 (0.59–1.96)	0.802	0.77 (0.38–1.54)	0.456
Serum α -tocopherol							<i>p</i> for trend	0.042
Low (<753.0)	53	594.2	34	68.8 (55.5–81.4)	1.00		1.00	
Medium (753.0–983.9)	49	718.2	19	43.5 (30.1–59.7)	0.47 (0.27–0.83)	0.009	0.53 (0.27–0.94)	0.03
High (>983.9)	40	499.7	19	66.7 (46.0–86.0)	0.64 (0.36–1.11)	0.114	0.76 (0.42–1.40)	0.383
Retinol intake							<i>p</i> for trend	0.58
Low (<190.2)	50	573.8	29	62.3 (48.3–76.4)	1.00		1.00	
Medium (190.2–313.1)	51	673.9	25	56.5 (42.1–71.9)	0.74 (0.43–1.26)	0.263	0.76 (0.42–1.37)	0.36
High (>313.1)	41	564.4	18	52.3 (36.2–70.6)	0.63 (0.35–1.13)	0.124	0.57 (0.29–1.13)	0.106
Carotene intake							<i>p</i> for trend	0.182
Low (<3,281.4)	64	730.7	34	59.8 (46.9–73.1)	1.00		1.00	
Medium (3,281.4–5,042.8)	43	632.0	22	58.7 (42.7–75.4)	0.72 (0.42–1.24)	0.238	0.71 (0.39–1.31)	0.272
High (>5,042.8)	35	449.4	16	52.9 (35.8–72.2)	0.73 (0.41–1.33)	0.309	0.55 (0.25–1.18)	0.122
Vitamin A intake							<i>p</i> for trend	0.268
Low (<2,398.8)	65	723.6	36	61.9 (49.1–74.9)	1.00		1.00	
Medium (2,398.8–3,466.7)	43	642.5	19	49.1 (34.4–66.2)	0.59 (0.34–1.03)	0.064	0.58 (0.31–1.07)	0.081
High (>3,466.7)	34	446.0	17	60.6 (42.2–79.4)	0.74 (0.42–1.32)	0.307	0.60 (0.28–1.32)	0.208
Vitamin E intake							<i>p</i> for trend	0.567
Low (<6.7)	61	684.0	32	56.7 (44.1–70.1)	1.00		1.00	
Medium (6.7–8.7)	45	720.6	19	49.0 (34.4–66.0)	0.56 (0.32–0.99)	0.047	0.51 (0.25–1.05)	0.066
High (>8.7)	36	407.5	21	67.3 (49.6–83.8)	1.02 (0.59–1.77)	0.947	0.56 (0.23–1.38)	0.211

Cox's proportional hazard model showing the hazard ratio for regression in a cumulative 24-month period in current smokers only. The adjusted model was identical to the model used in Table 3. The units of micronutrients are expressed as $\mu\text{g}/\text{dL}$.

Table 6 HR of progression from entire CIN1/2 according to the serum micronutrients and nutrient intake questionnaire

	n	Person-months	Events	Cumulative 5-year rate (95 % CI)	Hazard ratio for progression (95 % CI)			
					Unadjusted	p value	Adjusted model	p value
Serum retinol							<i>p</i> for trend	0.372
Low (<55.2)	128	4,588.2	7	8.7 (3.6–20.1)	1.00		1.00	
Medium (55.2–67.9)	132	5,048.8	17	17.1 (10.8–26.6)	2.25 (0.93–5.44)	0.071	2.35 (0.95–5.77)	0.063
High (>67.9)	131	5,210.1	14	14.3 (8.5–23.7)	1.82 (0.73–4.51)	0.198	2.23 (0.88–5.60)	0.089
Serum α -carotene							<i>p</i> for trend	0.669
Low (<5.1)	127	4,506.6	13	15.4 (8.7–26.2)	1.00		1.00	
Medium (5.1–9.7)	133	4,955.5	17	16.0 (10.0–25.0)	1.21 (0.59–2.49)	0.609	1.08 (0.51–2.31)	0.835
High (>9.7)	131	5,385.0	8	9.6 (4.7–19.0)	0.52 (0.22–1.27)	0.153	0.46 (0.18–1.15)	0.098
Serum β -carotene							<i>p</i> for trend	0.337
Low (<28.3)	129	4,245.0	18	21.8 (13.6–33.9)	1.00		1.00	
Medium (28.3–57.6)	131	5,208.1	7	7.0 (3.2–14.7)	0.32 (0.13–0.77)	0.011	0.28 (0.11–0.71)	0.007
High (>57.6)	131	5,394.0	13	13.2 (7.7–22.3)	0.58 (0.28–1.19)	0.14	0.52 (0.24–1.13)	0.098
Serum zeaxanthin/lutein							<i>p</i> for trend	0.772
Low (<42.9)	130	4,611.4	11	12.1 (6.7–21.4)	1.00		1.00	
Medium (42.9–57.3)	130	5,291.5	17	17.9 (11.2–28.0)	1.37 (0.64–2.94)	0.415	1.58 (0.71–3.53)	0.266
High (>57.3)	131	4,944.2	10	9.4 (5.1–17.1)	0.87 (0.37–2.06)	0.756	0.95 (0.39–2.32)	0.908
Serum cryptoxanthin							<i>p</i> for trend	0.618
Low (<11.2)	129	4,591.6	12	12.2 (6.9–20.9)	1.00		1.00	
Medium (11.2–22.1)	130	4,906.2	16	17.1 (10.6–27.0)	1.26 (0.60–2.67)	0.544	1.37 (0.61–3.06)	0.445
High (>22.1)	132	5,349.3	10	10.5 (5.5–19.7)	0.73 (0.32–1.69)	0.465	0.71 (0.29–1.72)	0.450
Serum lycopene							<i>p</i> for trend	0.286
Low (<19.8)	129	4,827.0	15	17.5 (10.5–28.3)	1.00		1.00	
Medium (19.8–35.8)	131	4,954.6	11	10.0 (5.6–17.6)	0.71 (0.33–1.55)	0.395	0.61 (0.27–1.36)	0.223
High (>35.8)	131	5,065.5	12	13.1 (7.3–22.9)	0.76 (0.36–1.63)	0.48	0.73 (0.33–1.59)	0.428
Serum α -tocopherol							<i>p</i> for trend	0.788
Low (<753.0)	128	5,143.1	11	12.0 (6.6–21.2)	1.00		1.00	
Medium (753.0–983.9)	132	5,052.6	11	13.3 (7.4–23.3)	1.01 (0.44–2.33)	0.983	0.91 (0.39–2.10)	0.820
High (>983.9)	131	4,651.4	16	15.7 (9.3–25.8)	1.60 (0.74–3.45)	0.232	1.87 (0.84–4.19)	0.126
Retinol intake							<i>p</i> for trend	0.666
Low (<190.2)	130	4,778.5	14	14.7 (8.6–24.4)	1.00		1.00	
Medium (190.2–313.1)	130	4,985.2	15	16.7 (9.8–27.7)	1.02 (0.49–2.12)	0.948	1.08 (0.51–2.32)	0.834
High (>313.1)	131	5,083.4	9	9.5 (4.9–17.7)	0.60 (0.26–1.40)	0.239	0.62 (0.23–1.68)	0.346
Carotene intake							<i>p</i> for trend	0.331
Low (<3,281.4)	130	4,578.9	9	10.8 (5.2–21.6)	1.00		1.00	
Medium (3,281.4–5,042.8)	131	4,789.0	16	17.6 (11.4–26.7)	2.02 (0.91–4.46)	0.083	2.30 (0.97–5.42)	0.058
High (>5,042.8)	130	5,479.2	10	11.6 (6.2–21.0)	0.94 (0.38–2.33)	0.901	1.19 (0.41–3.44)	0.746
Vitamin A intake							<i>p</i> for trend	0.493
Low (<2,398.8)	130	4,510.5	11	12.2 (6.3–22.9)	1.00		1.00	
Medium (2,398.8–3,466.7)	131	4,921.0	16	15.1 (9.4–23.9)	1.33 (0.62–2.87)	0.463	1.32 (0.59–2.97)	0.500
High (>3,466.7)	130	5,415.6	11	12.6 (3.8–22.2)	0.84 (0.36–1.95)	0.689	0.92 (0.33–2.54)	0.873
Vitamin E intake							<i>p</i> for trend	0.834
Low (<6.7)	130	4,431.0	12	13.8 (7.5–24.7)	1.00		1.00	
Medium (6.7–8.7)	130	5,128.1	15	14.1 (8.6–22.6)	1.08 (0.51–2.31)	0.842	1.06 (0.44–2.56)	0.892
High (>8.7)	131	5,288.0	11	12.5 (6.8–22.1)	0.78 (0.34–1.77)	0.55	1.00 (0.30–3.38)	0.998

Cox's proportional hazard model showing the hazard ratio for progression over a cumulative 60-month period. The adjusted model was identical to the model used in Table 3. The units of micronutrients are expressed as $\mu\text{g/dL}$

effects were weaker or not found with a higher level of serum beta-carotene (HR 0.52, 95 % CI 0.24–1.13, $p = 0.098$). In contrast, a high carotene intake did not show an inverse relationship, but rather a non-significant increase in progression (HR 2.30, 95 % CI 0.97–5.42, $p = 0.058$). There was no significant association between other serum micronutrients and risk for CIN progression.

Discussion

The role of environmental factors, including micronutrients and tobacco smoking, in cervical carcinogenesis has been discussed. Smoking status in particular interfered with serum levels and intake of carotenoids as shown in Tables 1 and 2. In smokers, food intake is intrinsically lower than in non-smokers [22]. From the questionnaires, the intake per day of all micronutrients, except retinol and tocopherol, was lower in current smokers than in non-smokers, suggesting an unbalanced diet resulting from either smoking or other lifestyle behaviors (Table 1). Serum levels of alpha-carotene, beta-carotene and cryptoxanthin were inversely correlated with smoking status, but alpha-tocopherol was not correlated with smoking status after adjusting for age, BMI and frequency of alcohol intake (Table 2). These data were consistent with a previous report in which smoking was shown to affect serum beta-carotene levels but to have no effect on alpha-tocopherol levels [23]. Though alpha-tocopherol and beta-carotene are well known as antioxidants, the antioxidant effect of alpha-tocopherol is not due to a reaction with oxygen. In contrast, beta-carotene does react with oxygen. This suggests that there is a difference in the mechanisms of antioxidant reaction [24].

In regression subjects, we expected to find a protective effect from high serum levels or intake of carotenoids; however, neither of these had protective effects. We assume that smoking status modulates dietary intake or serum levels of micronutrients. Therefore, we investigated the association between dietary intake or serum levels of micronutrients and CIN regression, taking into account smoking status (Tables 3, 4, 5). In non-smoking regression subjects, regression was significantly related to the serum levels of zeaxanthin/lutein. This relationship was not found in current smokers. In a similar example, an isoflavone has a protective effect for lung cancer, but the effect is abolished by smoking [25]. It was reported that zeaxanthin/lutein may be a useful marker of intake of leafy vegetables, spinach, green peas, broccoli and seaweed [26]. Zeaxanthin/lutein is chemically more hydrophilic than other carotenoids such as alpha- and beta-carotene, lycopene and beta-cryptoxanthin. The mechanisms of a potential protection against carcinogenesis may include: induction of

apoptosis, inhibition of angiogenesis, enhancement of gap junction intercellular communication, induction of cell differentiation, prevention of oxidative damage, and modulation of the immune system. Serum levels of lutein have been inversely associated with cytochrome CYP1A2 activity, a hepatic enzyme responsible for the metabolic activity of a number of putative human carcinogens [27]. High serum levels of alpha-tocopherol tend to have an inhibitory effect on regression in smokers (Table 4). There is a similar effect in that supplemental vitamin E, presumably causing a high concentration of alpha-tocopherol, is associated with an increased risk of lung cancer, which was confined to current smokers [28]. Alpha-tocopherol is considered to be an antioxidant, but it might act as a pro-oxidant [24].

Though a weak and non-significant protective effect of dietary intake or low serum concentration of beta-carotene has been observed previously [10, 15, 29, 30], we found that a medium serum level of beta-carotene showed a significant protective effect on CIN progression, whereas this protective effect at higher serum levels of beta-carotene was weaker or abolished (Table 6). These data appear to be consistent with in-vitro experiments reporting that very high concentrations of beta-carotene decreased antioxidant and/or induced pro-oxidant effects [31, 32]. Based on epidemiological studies that have shown an association between a low intake of carotenes and human cancers [33], an intervention study was conducted for the prevention of lung cancer [34]. However, it was paradoxically reported that high serum levels of beta-carotene induced by oral supplements promoted lung cancer in male heavy smokers aged 50–69 years. In CIN, oral beta-carotene supplementation did not enhance CIN regression in a randomized, double-blind phase III trial [35]. One explanation for these failures may be that oral supplements induced extremely high serum levels of beta-carotene. Taken together, these data suggest that medium serum levels of beta-carotene may interfere with CIN progression or cancer development.

There was a discrepancy between the results of dietary intake and serum levels of beta-carotene. Endogenous metabolic processes may influence the serum concentrations of micronutrients. In fact, inconsistent results of the serum levels and dietary intake of alpha-tocopherol in patients with prostate cancer, and contradictory results of retinol in patients with cervical cancer, have been reported previously [14, 36, 37]. Additionally, there is limited dietary intake information obtained from questionnaires because of inherent recall bias. We examined the residual confounding factors, including passive smoking, the number of sexual partners, and serum *Chlamydia* IgG antibody, in addition to the adjusted model. Despite confounding by other risk factors included for adjustments, the analyses did not change the conclusion.

To our knowledge, this is the first large-scale prospective cohort study for CIN outcome to report an association between serum levels of antioxidant micronutrients adjusted for potential confounders including CIN grade, HPV genotype, age, total energy intake and smoking. To make our comparisons, we investigated not only serum levels but also dietary intake of micronutrients, despite the fact that food-intake questionnaires contain limited information. It is known that the accuracy of recalling past dietary intake is influenced by current dietary habits [38]. There are inconsistent results between previous case-control and cohort studies. However, our discrepant results did not reach the conclusion that women with CIN received a benefit from consuming a beta-carotene-rich diet. However, not smoking and maintaining high serum levels of zeaxanthin/lutein, presumably by intake of leafy vegetables, spinach, green peas, broccoli, and seaweed, are advantageous for the prevention of cervical cancer.

This study has some potential limitations. We included only CIN patients with an available serum sample for measurement of serum nutrients [18]. The majority of CIN patients already had persistent HPV infection at enrollment in the present study. If these nutrients play an important role in preventing persistent HPV infection, we cannot determine that role in this cohort study. The food intake contains not only the micronutrients being investigated but also other nutrients and mixtures. The incident number of progression cases was small and it was difficult to analyze by smoking status. A large-scale cohort study with a longer period of observation is required to clarify the association between serum levels or dietary intake of micronutrients and the risk of developing cervical cancer.

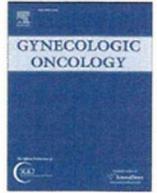
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Conflict of interest The authors declare that they have no conflict of interest.

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Effective treatment of pelvic lymphocele by lymphaticovenular anastomosis

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HIGHLIGHTS

- ▶ Pelvic lymphocele is a major complication after pelvic lymphadenectomy.
- ▶ We performed lymphaticovenular anastomosis (LVA) on pelvic lymphoceles, and found that LVA was highly effective regardless of the lymphoceles' size.
- ▶ LVA could be considered as an initial treatment for lymphoceles.

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ABSTRACT

Objective. Pelvic lymphocele can be a severe complication associated with surgical procedures such as pelvic lymphadenectomy. Lymphaticovenular anastomosis (LVA) is increasing in popularity as a surgical treatment for lymphedema. The aim of this study was to evaluate whether LVA is an effective treatment for lymphocele, which is caused by an obstruction of the lymphatic flow in a manner similar to the development of lymphedema.

Methods. Eleven female patients, who presented with lymphocele, were treated with LVA. Before the operation, 3 of them were treated with a percutaneous catheter. Lymphocele size and the volume of daily drainage were measured before and after LVA.

Results. The lymphocele was completely resolved in 6 patients and partially resolved in the remaining 5 patients. The mean size of the pelvic lymphocele changed from 400 ml (range 50–1050 ml) to 43 ml (range 0–120 ml) ($P < 0.01$). In the 3 patients who had percutaneous drainage catheters, the volume of fluid drained decreased from 340 ml/day to 20 ml/day after LVA.

Conclusions. Our technique is minimally invasive and is performed under local anesthesia. LVA is effective regardless of the size of the lymphocele. Therefore, LVA should be considered as a therapy for lymphocele because of its low invasiveness and its effectiveness in re-establishing circulation of lymphatic flow. Further studies should be performed to compare LVA with other minimally invasive techniques, such as percutaneous catheter and sclerotherapy.

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Introduction

A lymphocele is defined as an abnormal collection of lymph fluid, without an epithelial lining, at the site of lymphatic surgery [1,2]. A pelvic lymphocele can occur after surgical procedures such as pelvic lymphadenectomy for gynecologic or prostatic malignancies and renal transplantation [2–7] and has an incidence of 1–49% [1,2,8]. Most lymphoceles are small and asymptomatic, and they disappear spontaneously with time. However, when sufficiently large, they may lead to

symptoms such as abdominal pain, infection, increased urinary frequency, hydronephrosis, deep venous thrombosis, and lower extremity lymphedema [1,2,6,8].

Several treatment options are available for the management of pelvic lymphoceles; however, there is no consensus as to which is most effective. Needle aspiration and percutaneous catheter drainage, which are commonly used in the initial management of symptomatic lymphoceles, have reported initial cure rates of up to 80%, but treated lymphoceles are often complicated by infection (in up to 50% of cases) and recur in 80–90% of cases [1,2,9]. The cure rate of sclerotherapy is also reported to be between 77% and 98%, but the success of this treatment is inversely proportional to the size of the lymphocele—larger lymphoceles are more likely to be symptomatic and cause complications; thus, the effectiveness of this therapy is limited [1,9]. Laparoscopic or surgical fenestration is the most invasive of the current therapies

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for pelvic lymphoceles and is often reserved for refractory cases. Complications associated with this approach include perforation of the bladder, transection of the ureter, and injury of pelvic vessels [6,10].

Lymphaticovenular anastomosis (LVA) using supermicrosurgery has been reported as being a simple, minimally invasive, and effective treatment for secondary lymphedema of the upper and lower extremities [11–15]. This technique bypasses proximal lymphatic blockages, providing an alternative route for lymphatic fluid recirculation into the venous system. We reasoned that a similar principle could be used to treat pelvic lymphoceles. By providing an alternative route of lymphatic drainage into the venous system for lymphatic fluid from the lower limb, the flow of lymphatic fluid into the lymphocele would be reduced. Furthermore, we have previously demonstrated that valvular incompetence permits a reversal of the lymphatic flow in cases of lymphedema, and we hypothesized that a similar mechanism in the postsurgical pelvic lymphatic system of patients with lymphoceles would allow drainage of the lymphocele through the newly created LVAs. We have previously reported successful management of a pelvic lymphocele using this approach in a single patient [16]. In this study, we report our experience using this technique to treat pelvic lymphoceles in a series of 11 patients.

Materials and methods

Patients

Eleven female patients with pelvic lymphoceles were referred to our department between May 2010 and October 2011. All the patients had undergone treatment for gynecologic cancer (see Table 1). The presence of a pelvic lymphocele was determined in all cases by a CT scan. In 3 patients, a percutaneous catheter had been inserted prior to referral in an attempt to treat the lymphocele, but drainage had remained unacceptably high.

Preoperative preparation

All patients gave fully informed consent for the procedure, acknowledging that current outcome data on efficacy was unknown. One day before each operation, fluorescence lymphatic imaging, using a near-infrared fluorescence imaging device (Photodynamic Eye, Hamamatsu Photonics, Hamamatsu City, Japan), was performed after the injection of indocyanine green dye (ICG) to identify the lymphatic channels in both lower limbs, as previously described [17–19]. The location of the lymphatic channels was marked, facilitating the accurate placement of short incisions and thereby allowing the procedure to be performed under local anesthesia. In those patients with a percutaneous drainage device in situ, the drain was clamped after lymphatic mapping in order to increase pressure in the lower-limb lymphatics and facilitate LVA.

Table 1
Patient data.

Age	Site of primary cancer	Stage	Primary operation	Pre-LVA lymphocele volume [ml]	Post-LVA lymphocele volume [ml]	Catheter inserted
52	Cervical cancer	Ib	RH, BSO, PLA	60	0	–
63	Cervical cancer	IVb	RH, SILA, PALA	160	0	+
42	Endometrial cancer	Ic	RH, BSO, PLA	50	0	–
53	Endometrial cancer	Ib	RH, BSO, PLA	1050	0	+
53	Endometrial cancer	Ic	TAH, BSO, PLA	700	0	–
66	Endometrial cancer	Ic	RH, BSO, PLA, PALA	200	110	–
42	Ovarian cancer	Ic	TAH, BSO, PLA, PALA	460	50	–
53	Ovarian cancer	Ia	TAH, BSO, PLA, PALA, pOM	170	90	–
56	Ovarian cancer	IIIC	Secondary EILA	500	100	+
61	Ovarian cancer	Ic	TAH, BSO, PLA, PALA, pOM	700	0	–
69	Ovarian cancer	Ic	TAH, BSO, PLA, PALA, pOM	350	120	–

RH: radical hysterectomy, BSO: bilateral salpingo-oophorectomy, PLA: pelvic lymphadenectomy, SILA: superficial inguinal lymphadenectomy, TAH: abdominal total hysterectomy, pOM: partial omentectomy, EILA: external iliac lymphadenectomy, PALA: para-aortic lymphadenectomy.

Operative technique

Under local anesthesia, 2 or 3 incisions (2 cm each) were made on each lower limb—on the dorsum of the foot, the distal medial thigh, and the groin—overlying previously mapped lymphatic channels [20]. Dissection of superficial lymphatic channels and venules was performed under magnification using the operative microscope. Lymphaticovenular anastomosis was performed using either 11/0 or 12/0 nylon sutures in an end-to-end (Fig. 1A and B) or side-to-end configuration. The patency of anastomoses was confirmed by either washout of the venous lumen by lymphatic flow or venous backflow into the lymphatic channels. Wounds were closed with intradermal 4/0 PDS and interrupted 5/0 nylon sutures.

Postoperative management

Twice daily for 7 days after surgery, 60 µg of prostaglandin E1 (Prostandin; Ono Pharma. Co., Osaka, Japan) was injected intravenously. Prostaglandin is used for dilation of the vessels and seems to result in decreased occlusion of the anastomosis site. Compression therapy was started on postoperative day 14. All but one patient had follow-up CT scans.

Assessment

Assessment of the lymphocele was performed by either CT or ultrasonography. The volume of the lymphocele was calculated as an ellipsoid. Statistical analysis of the data was performed using a Wilcoxon test. A P value less than 0.05 was deemed significant.

Results

The demographic details of the patients and the details of their gynecologic treatment are shown in Table 1. We performed a mean of 8.2 lymphaticovenular anastomoses, with a mean venule diameter of 0.70 mm and a mean lymphatic diameter of 0.55 mm. In 6 of the 11 patients, the pelvic lymphocele was completely resolved after LVA, and in the remaining 5 patients, the lymphocele was partially resolved. The average pelvic lymphocele size was 400 ml (range 50–1050 ml) on preoperative CT scan and 43 ml (range 0–120 ml) on postoperative CT ($P < 0.01$). In the 3 patients who underwent preoperative placement of percutaneous drainage catheters, the mean volume of fluid drained each day was reduced from 340 ml to 20 ml after LVA (Fig. 2).

Prior to our operations, 10 patients had symptoms: 1 had hydro-nephrosis requiring a urinary stent, 1 had frequent pre-ileus, 2 had increased urinary frequency, 2 had infection of lymphoceles, 3 had abdominal pain, and 7 had lower-extremity lymphedema. All symptoms except for lymphedema were alleviated after the LVA operation, and the lymphedema was improved from the pre-LVA state. No patients in

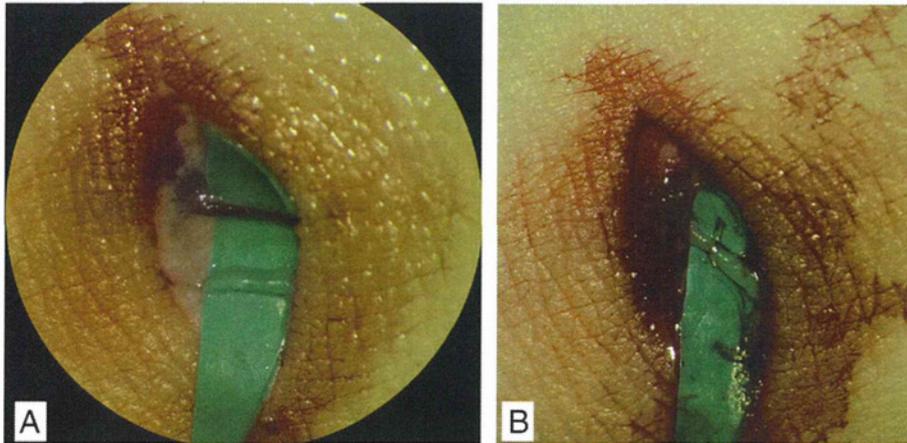


Fig. 1. Lymphaticovenular anastomosis. (A) Pre-anastomosis image. The upper vessel is a vein; the lower image is a lymphatic channel. (B) Post-anastomosis image. The anastomosis was performed with 5 sutures of 11–0 nylon. The left side of the anastomosis looks clear because the lymphatic fluid is under higher pressure than venous blood; therefore, flow from the lymphatic channel on the right washes out blood from inside the vein.

our series suffered any complications of LVA. Specifically, there were no infections and no wound-healing problems.

Representative case

The lymphocele was detected on CT 11 days before LVA (Fig. 3A). The patient had abdominal pain, urinary frequency, and lower-extremity lymphedema. A percutaneous catheter was inserted 3 days before the operation and the daily volume of drained fluid was recorded until the tube was removed (Fig. 3D). The operation site of LVA was noted (Fig. 3B). A CT image taken 3 days after the operation showed that the lymphocele had disappeared (Fig. 3C). The catheter was removed after confirming that the symptoms had disappeared.

Discussion

Since Teruel et al. [21] first reported successful sclerotherapy with povidone iodine for lymphoceles, several types of sclerotherapy with a variety of agents have been reported [1,6,9]. The cure rate for sclerotherapy is reported to be between 77% and 98% [1], and the recurrence rate is 31% [22]. However, the success of this treatment is inversely proportional to the size of the lymphocele [1]—larger lymphoceles are

more likely to be symptomatic and cause complications; thus, when the lymphoceles most require treatment, this therapy is likely to be relatively less effective.

Laparoscopic or open surgical fenestration can be used to open a pathway from the lymphocele into the peritoneal cavity, allowing the peritoneum to absorb lymphatic fluid [23]. These techniques enable lymphatic fluid to re-circulate into the venous system. However, they are more invasive than other therapies and have been associated with complications including bladder perforation, ureter transection, and injury of pelvic vessels [6,10]. Recurrence can occur with closure of the fenestrated window in 6–15% of cases [6,24].

The ideal therapy for lymphoceles would be more effective and less invasive than traditional treatment methods (including sclerotherapy and surgical fenestration), with fewer complications and a lower chance of recurrence. Moreover, restoration of lymphatic circulation, broken by lymphadenectomy, is desired.

LVA is emerging as the treatment of choice for lymphedema of the extremities. Before the LVA operation was available, only conservative therapies, such as massage and compression garments, could be used for lymphedema. These techniques do not enable re-establishment of lymphatic fluid circulation into the venous system, but simply release it into the trunk lesion. Therefore, patients are never able to discontinue the therapy if they wish to reduce the edematous lesion. LVA was

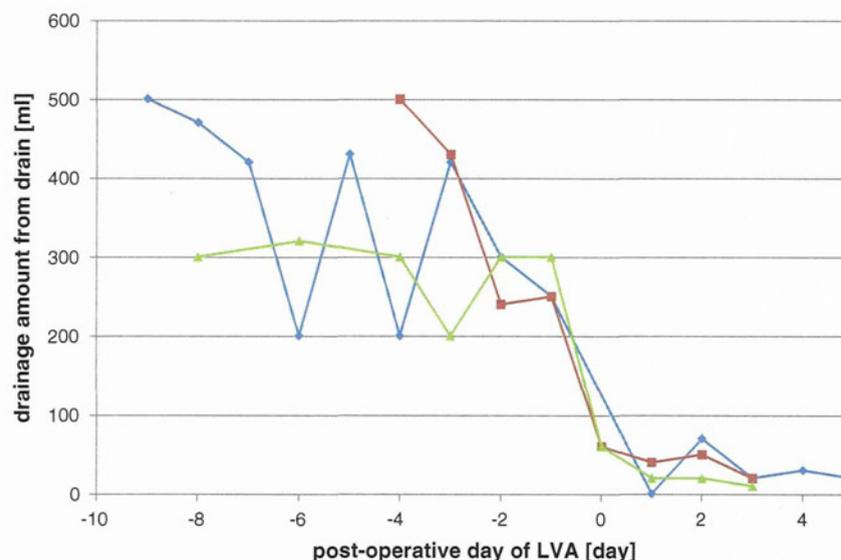


Fig. 2. Daily drainage from 3 patients who had preoperative placement of percutaneous drains. Note the dramatic decrease in drainage following LVA.

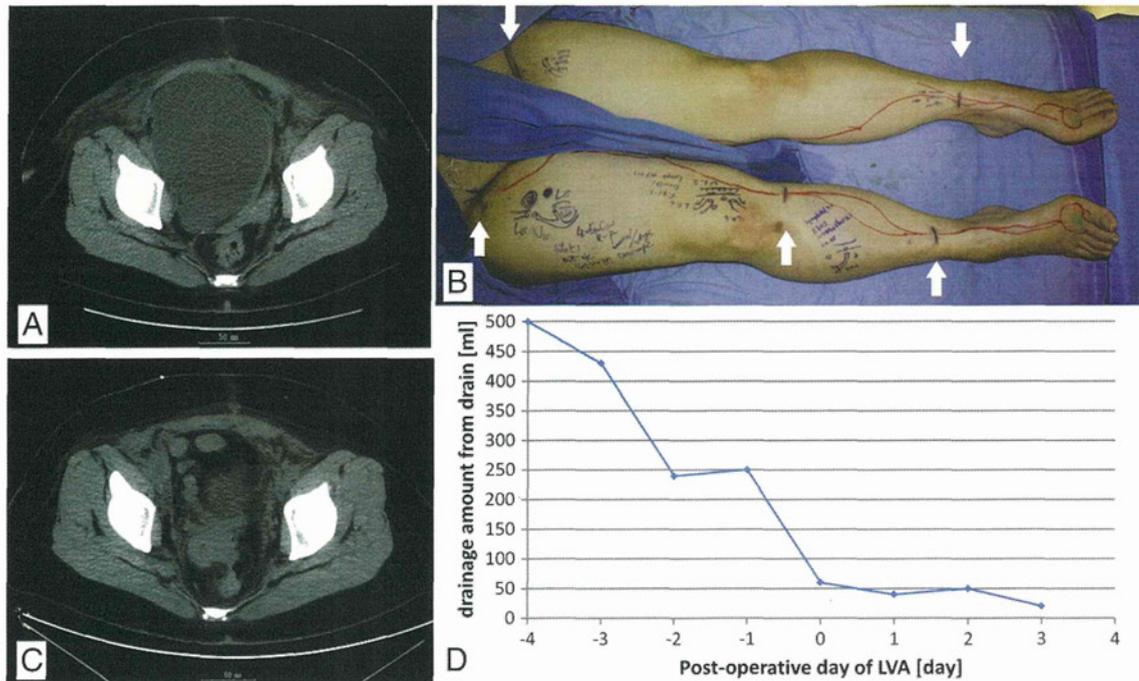


Fig. 3. Resolution of pelvic lymphocele after LVA. (A) CT scan of a large pelvic lymphocele after gynecologic surgery. (B) Immediate postoperative view. Seven anastomoses were performed through five 3-cm incisions under local anesthesia. (C) A CT scan of the same patient 3 days after LVA demonstrates complete resolution of the lymphocele. (D) The drainage chart of the same patient demonstrates large daily drainage volumes before LVA. Following LVA, the volume of fluid drained was dramatically reduced, and the drain was removed on postoperative day 4.

introduced as a new concept for lymphedema therapy [13–15]. The aim is to bypass proximal lymphatic blockages that cause congestion of lymphatic flow and thereby provide an alternative route for lymphatic fluid recirculation. Although the lymphatic channels normally have autokinetic movement because of smooth muscles, when the muscle damage due to lymphedema is irreversible, compression therapy is needed as an adjuvant therapy to direct lymphatic fluid into venules. However, when the damage is dormant, the muscles react by pushing lymphatic fluid into the venous system. In this case, the patients do not need to receive any further adjuvant therapy.

In lymphoceles, the lymphatic flow from the lower limbs is similarly interrupted at the surgical region, where it flows into the cavity.

This is illustrated in Fig. 4, where ICG injected into the dorsum of the feet is seen to escape into the percutaneous drainage catheter of a lymphocele. We reasoned that LVA would enable the lymphatic flow from the limbs to bypass the lymphocele, reducing its volume and preventing lymphatic flow into the lymphocele, thereby allowing spontaneous resolution. Our results supported this hypothesis, with total recovery in 6 of the 11 cases and improvement in the remaining 5 cases.

We believe that LVA has multiple advantages over the other methods currently used to treat lymphoceles. First, LVA is minimally invasive because it can be performed under local anesthesia and requires only 2 or 3 small skin incisions. Second, LVA is effective for

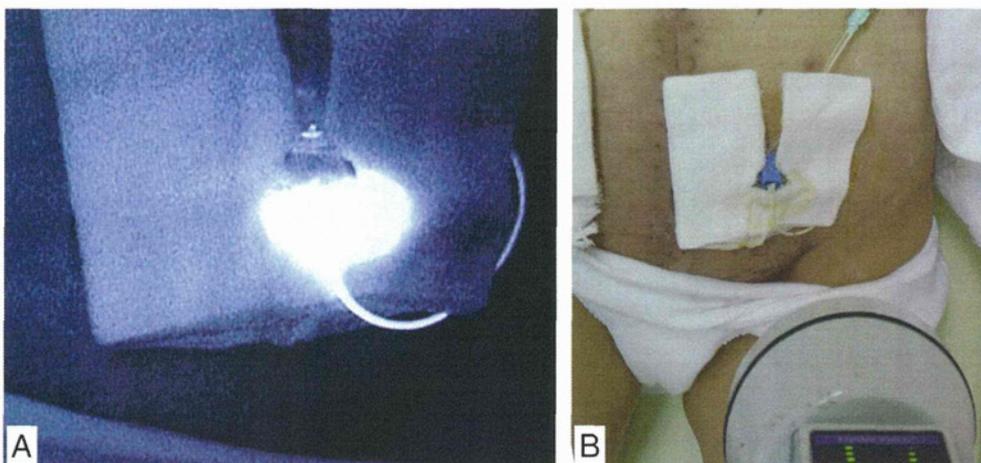


Fig. 4. Demonstration of lymphatic flow from the leg into a pelvic lymphocele. (A) Fluorescence lymphatic image of a percutaneous catheter, 5 min after injecting ICG into the first dorsal web space of the foot, indicates the lymphatic flow from the leg rapidly entering the lymphocele. It also indicates that the fluorescing root is the dominant lymphatic channel pouring into the lymphocele. (B) Conventional photograph of the same area. The Photodynamic Eye camera used to obtain the picture in (A) is seen at the bottom of the picture.

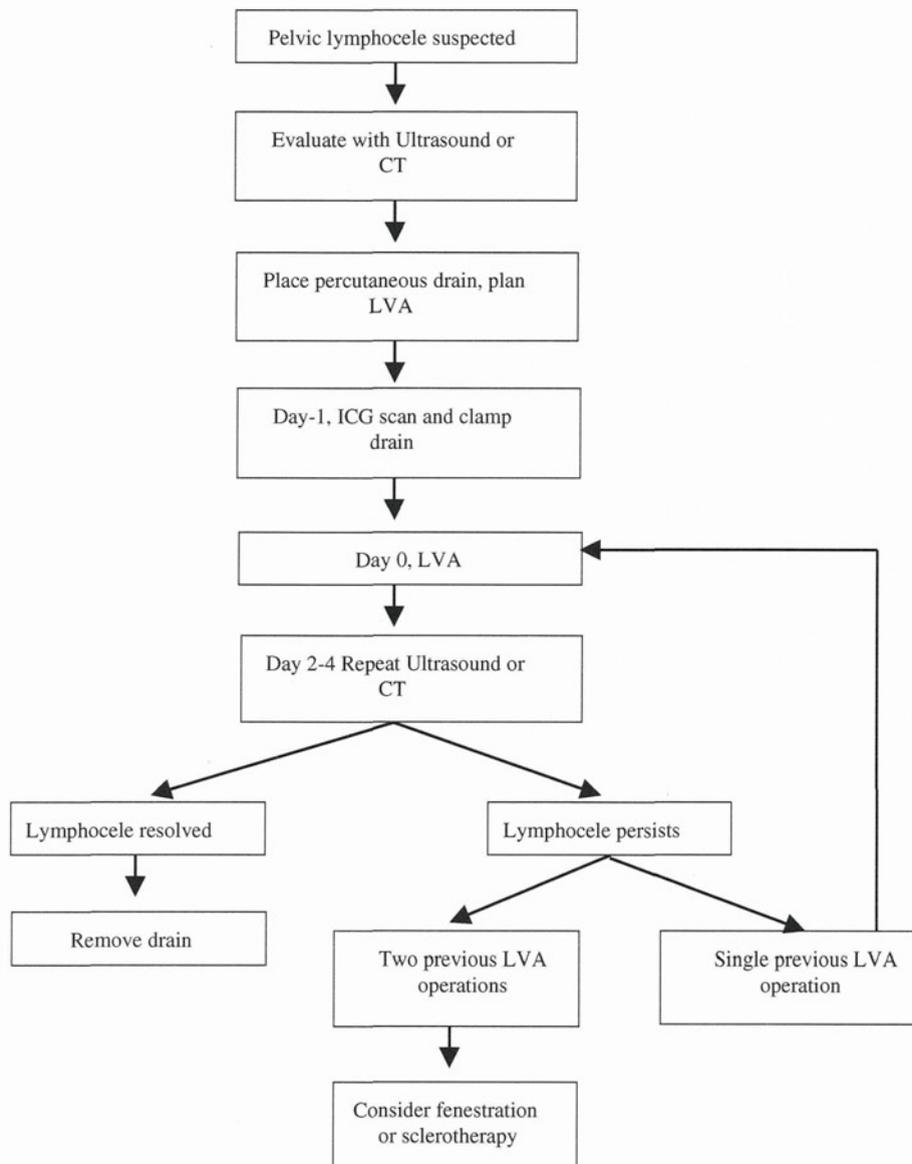


Fig. 5. Algorithm for the management of pelvic lymphocele.

all sizes of lymphocele. Third, LVA can prevent or improve lymphedema, which is a common complication of pelvic lymphadenectomy. This is in contrast to other techniques that resolve the fluid collection by blocking or sclerosing lymphatic channels, which may in itself provoke the development of lymphedema.

Our experience of reverse lymphatic flow with the valvular incompetence of lymphatic channels in lymphedema indicates that lymphatic flow into the lymphocele from places other than the leg may occur in a retrograde pattern into the leg's lymphatic channels and then into the venous system. Competent lymphatic valves may account for the partial failure of our technique, and we recommend that a percutaneous catheter be used to drain the remaining fluid if it is symptomatic.

Unfortunately, LVA is not perfectly effective for all patients. We suggest that the reason for this is that the lymphatic channels used for LVA are sometimes not the dominant lymphatic channels for the lymphoceles. In such cases, the lymphocele could diminish but not vanish. A second LVA might be able to locate the dominant lymphatic channel. Other therapies could also be used: LVA is an indirect approach to the lymphocele whereas other therapies approach

lymphoceles directly. We present our algorithm for management of pelvic lymphoceles in Fig. 5.

In conclusion, our technique is minimally invasive and is performed under local anesthesia. It is therefore suitable for patients who have recently undergone major pelvic surgery. LVA should be considered as an initial therapy for lymphoceles because of its low invasiveness, high effectiveness, and ability to re-establish circulation of lymphatic flow. Further studies should be performed to compare LVA with other minimally invasive techniques, such as percutaneous catheter and sclerotherapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Genome-Wide Single Nucleotide Polymorphism Arrays as a Diagnostic Tool in Patients With Synchronous Endometrial and Ovarian Cancer

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Objective: Synchronous carcinomas in the endometrium and ovaries can be a single primary tumor with metastasis (SPM) or dual primary tumors (DP). Although the prognosis of DP without any metastases is significantly better than that of SPM, pathological diagnosis is difficult in tumors with similar histological features.

Materials and Methods: In 10 tumors from 5 patients with synchronous endometrial and ovarian carcinomas, 250K single nucleotide polymorphism arrays were performed. The patients were genetically diagnosed according to the pattern of copy number alterations (CNAs), in addition to microsatellite status and mutational analysis of *PIK3CA*, *PTEN*, *K-Ras*, and *CTNNB1*.

Results: Of the 5 patients, 3 exhibited identical CNA patterns, including type, loci, and degree of each alteration in the endometrial and ovarian carcinomas. The other 2 exhibited CNAs only in either endometrial or ovarian carcinoma. All 5 tumors had 1 or more genetic mutations in the genes examined. One patient exhibited mutations both in *PIK3CA* and *PTEN* at discordant sites between endometrial and ovarian carcinomas, whereas the other 4 exhibited concordant mutations. Overall, 4 of the 5 patients were genetically diagnosed with SPM, and the remaining 1 with DP. The pathological diagnosis was not in agreement with the genetic diagnosis in 4 of the 5 patients.

Conclusions: Genome-wide genotyping diagnosis may represent a useful approach for distinguishing between SPM and DP in synchronous endometrial and ovarian carcinomas.

Key Words: Synchronous carcinomas, Endometrial cancer, Ovarian cancer, SNP arrays, Genetic diagnosis

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