The Influence of Infertility Treatment on the Prognosis of Endometrial Cancer and Atypical Complex Endometrial Hyperplasia

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Introduction: Many patients with endometrial cancer have no children when diagnosed, and thus are reluctant to undergo hysterectomy, hoping to preserve their fertility. Their requirement is met, at least partially, with high-dose medroxyprogesterone acetate that brings good response rate in the treatment of endometrial cancer in the early stage and atypical complex endometrial hyperplasia (EC/ACEH). Actually, a number of successful pregnancies after the conservative treatment have been reported. To conceive, many of them need infertility treatment because of ovulation disorders which might have induced the cancer with unopposed estrogens. However, on the other side, hyperestrogenic status caused by ovulation induction or controlled ovarian stimulation might promote the progression and the recurrence of the disease.

Objective: This study aimed to assess the effectiveness and safety of infertility treatment after conservative therapy for EC/ACEH, to confirm the significance of fertility-sparing therapy.

Methods: The patients with EC/ACEH who achieved complete response after high-dose medroxyprogesterone acetate were eligible for this retrospective study. Characteristics of the patients, whether they underwent infertility treatment, conceived, or relapsed, and the interval from complete response to conception or recurrence were retrospectively analyzed.

Results: The clinical outcomes of 36 patients were investigated. Twenty-six of them desired to conceive soon after complete response. All of them underwent infertility treatment, and 16 women delivered healthy babies. Kaplan-Meyer curve and log-rank test analysis revealed that women who achieved live birth had a significantly lower risk of recurrence than those without live birth. There was not a significant difference between the patients with and without infertility treatment.

Conclusions: Use of ovulation induction drugs after conservative treatment of endometrial cancer did not increase the recurrence of the disease. Moreover, resulting pregnancy seems to have an advantageous effect on the oncologic outcome.

Key Words: Endometrial cancer, Ovulation induction, Controlled ovarian stimulation, Recurrence

Received August 7, 2012, and in revised form October 23, 2012.
Accepted for publication November 1, 2012.

(*Int J Gynecol Cancer* 2013;23: 288–293)

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ISSN: 1048-891X
DOI: 10.1097/IGC.0b013e31827c18a1
The authors declare no conflicts of interest.

Endometrial cancer in women younger than 40 years represents approximately 5% of all cases.[1] The occurrence of the disease in women of reproductive age is related to long-term unopposed estrogen exposure, as in cases with polycystic ovary syndrome or other ovulation disorders. Atypical complex endometrial hyperplasia is a precancerous lesion and it can progress to endometrial cancer in several years.[2] Some of such cases might include endometrial cancer but cannot be detected at the first diagnosis.

The standard treatment of endometrial cancer in the early stage or atypical complex endometrial hyperplasia (EC/ACEH) includes hysterectomy and bilateral salpingo-oophorectomy. However, many patients younger than 40 years have no children at the diagnosis of the disease. They cannot accept the standard therapy and desire to preserve fertility. Recently, high-dose medroxyprogesterone acetate is reported as effective fertility-preserving therapy for early stage endometrial cancer and atypical complex endometrial hyperplasia.[3] Successful pregnancies after conservative treatment have been reported and most of them are the results of infertility treatment including assisted reproductive technology (ART).[4]

However, ovulation induction or controlled ovarian stimulation for infertility treatment can induce hyperestrogenic status, which might be implicated in the progression or recurrence of the disease. In the previous reports regarding successful pregnancies, several recurrent cases have been reported.[4,5] However, there has not been enough evidence about the influence of infertility treatment on the recurrence after conservative therapy for EC/ACEH.

Therefore, we retrospectively reviewed the clinical courses of 36 cases after complete response with high-dose medroxyprogesterone acetate. The purpose of this study was to assess the pregnancy rate after infertility treatment and the influence on the recurrence of EC/ACEH.

METHODS

This retrospective study was approved by the institutional review board of the University of Tokyo. We evaluated the patients with EC/ACEH who achieved complete response after high-dose medroxyprogesterone acetate and were followed up at the University of Tokyo hospital from January 1996 to January 2012.

After careful examination using ultrasound scan and magnetic resonance imaging to rule out the cases with myometrial invasion or adnexal involvement, total endometrial curettage was performed to determine the initial pathological diagnosis. Cases diagnosed as grade 1, stage Ia endometrial cancer, or atypical complex endometrial hyperplasia (FIGO classification in 1988), who were younger than 40 years and desired to preserve fertility were eligible for conservative treatment. They were given oral high-dose medroxyprogesterone acetate (600 mg/d) for 26 weeks. At 8, 16, and 26 weeks, cytodiagnosis or biopsy of the endometrium was conducted to check the efficacy of the treatment. Complete response was diagnosed when any hyperplastic or cancerous lesion was pathologically absent. Total endometrial curettage or hysteroscopic resection was performed again when the specimen showed remaining lesion. Cases diagnosed as progressive disease at any point stopped conservative treatment and hysterectomy was recommended. After 26 weeks, cases diagnosed as complete response received cyclic estrogen and progestin therapy for another 6 months to confirm complete response was maintained. Cases free of disease for 6 months could choose to attempt pregnancy. The patients who did not wish childbearing soon after achieving complete response continued cyclic estrogen and progestin or progestin only.

We recommended starting ovulation induction for women who wish to conceive soon after achieving complete response. Ovulation induction using clomiphene citrate or human menopausal gonadotropin, combined with timed intercourse or intrauterine insemination was initiated for the patients who had no history of infertility because many of them had ovulation disorder. The patients with other infertility factors, history of infertility treatment, or history of recurrence of EC/ACEH, were recommended to undergo ART to attain pregnancy as soon as possible. Oral contraceptive pills were given in the intervening cycles between ovulation induction cycles. The patients who no longer wish to conceive either after successful pregnancy or giving up infertility treatment were recommended to take oral contraceptive pills as long as any sign of recurrence was not detected or to undergo hysterectomy.

Characteristics of the patients, whether they underwent infertility treatment, conceived, or relapsed, and the interval from complete response to recurrence or pregnancy were retrospectively investigated. The duration of infertility was defined as the time from the first infertility treatment cycle to conception. The observational period [mean (SD)], from complete response to the latest follow-up, was 91.4 (46.2) months. Statistical analysis was performed using JMP 9 (SAS Institute, Cary, NC). The characteristics of the 2 groups—those with or without infertility treatment—were compared using Cochrane-Cox test. The characteristics of the 3 groups—those with infertility treatment resulting in live birth, those not resulting in live birth, and those without infertility treatment—were compared using Kruskal-Wallis test. The rate of recurrence was analyzed using Kaplan-Meier curve. The influence of infertility treatment and resulting live birth on recurrence was evaluated with log-rank test.

RESULTS

Thirty-six patients who achieved complete response after high-dose medroxyprogesterone acetate for 26 weeks followed by cyclic estrogen-progestin therapy for 6 months were eligible for this retrospective analysis. The age [mean (SD)] at the first diagnosis was 30.9 (5.8) years. Twenty-three cases were diagnosed as endometrial cancer and 13 cases as atypical complex endometrial hyperplasia. Only 1 woman had a child and all the others were nulliparous at the initial diagnosis. Body mass index (BMI) [mean (SD)] was 22.4 (4.5) kg/m^2 and 3 of 36 cases were obese (BMI $\geq$ 30 kg/m^2). Twenty-three cases had clinical background of irregular menstruation or amenorrhea.

Twenty-six of 36 patients desired to conceive soon after complete response and all of them underwent infertility treatment. Ten patients did not desire to conceive soon and

TABLE 1. Characteristics and pregnancy outcomes of the 2 groups—with or without infertility treatment after achieving complete response (n = 36)

	Infertility Tx	No Infertility Tx	
No. patients	26	10	
Age, mean (SD), y	31.8 (4.4)	28.4 (8.2)	NS
EC/ACH	16:10	7:3	NS
BMI, mean (SD), kg/m^2	22.1 (4.0)	23.2 (5.7)	NS
History of irregular menstruation	17 (65.4%)	6 (60.0%)	NS
No. patients who delivered babies	16 (61.5%)	—	
No. patients who delivered babies after ART	13	—	

NS, not significant; Tx, treatment.

continued to take oral conceptive pills. The characteristics and pregnancy outcomes of the 2 groups are shown in Table 1. The age at the first diagnosis, the ratio of endometrial cancer, BMI, and the ratio of patients with irregular menstruation were not significantly different between the 2 groups.

Eighteen (69.2%) of 26 patients who underwent infertility treatment conceived, and 16 of them delivered healthy babies. Live birth rate per patient was 61.5% and the duration [mean (SD)] of infertility was 12.2 (10.3) months. Fourteen (87.5%) of 16 successful cases conceived within 2 years. Of 23 women with history of irregular menstruation, 17 underwent infertility treatment, and 14 of them underwent ART. Meanwhile, 9 of 13 patients with regular menstruation underwent infertility treatment, and 7 of them underwent ART. In total, 21 women underwent ART, and 13 of them achieved live birth.

To investigate the relationship among infertility treatment, resulting pregnancy, and recurrence, we sorted the patients into 3 groups—women with infertility treatment resulting in live birth (group A), those not achieving live birth (group B), and those without infertility treatment (group C). Characteristics and oncologic outcomes of the 3 groups are shown in Table 2. The ratio of endometrial cancer and the duration to achieve complete response, both of which could be related to early recurrence of the disease, were not significantly different among the 3. Of 16 patients in group A, only 3 (18.8%) patients experienced recurrence, whereas 7 (70.0%) of 10 patients in group B did. In total, 10 (38.5%) of 26 patients who underwent infertility treatment experienced recurrence. Seven (70.0%) of 10 cases in group C experienced recurrence despite continuous administration of estrogen and progestin or progestin only. The recurrence rate of women in group A was significantly lower than that in group B or C ($P < 0.05$). The median time from complete response to recurrence was 20, 13, and 23 months in groups A, B, and C, respectively, not significantly different. Two of 3 women in group A, all 7 cases in group B and 5 of 7 patients in group C experienced recurrence within 2 years.

Of 17 recurrent cases, 9 patients underwent hysterectomy and 8 cases desired to preserve fertility. After close check-up with ultrasound scanning and magnetic resonance

TABLE 2. Oncologic outcomes of 3 groups—women achieving live birth, not achieving live birth after infertility treatment, and those who did not undergo infertility treatment

	Group A, Infertility Tx With Live Birth	Group B, Infertility Tx No Live Birth	Group C, No Infertility Tx	
Total no. patients	16	10	10	
Follow-up period (min-max, median)	31–158 (98)	15–138 (91)	28–186 (73)	NS
EC/ACH	10:6	6:4	7:3	NS
The duration to achieve CR (min-max, median)	8–56 (16)	8–26 (16)	8–26 (10)	NS
No. recurrent cases	3 (18.8%)	7 (70.0%)	7 (70.0%)	$P < 0.05$
The duration from CR to recurrence (min-max, median)	15–45 (20)	8–20 (13)	12–86 (23)	NS
No. recurrence within 2 y after CR	2	7	5	
No. recurrence more than 2 y after CR	1	0	2	
Additional conservative therapy for recurrent tumors	2	3	3	
CR after additional therapy	2	3	3	
No. successful pregnancies after recurrence	2	0	0	

CR, complete response.

imaging, they were given an additional high-dose medroxyprogesterone acetate therapy for 6 months. All 8 cases achieved complete response again. Five women attempted to conceive, and 2 of them achieved successful pregnancies after ART.

The remaining 1 case with recurrence in group A relapsed 45 months after complete response, 19 months after live birth and during oral estrogen and progestin therapy. She underwent hysterectomy.

Kaplan-Meier recurrence free curve of the 3 groups is shown in Figure 1A. Log-rank test analysis revealed that there is a significant difference of recurrence rate among the 3 groups ($P < 0.01$).

Kaplan-Meier recurrence free curve of those with or without live birth is shown in Figure 1B. Log-rank test analysis revealed that the recurrence rate of women with live birth (group A) is significantly lower than that of those without live birth, irrespective of infertility treatment (group B + group C) ($P < 0.01$).

Kaplan-Meier curve of those with or without any infertility treatment is shown in Figure 1C. Disease-free rates in both groups were apparently similar within 2 years. In the group with infertility treatment, recurrence after 2 years occurred in only 1 case, and disease-free rate remained at the level of 62%, whereas that in the group without any infertility treatment declined to 16.7%. However, log-rank test analysis revealed that there was no statistically significant difference between the 2 groups ($P = 0.17$).

The overall recurrence rate of group B (cases with infertility treatment not resulting in live birth) and group C (cases without infertility treatment) was the same (7/10, 70.0%). Although the disease-free rate in group B declined in a shorter period than that in group C, log-rank test analysis revealed that there was no statistically significant difference (Fig. 1A).

To investigate the relationship between the kind of infertility treatment and recurrence, clinical courses of 26 women who underwent any infertility treatment were investigated, regardless of whether pregnancy was obtained or not. Thirteen of them underwent ovulation induction other than ART at the beginning, including 8 women who proceeded to ART. Five of 13 patients experienced recurrence, 4 during clomiphene citrate administration and 1 during hMG administration. Of 18 women who underwent ART, excluding 3 patients who were submitted to ART after recurrence, 4 patients experienced recurrence. There was not a statistically significant difference of recurrence rate between ART and non-ART treatment groups.

Between 4 patients who experienced recurrence during ART and 14 patients who did not during ART, there was not a statistically significant difference of peak estradiol on day of hCG administration (2783 vs 3011 pg/mL) or maximum hMG dose during 1 cycle (1950 vs 2967 IU).

DISCUSSION

To the best of our knowledge, this is the first report comparing the recurrence rate of endometrial cancer or atypical complex endometrial hyperplasia between the patients with and without infertility treatment after conservative management.

Only a few studies have assessed the influence of infertility treatment on the development of endometrial cancer.[6] In the previous 2 cohort studies of infertile women, standardized incidence ratio for endometrial cancer was higher among women with infertility treatment than those with no treatment.[7,8] Althuis et al[9] showed that clomiphene citrate might increase endometrial cancer risk. However, most of the patients in their studies undergoing infertility treatment have ovulatory dysfunction, which is a well-known risk factor for the incidence of endometrial cancer.[7] Chao et al[10] compared clinical outcomes of those who achieved pregnancy after conservative treatment of endometrial carcinoma between cases with and without in vitro fertilization. The rate of metastasis or recurrence was not increased in cases with in vitro fertilization. However, those who failed to conceive or did not desire to conceive were not included in their study. Therefore, the role of ovulation induction drugs in the development of endometrial cancer remains to be fully elucidated.

Recently, many women younger than 40 years diagnosed as EC/ACEH desire to preserve fertility and choose

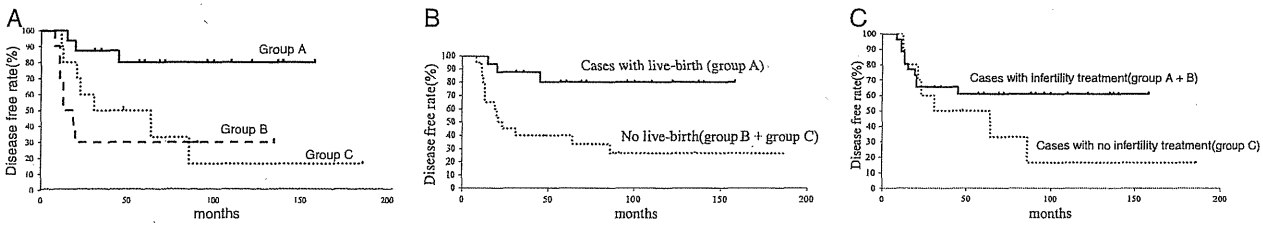

FIGURE 1. A, Kaplan-Meier disease-free curve of the 3 groups—women with infertility treatment resulting in live birth (group A), those not achieving live birth (group B), and those without infertility treatment (group C). There is a significant difference of recurrence rate among the 3 groups ($P < 0.01$), whereas there is no significant difference between groups B and C. The comparison of the disease-free curve was performed using log-rank test. B, Kaplan-Meier disease-free curve of the patients with and without live birth. Patients with live birth (group A, n = 16) present significantly better prognosis ($P < 0.01$), compared with those without live birth (groups B + C, n = 20), regardless of infertility treatment. C, Kaplan-Meier disease-free curve of cases with (groups A + B, n = 26) and without (group C, n = 10) infertility treatment, regardless of live birth. There is no statistically significant difference between 2 groups ($P = 0.17$).

conservative treatment. Several both successful pregnant and recurrent cases after conservative treatment have been reported but there have been no data regarding the influence of infertility treatment on the recurrence of endometrial cancer.

The recurrence rate after conservative treatment of endometrial cancer or atypical complex endometrial hyperplasia is reported to be 33.8% to 47% according to the multicenter trial in Japan[3] and previous reviews.[11,12] Mean duration to recurrence is 20 to 47.9 months.[3,11] In this study, including 26 patients undergoing infertility treatment and 10 patients who had continuous estrogen and progestin pills, the overall recurrence rate was 47.2%, a comparable result to previous reports. Kaplan-Meier analysis revealed that there is no apparent difference of recurrence rate between the 2 groups within 2 years after initiating infertility treatment (Fig. 1C). After 2 years, recurrence occurred more often among the women without infertility treatment, probably because live births after infertility treatment had inhibitory effects on the recurrence, as shown in Figure 1B. Log-rank test analysis revealed no significance possibly because of the small number of patients. The overall recurrence rate was 70% in cases without pregnancy regardless of whether they were treated with infertility treatment, although the disease-free rate in cases with infertility treatment seemed to decline in a shorter period. Clinical backgrounds related to recurrence, such as pathological diagnosis and the duration to achieve complete response were not significantly different between these groups (Table 2). The recurrence risk seemed not to be influenced by the kind of infertility treatment, peak estradiol level, or total gonadotropin dose per cycle in our limited number of cases. These results indicate that ovulation induction drugs seem not to increase the recurrence rate. To confirm the favorable effect of infertility treatment on the recurrence of EC/ACEH in the long-term, a larger scale study including a large number of pregnant women will be necessary.

We did not use tamoxifen or letrozole for ovulation induction drugs in this study. These drugs have been reported to be useful in ovulation induction at the same time as antitumor effect against estrogen-sensitive cancer.[13,14] They can induce follicle growth with relatively low estradiol levels, and may be useful as ovulation induction drugs in cases with infertility treatment after conservative therapy for endometrial cancer.

We achieved a high live birth rate per patient (61.5%) for the patients who desire to conceive after conservative treatment. Thirteen (81.3%) of 16 successful cases achieved live birth after ART. In the previous results regarding clinical pregnancies, successful pregnancy rate per patient was around 50%, and most of the cases were treated with infertility treatment.[4,5,15–17] In these reports, 12 (54.5%) of 22 successful cases conceived after ART (data not shown in 1 report). We recommended undergoing ART soon after achieving complete response for the patients with other infertility factors, history of infertility treatment, or history of recurrence. Our treatment protocol could achieve a high live birth rate compared with the previous reports.

Additional medroxyprogesterone acetate therapy for recurrence achieved good response rate in this study. Ushijima et al[3] reported medroxyprogesterone acetate therapy was conducted again for 8 in 14 recurrent cases and 6 cases achieved initial response, but lesion recurred in 5 patients. We here showed 2 successful pregnant cases after recurrence and additional medroxyprogesterone acetate therapy. Both cases experienced recurrence after infertility treatment other than ART. After complete response again, they underwent ART and achieved successful pregnancy. Only a few pregnant cases after recurrence have been reported so far.[18,19] Our result shows that conservative therapy for recurrent EC/ACEH and subsequent infertility treatment is feasible in carefully selected patients.

Recurrence after successful delivery is considered to be rare. We here report a patient who relapsed 19 months after normal singleton delivery with clomiphene citrate therapy. Elizur et al[4] reported a recurrent case 12 months after delivery and Niwa et al[16] also reported 2 recurrent cases 18 and 51 months after delivery, respectively. After finishing breastfeeding, we need to recommend prompt initiation of cyclic estrogen and progesterone therapy and close follow-up in the same way as before pregnancy.

In summary, our results suggest that hyperestrogenic state after controlled ovarian stimulation or ovulation induction does not increase the overall recurrence of EC/ACEH after conservative treatment and that women with live birth has significantly lower risk of recurrence than those without live birth. Therefore, improvement of pregnancy rate with the aid of infertility treatment including ART can improve long-term prognosis of EC/ACEH.

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Gynecologic Oncology 128 (2013) 209–214

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

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.

ARTICLE INFO

Article history:
Received 26 September 2012
Accepted 11 November 2012
Available online 16 November 2012

Keywords:
Lymphaticovenular anastomosis
Lymphocele
Pelvic lymphadenectomy
Supermicrosurgery

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.

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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0090-8258/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygyno.2012.11.014

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.

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 hydronephrosis 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

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.

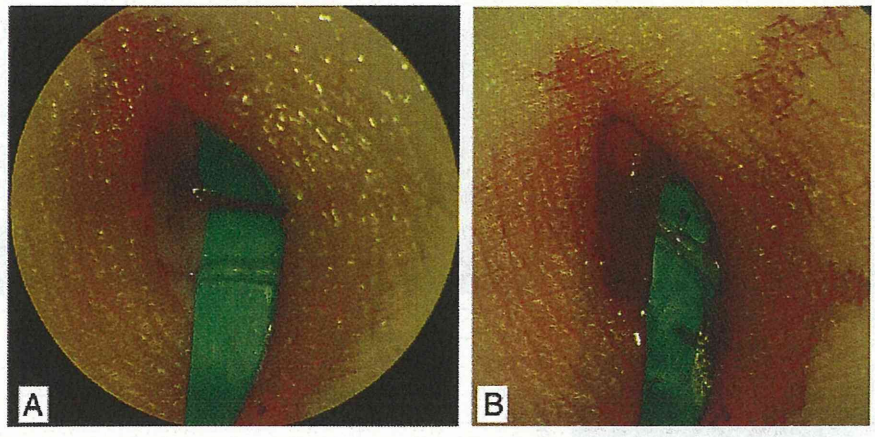

Fig. 1. Lymphaticovenular anastomosis. (A) Pre-anastomosis image. The upper vessel is a vein; the lower image is a lymphatic channel. (B) Post-anastomosis image. The anastomosis was performed with 5 sutures of 11–0 nylon. The left side of the anastomosis looks clear because the lymphatic fluid is under 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

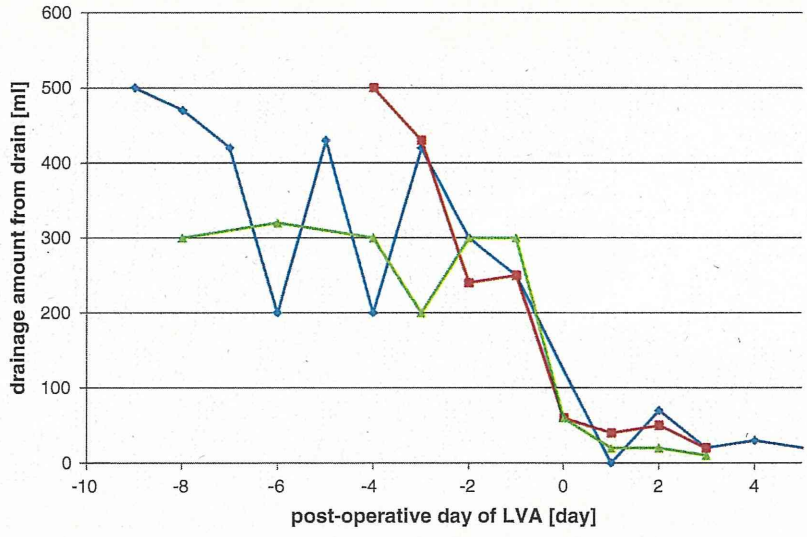

Fig. 2. Daily drainage from 3 patients who had preoperative placement of percutaneous drains. Note the dramatic decrease in drainage following LVA.

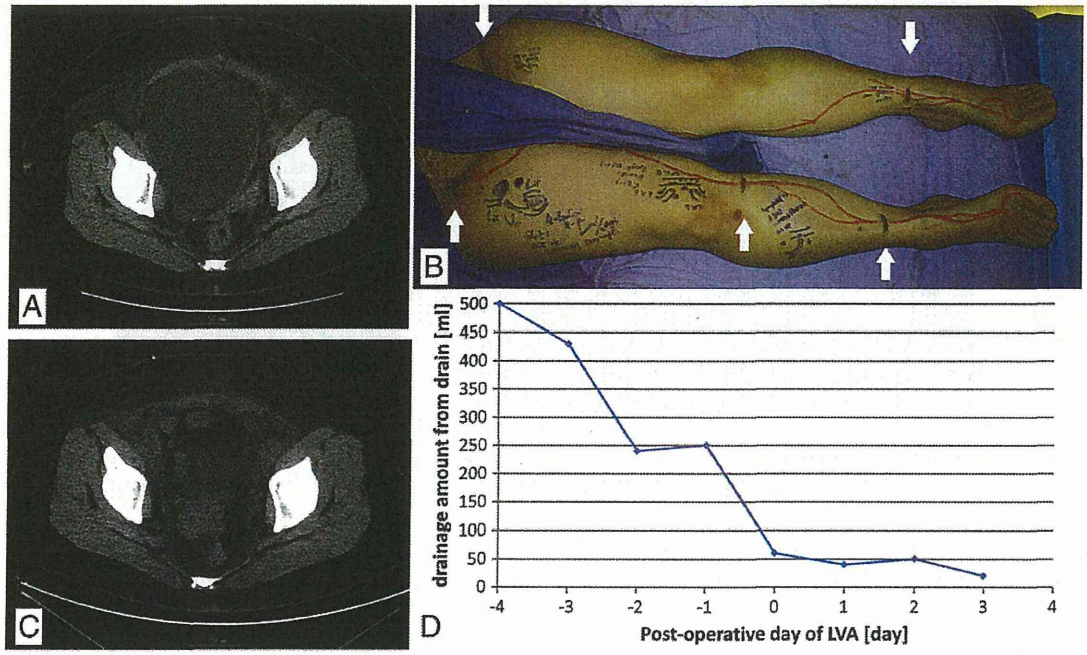

Fig. 3. Resolution of pelvic lymphocele after LVA. (A) CT scan of a large pelvic lymphocele after gynecologic surgery. (B) Immediate postoperative view. Seven anastomoses were performed through five 3-cm incisions under local anesthesia. (C) A CT scan of the same patient 3 days after LVA demonstrates 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 venulae. 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

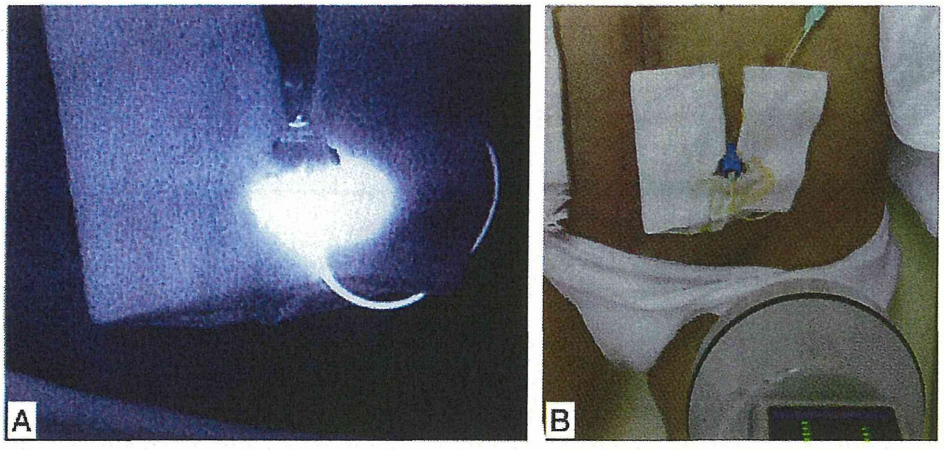

Fig. 4. Demonstration of lymphatic flow from the leg into a pelvic lymphocele. (A) Fluorescence lymphatic image of a percutaneous catheter, 5 min after injecting ICG into the first dorsal web space of the foot, indicates the lymphatic flow from the leg rapidly entering the lymphocele. It also indicates 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.

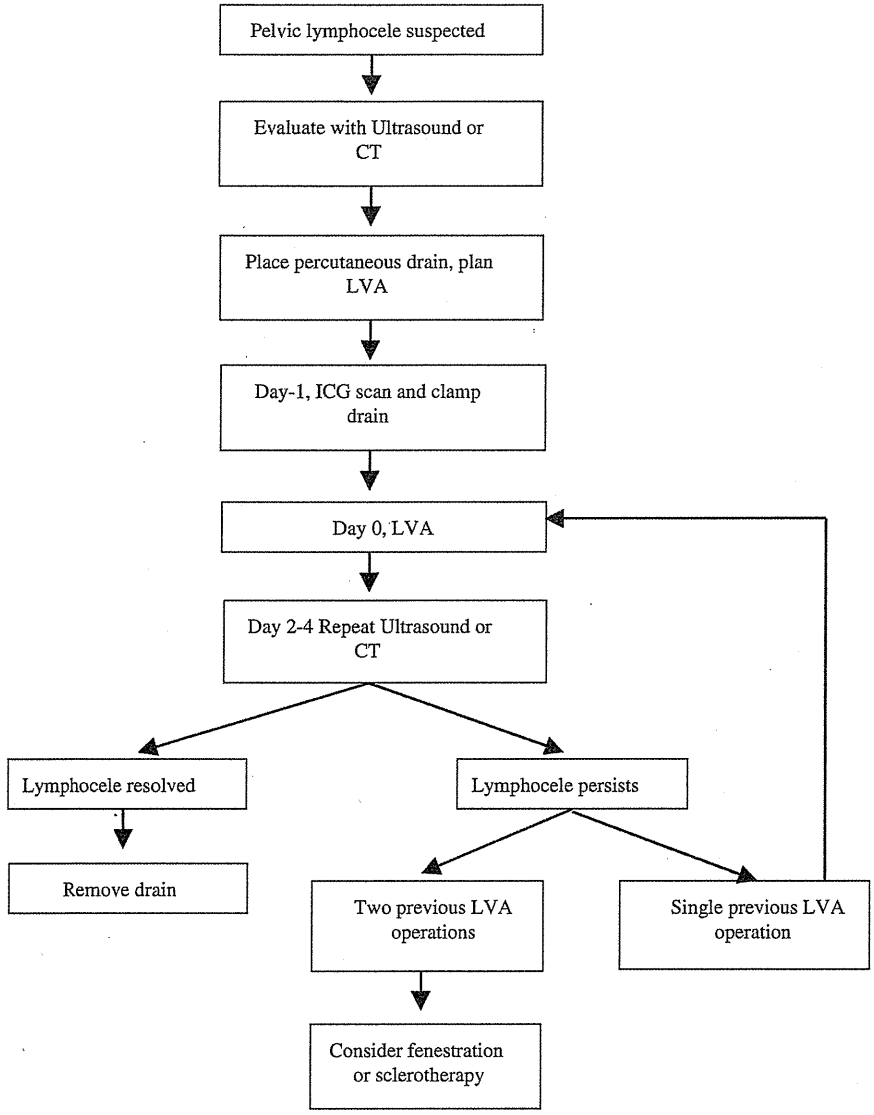

Fig. 5. Algorithm for the management of pelvic lymphocele.

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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Int J Clin Oncol (2013) 18:1091–1101
DOI 10.1007/s10147-012-0486-5

ORIGINAL ARTICLE

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

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Received: 14 May 2012 / Accepted: 1 October 2012 / Published online: 25 October 2012

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 °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).

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 and . In non-smokers (Table), 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 . 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).

Effect of serum micronutrients and nutrient intake in progression subjects

In Table , 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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 μg/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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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 . The units of micronutrients are expressed as μg/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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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 . The units of micronutrients are expressed as μg/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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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							*p* 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 μg/dL