Folate and vitamin B12 levels in abnormal pap smears: a case control study

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Summary

Objective: This study aimed to explore the risk of abnormal cervical cytology in relation to serum folate and vitamin B12 levels, and demographic characteristics. *Patients and Methods:* A case-control study was carried out among women attending the gynecology clinic for cervical cytology screening. At the follow-up clinic visit, fasting blood samples were collected from 103 cases with abnormal cervical cytology of the cervix and 175 controls with cytological normal smears and serum folate and vitamin B12 levels were measured. Data from cases and controls were compared. Logistic regression analysis was used to estimate the relative odds of abnormal cytology with increasing vitamin B12 levels. *Results:* There were no significant differences between cases and controls in demographic characteristics and mean folate levels ($p > 0.05$). The mean vitamin B12 level of cases was significantly lower than that of controls ($p < 0.001$). Dichotomized logistic regression analyses of vitamin B12 levels were significantly different ($p < 0.001$). The predicted percentages at a cut-off value of 0.5 were as follows: sensitivity 70.0%, specificity 74.6%, positive predictive value 71.8% and negative predictive value 72.6%. The logistic regression analysis of the 1st and the 4th quartiles of vitamin B12 levels showed a significant difference $p < 0.001$, OR: 1.525 (CI, 1.175-1.875). *Conclusion:* The results of this study suggest that lower vitamin B12 levels are associated with abnormal cervical cytology. It is recommended that women should consume not only folate-rich foods such as fruits and vegetables but also vitamin B12-rich foods such as meat, fish, milk products and eggs in a balanced way.

Key words: Pap smear; Preinvasive lesion; Cancer; Folate; Vitamin B12.

Introduction

Cancer of the cervix remains the second most common cancer among women in the world, accounting for about 10% of all new cancer cases. Approximately 471,000 cases are newly diagnosed and 233,000 women die each year from cervical cancer, most of them in less-developed countries [1].

From a clinical perspective, it is important to determine cervical cytological abnormalities and treat them before invasive cancer occurs. Mortality rate has decreased by 75% due to screening programs [2]. It is better to determine the etiology of abnormal cytology and remove the risk.

There have been many risk factors blamed for the etiology of cervical precancerous and cancerous lesions of the cervix. Among them is human papillomavirus (HPV) infection, younger age at first sexual activity, multiple sexual partners, oral contraceptives, cigarette smoking, lack of hygiene and no usage of barrier methods [3]. When HPV is present, smoking doubles the risk of progression to cervical intraepithelial neoplasia (CIN)-3 [4].

Concerning cervical cancer and diet, early epidemiologic research on diet and cervical cancer focused mainly on invasive disease. Available evidence was reviewed in the 1990s [5, 6]. There is evidence that folate, retinol and vitamin E is probably protective against cervical neoplasia and that vegetables containing vitamin C, vitamin B12, alpha-carotene, beta-carotene, lycopene, lutein/zeaxanthin and cryptoxanthin possibly protect against it. However, it is expected that risk factors may change depending on the eating habits and sociocultural differences of societies. Therefore, it is important to determine the association between abnormal cervical cytological findings and nutrients and sociocultural characteristics in terms of preventive medicine.

It was assumed that people living in the region where the study was conducted ate mostly vegetables rather than foods derived from animals. With the folate from vegetables and vitamin B12 from animal-based foods, diminished folate status may disrupt DNA synthesis and repair mechanisms, and may influence gene expression through abnormal DNA and RNA methylation [7]. Furthermore, the metabolic pathway involved in DNA methylation requires the presence of other micronutrients like vitamin B12 as cofactors [8]. We therefore aimed to explore the risk of abnormal cervical cytology in relation to serum nutrient factors including folate and vitamin B12 and demographic characteristics.

Patients and Methods

We conducted a case-control study among women attending the Obstetric and Gynaecology Clinic of Adnan Menderes University Hospital in the south-west of Turkey for cervical cytological screening between May 2003 and January 2005. Eligible cases and controls included women aged 18 years and older and residing in this region. Women who had a history of chronic illnesses, a prior hysterectomy, multipartnership and a previous

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diagnosis of cervical dysplasia within the past three years or who were currently pregnant, in more than six months of their postpartum period or lactating, taking vitamins or mineral supplements or having a special diet within the past six months or consumed alcohol were ineligible to participate. One hundred and three women with abnormal cervical cytology and 175 women attending the same clinic with negative cytological smears participated in the study. Written informed consent was obtained from all subjects.

Through face-to-face questionnaires conducted at baseline, data on age, parity, age of first intercourse, education level, oral contraceptive and intrauterine device usage and duration, route of deliveries, and history of cigarette smoking were obtained. During baseline gynecological examinations, exfoliated cervical cells were collected from the subjects.

Subjects were advised not to take vaginal medications, have vaginal douches or have sex 24 hours before smear collection. All smears were collected between 10 and 18 days of the menstrual cycle. Thin sections of the smears were spread on the slides. The 2001 Bethesda System of nomenclature was used throughout this study to describe the categories of epithelial cell abnormalities. These were atypical squamous/glandular cells of undetermined significance (ASCUS/AGUS), low-/high-grade squamous intraepithelial lesion (LSIL/HSIL), atypical squamous cells- high-grade squamous intraepithelial lesion (ASC-H) and cervical cancer.

At the follow-up clinic visit, fasting blood samples from the participants were collected, coagulated and then centrifuged to obtain sera. All samples were processed within one hour of collection and stored at -70°C until nutrient analyses were conducted. Serum folate and Vit-B12 levels were measured by the Corning Magic Lite Folate Immunoassay System (serum folate and vitamin B12 levels were determined with the CIBA Corning Magic Lite Folate Immunoassay System (Norwood, MA, USA).

Chi-square and independent sample t-tests were used when indicated. Logistic regression analysis was used to estimate the relative odds of abnormal cytology with increasing levels of vitamin B12 levels (cut-off value of 0.5). Also, vitamin B12 levels were categorized into quartiles and the quartile cut-off points were based on their distributions in controls with 95% confidence intervals (CIs) for the highest-to-lowest quartile comparison. $p < 0.05$ was accepted as significant in this study. SPSS 13.0 (Statistical Package for Social Sciences13.0) for Windows was used for statistical analyses.

Results

The distributions of abnormal cytologies are given in Table 1. The subject characteristics and comparisons of serum folate and vitamin B12 levels between cases and controls are shown in Table 2. There were no significant differences between cases and controls in age, parity, age at first intercourse, education, history of taking oral contraceptive and using intrauterine devices, route of delivery, cigarette smoking and mean folate levels ($p > 0.05$). The mean vitamin B12 level of cases was significantly lower than that of controls ($p < 0.001$).

Table 1. — *Distribution of abnormal Pap smears.*

Cytology	n = 103 (%)
ASCUS	47 (45.7)
LSIL	40 (38.9)
HSIL	6 (5.8)
ASC-H	2 (1.9)
AGUS	6 (5.8)
Cervix cancer	2 (1.9)

Table 2. — *Comparison of the demographic characteristics, serum folate and vitamin B12 levels in cases with abnormal cervical cytology and controls.*

Variables	Cases (n = 103)	Controls (n = 175)	p value
Mean age (years)	44.3 (± 8.8)	45.5 (± 9.5)	NS
Parity			NS
0	4 (3.9)	13 (7.4)	
1-2	71 (69)	107 (61.2)	
≥ 3	28 (26.1)	55 (31.4)	
Age of first intercourse			NS
≤ 15	5 (4.9)	18 (10.2)	
16-19	31 (30)	48 (27.5)	
≥ 20	67 (65.1)	109 (62.3)	
Education (graduated from)			NS
Illiterate	5 (4.9)	8 (4.6)	
Primary school	36 (35)	66 (37.7)	
High school	36 (35)	54 (30.9)	
University	26 (25.1)	47 (26.9)	
History of taking oral contraceptives			NS
> 1 year	26 (25.2)	30 (17.1)	
< 1 year	15 (14.6)	28 (16.0)	
Never	62 (60.2)	117 (66.9)	
History of using intrauterine devices			NS
> 6 months	38 (36.9)	59 (33.7)	
< 6 months	7 (6.8)	15 (8.6)	
Never	58 (56.3)	101 (57.7)	
Route of delivery			NS
Vaginal	92 (92.9)	144 (88.9)	
Cesarean	7 (7.1)	18 (11.1)	
Cigarettes (current, > 1 year)			NS
≥ 5 /days	7 (6.8)	11 (6.3)	
< 5 /days	24 (23.3)	31 (17.7)	
Never	72 (69.9)	133 (76)	
Mean serum nutrients			
Folate (ng/ml)	8.97 (± 3.5)	9.22 (± 2.6)	NS
Vitamin B12 (pg/ml)	232.6 (± 60.9)	348.4 (± 134)	< 0.001[a]
Extreme quartiles of vitamin B12			< 0.001[b]
1st (< 261 pg/ml)	73 (71)	44 (25)	
4th (> 386 pg/ml)	0 (0)	44 (25)	

Data are expressed in numbers (%) and mean (± SD). The independent sample t-test was used to compare means and the chi-square test was used to compare categorical variables.
NS: not significant; [a]The value both for the t-test and logistic regression analysis; [b]Logistic regression analysis.

The number of births, education levels and folate and vitamin B12 levels of the groups were analyzed by logistic regression and there was a significant difference only in vitamin B12 levels between the groups. Dichotomized logistic regression analyses of vitamin B12 levels were significantly different $p < 0.001$, OR: 1.017 (CI, 1.012-1.023). The predicted percentages at the cut-off value of 0.5 were as follows: sensitivity 70.0%, specificity 74.6%, positive predictive value 71.8%, and negative predictive value 72.6%.

The logistic regression analysis of the 1st and the 4th quartiles of vitamin B12 levels revealed that cut-off points based on their distributions in controls showed a significant difference $p < 0.001$, OR: 1.525 (CI, 1.175-

1.875). It was noted that there was not any case with cytologic abnormality in the highest quartile of the controls.

The analysis of the relatively more populous subgroups of cases documented as ASCUS and LSIL showed no significant difference in terms of mean folate and vitamin B12 levels between each other ($p > 0.05$) whereas the mean vitamin B12 values of both these subgroups were also significantly lower than that of controls ($p < 0.001$).

Discussion

This case-control study was conducted to investigate the role of folate, vitamin B12 and demographic characteristics in cytologic abnormalities on Pap smears among women living in the south-west of Turkey. The results suggested that age, parity, education level, age of first intercourse, route of delivery, taking oral contraceptives and using intrauterine devices, cigarette smoking and folate levels were not associated with an increased risk of an abnormal Pap test in this population. However, we observed an inverse association between circulating concentrations of vitamin B12 and abnormal Pap test results. The first and the last quartiles of vitamin B12 levels based on the controls showed a more obvious estimated risk of abnormal cervical cytology.

Protooncogenes and oncogenes play an important part in cancerogenesis of the cervix. In the remethylation pathway, folate and vitamin B12 serve as substrate and cofactor, respectively, whereby 5-methyltetrahydrofolate provides the methyl group to remethylate homocysteine into methionine [9]. Lower levels of folate and vitamin B12 in the diet may therefore result in reduced levels of DNA methylation [9] as well as elevated levels of homocysteine. Deficiencies in methyl donors, such as folate, vitamin B12 and methionine, may lead to DNA methylation aberrations and DNA fragility [10].

There is consistent evidence that low folate intake may have procarcinogenic effects [11, 12]. Folate depletion and concomitant elevations in plasma concentrations of homocysteine may be related to the risk of ovary [13] and perhaps cervical carcinomas [11, 14]. In addition, a population-based case-control study suggested that if folate intake is to have a protective effect against breast cancer, an adequate intake of vitamin B12 may also be necessary [15]. Also, folate and vitamin B12 supplementation induced the regression of bronchial squamous metaplasia [16].

On the other hand, a role for folate deficiency and for other alterations of methionine cycle metabolites (homocysteine, vitamin B12) as a risk factor for cervical carcinoma has never been demonstrated definitively [17-19]. The studies addressing the association between cervical neoplasia and folate and other nutrients such as vitamin B12 and homocysteine have been inconclusive. A positive, stepwise relation of DNA hypomethylation to increasing severity of cervical dysplasia and carcinoma has been reported, [14] and serum and cervical tissue folate levels have been shown to be weakly associated with the degree of DNA methylation [12]. The effect of folate status may be restricted to early preneoplastic cervical lesions and not to more advanced disease [5]. The investigation by Hernandez et al. yielded evidence that dietary thiamin, riboflavin, folate, and vitamin B12 may protect against premalignant cervical lesions, particularly high-grade SIL compared to low-grade dysplasia [20]. Intake of these vitamins reduced the OR for HSIL by 50–90% for the highest compared to the lowest levels of consumption [20].

Epidemiological studies have shown evidence that folate probably protects against cervical neoplasia and that vitamin B12 is possibly protective against it. Some of the case-control studies on the association between dietary folate intake and cervical neoplasia revealed a protective effect of folate against cervical dysplasia [17, 21], but others did not find any association [22-25]. Similar results were reported in the case-control studies that assessed the association between cervical neoplasia and circulating folate concentrations; only two of them reported a protective effect [24, 26], while the others found no association [18, 19, 27, 28].

Folic acid supplementation in women with cervical dysplasia was investigated, and only one trial revealed cytologic improvement [28-30]. While protective associations were observed for dietary vitamin B12 and cervical neoplasia [21], previous investigations of circulating vitamin B12 and homocysteine levels found no significant associations [18, 27, 31]. To our knowledge, there is no prospective trial evaluating the effects of vitamin B12 supplementation for the treatment of abnormal Pap test.

The conflicting results from the studies investigating the associations between Pap test abnormalities and methyl donors such as folate and vitamin B12 might be due to the complexity of carcinogenesis, genetic and demographic characteristics, interaction between nutrients, eating habits and sample size. For example, it was suggested that polymorphisms in the Methylenetetrahydrofolate reductase T allele may be an independent risk factor for cervical dysplasia [21].

Free radicals and oxidants, which are produced during normal metabolism and the inflammation process, and which are also present in high amounts in tobacco smoke, could lead to extensive damage of DNA, proteins and lipids if not counteracted by antioxidant molecules [32]. Products arising from lipid peroxidation and protein modification can interact in turn with cigarette smoke products, creating additional toxic products. These products are thought to activate inflammatory immune responses which may play a relevant role in oxidative tissue damage [32]. On the other hand, antioxidant nutrients could modulate immune response and decrease viral replication and gene expression [33]. It is worth noting that cigarette smoking and use of oral contraceptives, both established cofactors for cervical cancer, could decrease circulating levels of carotenoids, vitamin C and folate independently of intake [33]. These findings suggest that low levels of these antioxidants may play a role in the pathogenesis of cervical cancer.

It has been reported that, with respect to current use, the risk for cervical dysplasia increased for women who had been using oral contraceptives for longer than ten years [34]. The correlation between cervical dysplasia and oral contraceptives is based on the content of steroid hormones, such as estrogen and progesterone, which are thought to play a role in the progression of disease. Progesterone has been reported to increase HPV-16 and HPV-18 gene expression at the levels of transcription and mRNA stability [35]. Most cases of cervical cancer are in the most estrogen-sensitive region of the cervix known as the transformation zone [36], an area that displays a high level of conversion of estradiol to 16-hydroxyestrone. HPV, a necessary cause for cervical cancer, is integrated into the host DNA, especially at fragile sites, and activation of specific oncogenes appears to be a prerequisite for cervical carcinogenesis. Nutrition status, in particular status of nutrients involved in DNA methylation such as folate and vitamin B12, may influence HPV persistence [37].

Demographic factors such as older age, residence and low educational level, behavioral and sexual factors such as early age at first coitus, cigarette smoking, long-term oral contraceptive use and diet, and gynecologic factors such as multi-parity are risk factors for cervical cancer and its precursor lesions [3]. The women in this study noted that they had a single partner and had their first intercourse at marriage. The similarity of demographic risk factors between case and controls in the present study might be related to the sample size and population characteristics. The small number of women in this study limited our ability to perform subgroup analyses (other than ASCUS and LSIL) of serum nutrients by disease severity (HSIL, carcinoma, etc.) or to examine the form of the joint association of HPV infection and serum nutrients with the risk for cervical dysplasia. Furthermore, because of the study design, we were unable to evaluate the dietary intake of the nutrients and the effects of supplementation of vitamin B12 on abnormal cervical cytology.

In the future, clinical chemoprevention will require further development of trials based on a mechanistic understanding of carcinogenesis. Also, chemoprevention would aim to accelerate clearance, and in particular to reduce the proportion of HPV-infected women who are left with a persistent infection. It is widely agreed that the endpoint for evaluation of the efficacy of a prophylactic HPV vaccine should be high-grade CIN (together with type-specific persistent HPV infection). ZYC101a is a novel therapeutic containing plasmid-DNA-encoding fragment derived from the E6 and E7 proteins of HPV. As cervix cancer is seen mainly in developing countries, HPV vaccine may not be cost-effective for these countries. Today great attention is given to chemoprevention of cervical cancer and dysplasia based on eating habits. However, the time of intervention, dosage and duration is not known and further studies are needed.

A woman with poor folate status or vitamin B12, coupled with coexisting factors such as HPV infection or factors that affect utilization or intake may especially benefit from both increased folate and vitamin B12 in the diet [37]. While data relating to folate and vitamin B12 to cervical cytologic abnormality risk remains equivocal, when coupled with the other potential health benefits associated with folate and vitamin B12, it can be recommended that people living in this region, working in soil-based rather than animal-based agriculture, consume not only folate-rich foods such as fruits and vegetables but also vitamin B12-rich foods such as meat, fish, milk products and eggs in a balanced way.

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