VITAMIN B_{12} STATUS OF THAI WOMEN WITH NEOPLASIA OF THE CERVIX UTERI

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Abstract. The vitamin B_{12} statuses of Thai women with high- and low-grade cervical dysplasia were studied and compared with women with normal cytological smears. Serum vitamin B_{12} and vitamin B_{12} intakes were assessed, as well as demographic characteristics, sexual behavior, reproductive and menstrual history, exogenous hormone use, personal and familial medical history, smoking habit, and other risk factors. The presence or absence of genital HPV DNA was determined by polymerase chain reaction (PCR). Serum vitamin B_{12} levels in women with normal cytological smears were significantly higher than those with both high- and low-grade cervical dysplasia ($p<0.001$). Low vitamin B_{12} serum levels were significantly statistically associated with increased low-grade (OR = 4.08; 95% CI = 1.41-11.79; $p< 0.05$) and increased high-grade cervical dysplasia risk (OR = 3.53; 95% CI = 1.24-10.04; $p< 0.05$) for the highest *vs* lowest quartiles of serum vitamin B_{12}. This study indicated a relationship between low vitamin B_{12} status and increased risk of cervical cancer.

INTRODUCTION

Cervical cancer is an important health problem for women. Despite a substantial decrease in the incidence and mortality of cervical carcinoma over the past 40 years, it remains a significant health problem in Thailand (Siriraj Cancer Center, 1999). Identified risk factors for cervical neoplasia suggest a multifactorial etiology, *ie* parity, oral contraceptive use, multiple sexual partners, early age at first intercourse, cigarette smoking, with several human papillomaviruses (HPV) as the central cause (Miller, 1992; IARC, 1995). Evidence suggests that various factors, including diet, are likely to contribute to the risk of persistent HPV infection and the progression of neoplasia to cervical cancer. Research of the past two decades has suggested a role for nutrients in preventing cancer. Several studies have identified various dietary substances with antioxidant properties that have an inhibitory effect on the development and/or progression of cancer. Moreover, these studies have shown that many substances *eg* carotenoid, lycopenes, vitamins A, E, and C, selenium and phenol-containing dietary substances, have effects that extend beyond their antioxidant properties (Liebler and McClure, 1996; Potishman and Brinton, 1996; Glade, 1999; Willis and Wians, 2003).

Low vitamin C and carotenoid statuses are associated fairly consistently with both cervical cancer and precursor, whereas the results of vitamin E status are less constituent (Buckley *et al*, 1992; Van Eenwyk *et al,* 1992; Giuliano *et al*, 1997). It has been hypothesized that low folate may modulate cancer risk, notably the risk of cervical and colorectal cancer, breast cancer, including a rapidly growing number of other cancer sites, such as the lung, pancreas, stomach, esophagus, blood-forming organs, skin, and endometrium (Glynn and Albanes 1994; Duthie, 1999). In cervical cancer associated with HPV, it is suggested that folate deficiency facilitates the incorporation of viral genetic material into the host cells. A preliminary trial in smokers with precancerous lesions of the lung indicated that folic acid (10 mg) and vitamin B_{12} (500 μg) supplementation may be useful in the treatment of this kind of dysplasia (Basu and Dickerson, 1996; Heimburger *et al*, 1988). Shannon *et al* (2002) found a high intake of food rich in vitamin A, particularly high retinal foods, were associated with a reduced risk of *in situ* cervical dysplasia and invasive cervical cancer in Thai women, but no association between folate intake and the risk of this disease has been substantiated.

A relationship between low folate status and risk of cervical cancer was found in our study with the same study group (Kwanbunjan *et al*, 2005). In this study we conducted an association between vitamin B_{12} status and risk of cervical neoplasia in Thai women. The study population was out-patients registered at the National Cancer Institute, Bangkok, Bangkok Metropolitan

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MATERIALS AND METHODS

Subjects

Women, aged 17-70 years, who obtained gynecological examinations and Papanicolaou (Pap) smear outcomes constituted the screening basis for identifying healthy controls and cases. All cases were defined with positive Pap smear and obtained additional colposcopy and biopsy examinations after a repeat positive Pap smear, whereas control subjects in this study were screened as the subset of women free from any prior gynecologic disease or known dysfunction, and particularly found negative by Pap smear and had no other serious illness.

Study methods

Heparinized blood samples from all of the studied groups were collected to determine hemoglobin and hematrocrit. Serum vitamin B_{12} and folate were measured in duplicate with radio-binding assay using commercial kits (Dualcount Solid Phase No Boil Assay for Folate, Diagnostic Products Corporation DPC, Los Angeles). Well-trained academic staff conducted interviews with structured questionnaires to obtain detailed information on demographic characteristics, sexual behavior, reproductive and menstrual history, exogenous hormone use, personal and familial medical history, smoking and diet. Twenty-four hour recall was used to estimate vitamin B_{12} intake, and was calculated by the computer program WINNBIS 1997, the food composition database was provided by the Nutrition Division, Department of Health, Ministry of Public Health (2001) and the Folate Food Table of Areekul (1986). The subjects were also asked to complete the 85-item Food Frequency Questionnaire. Cervical smear cytology obtained for histological diagnosis and colposcopy directed biopsy investigations provided confirmation. A physician performed a cervicovaginal scraping for all subjects for test cells for the existence of genital human papillomavirus DNA using polymerase chain reaction (PCR).

Statistical analysis

Statistical analyses were conducted using Strata version 5.0 for Windows. Correlations were measured with Spearman Rank correlation coefficient Kruskal Wallis analysis of variance and multiple comparison were used to compare characteristic variable medians. The odds ratio (OR) was the measure of association used to estimate the relative risk of cervical cancer. The highest quartile was used as the referent, or

Table 1
Vitamin B_{12} status and hematological parameters of the study group.

Variable	Low-grade				High-grade				Control		
	Median (range)	95%CI	n	p^a	Median (range)	95%CI	n	p^a	Median (range)	95%CI	n
Serum Vitamin B_{12} (pmol/l)	279.97 (12.68-806.47)	264.74-371.42	44	0.000	292.38 (47.15- 910.75)	294.25-388.62	70	0.000	445.22 (75.90-1491.14)	428.92-529.40	95
Vitamin B_{12} intake (µg/d)	3.20 (0.44-19.20)	3.08-5.10	44	0.000	3.60 (0.29-10.92)	3.50-4.84	70	0.000	1.97 (0-38.87)	1.98-4.21	95
Hb(g/l)	129.5 (100.0-152.0)	123.6-131.4	44	0.791	128.0 (71.0-148.0)	120.9-129.1	69	0.460	129.5 (81.0-160.0)	123.6-130.7	95
Hct	38.8 (31.0-44.0)	37.2-39.4	44	0.574	38.0 (24.0-44.0)	36.6-38.8	69	0.055	39.8 (27.1-46.0)	38.0-39.8	95

Kruskal-Wallis analysis of variance and multiple comparison; [a]p-value for comparison of case and control group; NS between low and high grade CIN in serum Vitamin B_{12}, Hb, Hct; and NS between control and low; control and high grade CIN in Hb, Hct comparison group. Logistic regression was used to obtain maximum likelihood estimates of the OR and 95% confidence intervals (CI), while adjusting for potential confounders. The comparison of food frequency among the study groups was performed by chi-square.

RESULTS

The study groups were compared; the case group was divided into high- and low-grade CIN according to Pap smear classification. Serum vitamin B_{12}, daily vitamin B_{12} intake and hematological data were expressed as median, range and 95% confidence interval (CI), as shown in Table 1. The serum vitamin B_{12} levels of both case groups were significantly lower than the control group. By contrast, the daily vitamin B_{12} intake was significantly higher than the control group. The medians for vitamin B_{12} for all study groups was >150 pmol/l, the cut-off point suggested by WHO (1972). The criterion for vitamin B_{12} deficiency is a serum vitamin B_{12} level lower than this cut-off point. However, the percentages of vitamin B_{12} deficiency in both case groups were relatively high, and vitamin B_{12} deficiency was found to be 11.27% in high grade CIN and 18.18% in low grade CIN, whereas only 3.16% was found in the control group. Hemoglobin and hematocrit showed no statistical difference among the study groups. The dietary reference intake for Thais (2003) is recommended as 2.4 μg/d for male and female adults (Nutrition Division, Department of Health, Ministry of Public Health, 2003). The socioeconomic levels and educational backgrounds of the control group were considerably higher than both case groups; hence, they had better jobs and earned more. Most of the subjects did not smoke and there were no significant differences between the control and case groups. Regarding the reproductive risk factors of the case groups, contraceptive use was markedly higher, more had sexual intercourse and gave birth earlier, and were more likely to have a number of sexual partners, than the control group. The incidence of abortion among the case groups was not significantly different from the control. The percentage of HPV infection confirmed positive was higher in both cases, and cases with high-grade CIN were statistically higher than the control group (p=0.010).

Vitamin B_{12} is a water-soluble vitamin, found only in animal foods. Table 2 shows the vitamin B_{12} food sources consumed by the study group. There were no significant differences in these food sources among the study groups, except for fish, which the control group consumed much more frequently than both case groups. The Odds ratios (OR) with the multivariate-adjusted model were calculated and the results are shown in Table 3. The risk of low-grade CIN was markedly elevated in the lower class serum vitamin B_{12} tertile compared with the highest serum vitamin B_{12} tertile (serum vitamin B_{12} = 276.62-452.36 pmol/l, OR=3.11, 95%CI=1.04-9.28 and serum vitamin B_{12} <276.62 pmol/l, OR=4.08, 95%CI=1.41-11.79). Likewise, the high-grade CIN group with lower serum vitamin B_{12} indicated increased cervical dysplasia risk, but the OR in the lowest class serum vitamin B_{12} tertile was slightly less (serum vitamin B_{12} = 276.62-452.36 pmol/l, OR=2.82, 95%CI=1.0-8.25 and serum vitamin B_{12} <276.62 pmol/l, OR=3.53, 95%CI=1.24-10.04). Statistical significance was also found in the Odds ratios (OR) with the multivariate-adjusted model of low and high class dietary vitamin B_{12}.

DISCUSSION

Our findings indicated a relationship between low vitamin B_{12} status and risk of cervical change in this study group, although not found in other cervical cancer studies (Butterworth *et al*, 1992; Thomson *et al*, 2000). Nevertheless, population-based observations that have implicated diminished vitamin B_{12} status as a potential risk factor for certain cancers are generally recent. The animal experimental model of Choi *et al* (2004) suggested that a vitamin B_{12} deficient diet, which was of insufficient severity to cause anemia or illness, created aberrations in both base substitution and methylation of colonic DNA, which might increase susceptibility to carcinogenesis. Lower plasma levels of vitamin B_{12} were identified as an independent risk factor in large epidemiologic studies of breast cancer (Wu *et al*, 1999; Zhang, 2003); in addition, in an examination of lung tissue from squamous cell carcinomas compared with adjacent normal tissue, tissue concentrations of vitamin B_{12} were diminished in the carcinoma (Piyathilake *et al*, 2000). Evidence to date for such a relation in the colorectum is modest, but suggestive. A population-based cohort study observed that patients with pernicious anemia had a fourfold increased risk for developing colorectal adenocarcinoma, but only in the 5 years after initial diagnosis (*ie*, the period immediately after correction of their vitamin B_{12} deficiency) (Talley *et al*, 1989). More recently, a large prospective cohort study observed that the occurrence of cancer in the proximal colon was significantly lower among those with both high folate and high vitamin B_{12} intakes, an effect not observed with high folate intake alone (Harnack *et al*, 2002).

Both case groups had significantly lower serum vitamin B_{12} levels than the control group and the percentages were higher, whereas the daily intake

Table 2
Frequency of animal foods as vitamin B_{12} sources consumed by the study group.

Food/frequency	Low grade CIN			High grade CIN			Control	
	No.	%	p[a]	No.	%	p[a]	No.	%
Pork		0.114			0.008			
Every day	9	20.5		1	1.4		30	31.6
Every 2-3 days	24	54.5		12	17.1		49	51.6
Every week	4	9.1		34	48.6		8	8.4
Every month	4	9.1		11	15.7		6	6.3
Several months	1	2.3		12	17.1		2	2.1
Do not eat	2	4.5		-	-		-	-
n	44			70			95	
Beef			0.918			0.321		
Every day	1	2.3		-	-		2	2.1
Every 2-3 days	2	4.5		3	4.2		5	5.3
Every week	2	4.5		5	7.0		4	4.2
Every month	5	11.4		13	18.3		19	20.0
Several months	6	13.6		3	4.2		6	6.3
Do not eat	28	63.6		46	64.8		59	62.1
n	44			70			95	
Poultry		0.539			0.565			
Every day	-	-		-	-		-	-
Every 2-3 days	20	45.5		1	1.4		3	3.2
Every week	12	27.3		30	42.3		39	41.1
Every month	5	11.4		15	21.1		28	29.5
Several months	3	6.8		23	32.4		16	16.8
Do not eat	4	9.1		1	1.4		9	9.5
n	44			70			95	
Fish		0.023			0.002			
Every day	5	11.4		6	8.5		27	28.4
Every 2-3 days	28	63.6		49	69.0		53	55.8
Every week	7	15.9		7	9.9		8	8.4
Every month	3	6.8		5	7.0		4	4.2
Several months	1	23		1	1.4		2	2.1
Do not eat	-	-		2	2.8		1	1.1
n	44			70			95	
Shrimp		0.081			0.884			
Every day	2	4.5		-	-		-	-
Every 2-3 days	5	11.4		13	18.3		25	26.3
Every week	9	20.5		7	9.9		18	18.9
Every month	21	47.7		37	52.1		42	44.2
Several months	5	11.4		5	7.0		7	7.4
Do not eat	2	4.5		8	11.3		3	3.2
n	44			70			95	
Squid		0.954			0.043			
Every day	3	6.8		10	14.1		-	-
Every 2-3 days	5	11.4		9	12.7		12	12.6
Every week	10	22.7		38	53.5		20	21.1
Every month	15	34.1		8	11.3		38	40.0
Several months	6	13.6		5	7.0		9	9.5
Do not eat	5	11.4		-	-		16	16.8
n	44			70			95	

[a]p-value for the chi-square test for a difference between case and control

Table 3
Relative risk (OR) and 95% confidence interval (95% CI) of the study group in relation to vitamin B_{12} status.

Variable	Cases/ controls	Low grade CIN OR (95%CI)	Cases/ controls	High grade CIN OR (95%CI)
Serum vitamin B_{12} (pmol/l)				
> 452.36	8/46	1	16/46	1
276.62-452.36	15/30	3.11 (1.04-9.28)	24/30	2.82 (1.0-8.25)
<276.62	21/19	4.08 (1.41-11.79)	31/19	3.53 (1.24-10.04)
Trend OR (p)		0.026		0.048
Vitamin B_{12} intake (μg/d)				
> 4.58	14/12	1	26/12	1
1.22-4.58	25/48	0.43 (0.14-1.33)	32/48	0.28 (0.09-0.85)
<1.22	5/35	0.11 (0.02-0.48)	12/35	0.1 (0.02-0.38)
Trend OR (p)		0.013		0.004

OR adjusted for age, sexual partner, frequency of sexual activity, age at first intercourse and first child, contraceptive use, socioeconomic status, HPV infection and serum folate.

of vitamin B_{12} was statistically higher. Vitamin B_{12} deficiency or low vitamin B_{12} levels can be found in strict vegetarians or vegans, but none of this study group practiced vegetarianism. Low vitamin B_{12} status may be due to low vitamin B_{12} absorption and its bioavailability in some groups with cervical dysplasia. Failure of B_{12} absorption may result from atrophy of the gastric mucosa with evidence of IF deficiency, while some patients have antibodies that react with IF. Situations such as chronic gastritis, gastrectomy, iron deficiency and thyroid dysfunction could affect IF secretion, leading to low vitamin B_{12} and deficiency of this vitamin (Basu and Dickerson, 1996). The major limitation of our study was that we did not investigate vitamin B_{12} absorption and its bioavailability.

Vitamin B_{12} deficiency is often accompanied by folate deficiency. In the same instance as folate deficiency, vitamin B_{12} deficiency causes hematological damage resulting from lack of adequate N^5N^{10}-methyl FH_4, which delivers its methyl group to deoxyuridylate to synthesize thymidylate, and thus to synthesize DNA. Another possible interrelationship of folate and vitamin B_{12} appears to be vitamin B_{12} aiding in the cellular retention of folate. The decreased synthesis of folypoly glutamates, folate tissue storage, has been reported to be associated with vitamin B_{12} deficiency (Shane and Stokstad, 1985; Basu and Dickerson, 1996). Our study with the same study group indicated that low folate status was related to the risk of cervical change (Kwanbunjan *et al*, 2005). This study also suggested that promotion of cervical dysplasia may result from low vitamin B_{12} and folate status.

In conclusion, besides risky sexual behaviors, smoking, contraceptive use, HPV infection and low folate status as potential risk factors for cervical cancer, low vitamin B12 status was found to be associated with the risk of cervical dysplasia in Thai women in this study. Thai women should be educated and encouraged to consume more foods with available folate and vitamin B12, as well as other antioxidant vitamins.

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