International Journal of Women's Health

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ORIGINAL RESEARCH

Indian women with higher serum concentrations of folate and vitamin B12 are significantly less likely to be infected with carcinogenic or high-risk (HR) types of human papillomaviruses (HPVs)

This article was published in the following Dove Press journal:
International Journal of Women's Health
16 January 2010
Number of times this article has been viewed

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Background: Studies conducted in the USA have demonstrated that micronutrients such as folate and vitamin B12 play a significant role in modifying the natural history of high-risk human papillomaviruses (HR-HPVs), the causative agent for developing invasive cervical cancer (CC) and its precursor lesions.

Objective: The purpose of the current study was to investigate whether these micronutrients have similar effects on HR-HPV infections in Indian women.

Methods: The associations between serum concentrations of folate and vitamin B12 and HR-HPV infections were evaluated in 724 women who participated in a CC screening study in the southern state of Andhra Pradesh, India. Serum folate and vitamin B12 concentrations were measured by using a competitive radio-binding assay. Digene hybrid capture 2 (HC2) assay results were used to categorize women into two groups, positive or negative for HR-HPVs. Unconditional logistic regression models specified a binary indicator of HC2 (positive/negative) as the dependent variable and serum folate concentrations combined with serum vitamin B12 concentrations as the independent predictor of primary interest. Models were fitted, adjusting for age, education, marital status, parity, type of fuel used for cooking and smoking status.

Results: Women with higher concentrations of serum folate (>6 ng/mL) and vitamin B12 (>356 pg/mL) were at lower risk of being positive for HR-HPVs compared to those with serum folate ≤6 ng/mL and serum vitamin B12 ≤ 356 pg/mL (odds ratio = 0.26; 95% confidence interval: 0.08–0.89; $P = 0.03$).

Conclusions: These results demonstrated that improving folate and vitamin B12 status in Indian women may have a beneficial impact on the prevention of CC. Micronutrient based interventions for control of HR-HPV infections may represent feasible alternatives to vaccine based approaches to HPV disease prevention, which are currently unaffordable for use in resource limited areas in rural India.

Keywords: folate, vitamin B12, human papillomavirus, cervical cancer

Introduction

Folate and vitamin B12 play important roles as inhibitors of human carcinogenesis in several organs. These micronutrients are particularly important for cancers in the cervix because high-risk human papillomaviruses (HR-HPVs), classified as a human carcinogen, play a causative role in cervical carcinogenesis. Because millions of women worldwide continue to be exposed to at least 13 types of known HR-HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), any novel and practical interventions

that are able to control these infections should make a significant contribution to prevention of CC.

Although the HPV vaccine development holds promise for reducing the incidence of CC, a type-specific prophylactic HPV vaccine (HPV 16/18) may reduce but not eliminate the risk of CC.[1,2] Further, prophylactic HPV vaccines will not alter the course of existing HPV infections and no therapy has yet been identified to treat HPV infections. Therapeutic HPV vaccines have shown minimal impact on inducing disease regression.[3] This leaves millions of women who are already infected with HR-HPVs with no options for receiving protection against HPV associated risk of developing CC other than participating in routine screening. Organized routine CC screening programs are largely unavailable in developing countries including India. In addition to these concerns, even considerations for use of HPV vaccine in developing countries include the affordability and cost-effectiveness and the likelihood of cultural acceptability, political will, and public support. Therefore, nonvaccine approaches for control of HPV infections and ultimately the prevention of CC by these means are likely to be more practical, affordable and acceptable, especially in developing countries. Even with eventual availability, because of the higher cost, it is important to find adjuncts that are able to maximize the cost-effectiveness of HPV vaccines. Micronutrients such as folate and vitamin B12 are likely to be efficient as adjuncts as they have a positive influence on immune response in humans.[4–7]

Our recent studies conducted at the University of Alabama at Birmingham (UAB) in the USA have demonstrated that higher circulating concentrations of folate (a B vitamin naturally present in fruits and vegetables as folate and in fortified foods as folic acid) are associated with a lower likelihood of becoming HR-HPV positive and of having a persistent HR-HPV infection, and when infected, a greater likelihood of clearing HR-HPV.[8] Recent studies have reported similar findings.[9] Our studies have also demonstrated that women with lower folate status and positive for any of the 13 types of HR-HPV were twice more likely to have higher grades of cervical intraepithelial neoplasia (CIN 2+) which are precursor lesions for CC. More importantly, we also demonstrated that women with lower folate and positive for HPV-16 were 9× more likely to have CIN 2+, strongly suggesting a specific effect of folate on HPV 16,[10] the most commonly found and most carcinogenic type of HPV. Findings from our recent studies also demonstrate that in populations exposed to higher folate as a result of population-wide folic acid fortification programs, women with sufficient vitamin B12 status benefit most from higher folate status compared to women with lower/insufficient circulating concentrations of both micronutrients.[11] These associations held after controlling for other micronutrients and other known risk factors for CC strongly suggesting a positive role for folate and vitamin B12 in early cervical carcinogenesis.

These micronutrients are likely to be more important in countries such as India where deficiencies of these micronutrients are more frequent than in developed countries. The purpose of the current study was to investigate whether there are associations between folate and vitamin B12 and the likelihood of being positive for HR-HPVs in Indian women.

Material and methods

Study population

The current study was conducted on the first 724 women enrolled in the Community Access to Cervical Health (CATCH) study, in a population-based sample of women living in the southern state of Andhra Pradesh, India, which was designed to compare three methods of CC screening.[12] The study was approved by the SHARE Research/Mediciti Institute of Medical Sciences and Johns Hopkins Bloomberg School of Public Health.

Methods

A questionnaire was administered to gather information regarding age, education, marital status, parity, type of fuel used for cooking (gas or firewood, thought to be not only an indicator for socioeconomic status, but also a known risk factor for CC[13]) and exposure to tobacco smoke (by smoking or by exposure to second-hand smoke, but largely due to second-hand exposure). The questionnaire was administered after consent and before the pelvic exam was performed (ie, prior to knowing HPV status). Blood samples were collected from women between 10 am and 2 pm and we did not gather information on fasting/nonfasting status. Samples were clotted at room temperature, centrifuged and aliquotted for −80 °C storage within 8 hours. Serum folate and vitamin B12 concentrations were measured by using a competitive radio-binding assay (SimulTRAC-SNB; MP Biomedicals, Orangeburg, NY). Cervical samples were collected using the Digene cervical brush sampler and placed in standard transport medium (STM) which was stored at −20 °C until tested. Digene Hybrid Capture 2 (HC2) assay (Qiagen Inc. Valencia, CA) detects 13 HR-HPV infections (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and was performed according the manufacturer's instructions.

Women were considered positive for HR-HPV if the relative light unit/cutoff (RLU/CO) was ≥1.0.

Statistical analysis

Serum folate and vitamin B12 concentrations of the study population were analyzed using standard cutoff points: folate, < 3 ng/mL as deficient and ≥3 ng/mL as sufficient, and vitamin B12, <203.3 pg/mL as deficient and ≥203.3 pg/mL as sufficient. Descriptive statistics were used to characterize the 79 women who tested positive for HR-HPVs and 645 women who tested negative for HR-HPVs. The Pearson chi-square statistic was used to assess differences between observed and expected frequencies. Proportions were compared using a two-sided chi-square test.

Unconditional logistic regression models specified a binary indicator of HC2, positive or negative as the dependent variable. Serum concentrations of folate and vitamin B12 were examined as potential independent risk factors, adjusting for age (in increments of 5 years from 25 years to greater than 60 years), education (1st–9th standard or greater [9+] vs none), marital status (married vs divorced/separated/widowed), parity (3, 1–2 or 0 live births), type of fuel used for cooking (firewood vs gas) and exposure to tobacco smoke (yes vs no). In a variation of the model described above, we specified combined categories of serum folate and vitamin B12 as independent predictors of primary interest. Study population-based cutoff points (comparing the highest tertile to the remaining lower 2 tertiles) for folate and vitamin B12 were 6 ng/mL and 356 pg/mL, respectively. Individual effects of folate and vitamin B12 on the likelihood of being HR-HPV-positive was tested against these cutoff points in the regression models. We then categorized the women into four categories to examine the joint effects of folate and B12: higher folate (>6 ng/mL) and higher vitamin B12 (>356 pg/mL) (n = 88), lower folate (≤6 ng/mL) and higher vitamin B12 (>356 pg/mL) (n = 154), higher folate (>6 ng/mL) and lower vitamin B12 (≤356 pg/mL) (n = 155), and lower folate (≤6 ng/mL) and lower vitamin B12 (≤356 pg/mL) (n = 327). The referent group was women who had low concentrations of both micronutrients. We evaluated the strength of each association by estimating the odds ratio (OR) and its 95% confidence interval (CI), and its statistical significance using Wald's chi-square statistic of the null hypothesis that the OR = 1. All statistical analyses were conducted using SAS® Version 9.1.3 (SAS Institute, Cary, NC).

Results

Compared to standard cutoff points, vitamin B12 deficiency was more common than folate deficiency in this population of women, according to established cutoff points for the normal ranges of these micronutrients. We also observed that almost half the population (47%) was sufficient in both micronutrients. However, 42% were deficient for one of the micronutrients and 11% were deficient in both micronutrients.

The prevalence of HR-HPV by demographic and lifestyle factors and serum concentrations of folate and vitamin B12 is shown in Table 1. Women above the age of 45 years had a higher prevalence of HR-HPV compared to women of child bearing age (25–44 years) but it was statistically non-significant (P = 0.08). Prevalence of HR-HPV was

Table 1 Prevalence of HR-HPV by demographic/lifestyle factors and serum concentrations of folate and vitamin B12

Details	Number		%	*P*–
	HPV+	Total	HPV+	value
Age at enrollment (years)				
25–44	59	593	9.9	0.08
45+	20	131	15.3	
Education				
None	58	474	12.2	0.12
1st–9th standard or greater (9+)	21	250	8.4	
Marital status				
Married	69	646	10.7	0.57
Divorced/separated/widowed	10	78	12.8	
Parity				
0 live birth	3	37	8.1	0.58
1–2 live births	28	285	9.8	
≥3 live births	48	402	11.9	
Cooking fuel				
Firewood	38	358	10.6	0.80
Gas	41	366	11.2	
Exposure to tobacco smoke				
Yes	48	356	13.5	0.03
No	31	368	8.4	
Micronutrient concentrations by cut point serum folate (ng/mL)				
>6	22	243	9.1	0.25
≤6	57	481	11.9	
Serum vitamin B12 (pg/mL)				
>356	24	242	9.9	0.54
≤356	55	482	11.4	

Abbreviations: HR-HPVs, high-risk human papillomaviruses.

more common among women who were exposed to smoke (from tobacco use or second-hand smoke) compared to women who were not exposed ($P = 0.03$). Prevalence of HPV did not significantly differ by the categories of other variables including serum concentrations of folate and vitamin B12.

When the association between the two micronutrients and likelihood of being positive for HR-HPV was tested individually, we observed that only the highest tertile values for folate (>6 ng/mL) and vitamin B12 (>356 pg/mL) showed a lower risk of being positive for HR-HPVs (20% reduced risk for both micronutrients and statistically nonsignificant). Therefore, in the logistic regression models the combined effects of serum folate and vitamin B12 were tested with these two combinations against the reference category of folate ≤6 ng/mL and vitamin B12 ≤ 356 pg/mL (Table 2). Results demonstrated that women with higher concentrations of serum folate (>6 ng/mL) and vitamin B12 (>356 pg/mL) were at lower risk of being positive for HR-HPVs compared to those women with serum folate ≤6 ng/mL and vitamin B12 ≤ 356 pg/mL (OR = 0.26, 95% CI 0.08–0.89, $P = 0.03$). Our results also suggested that higher concentrations of folate or vitamin B12 in isolation do not appear to reduce the likelihood of being infected with HR-HPVs. In addition, we also observed that exposure to tobacco smoke was associated with higher risk of being positive for HR-HPVs.

Table 2 Relationship of serum folate and serum vitamin B12 interaction and other factors with HR-HPV infections

Risk factors	OR (95% CI)	*P*-value
Age at enrollment (increments of 5 years from 24–>60)	1.02 (0.89–1.18)	0.75
Education		
None	1.00 (reference)	0.30
1st–9th standard or greater (9+)	0.72 (0.38–1.34)	
Marital status		
Divorced/separated/widowed	1.00 (reference)	0.66
Married	0.84 (0.38–1.84)	
Parity (≥3, 1–2 , 0 live births)	1.09 (0.70–1.71)	0.70
Cooking fuel		
Gas	1.00 (reference)	0.46
Firewood	0.82 (0.48–1.39)	
Smoking exposure		
No	1.00 (reference)	0.05
Yes	1.70 (1.01–2.85)	
Folate and vitamin B12 combinations		
Folate ≤ 6 ng/mL, vitamin B12 ≤ 356 pg/mL (n = 327)	1.00 (reference)	
Folate > 6 ng/mL, vitamin B12 > 356 pg/mL (n = 88)	0.26 (0.08–0.89)	0.03
Folate ≤ 6 ng/mL, vitamin B12 > 356 pg/mL (n = 154)	1.22 (0.67–2.22)	0.53
Folate > 6 ng/mL, vitamin B12 ≤ 356 pg/mL (n = 155)	1.15 (0.63–2.10)	0.64

Abbreviations: HR-HPVs, high-risk human papillomaviruses; OR, odds ratio; CI, confidence interval.

Discussion

To our knowledge, our study is the first large scale community-based study to determine folate and B12 concentrations among Indian women in relation to risk of HR-HPV infections. We observed that vitamin B12 deficiency is much more common than folate deficiency in this study population. These results, however, are in concordance with the findings from small studies in selected or hospital-based Indian populations. In a study conducted in Pune, nearly 50% of the population had vitamin B12 deficiency while only 5% of the population had folate deficiency.[14] Similarly, another small study observed that 46% of women had deficient circulating concentrations of vitamin B12 while only 12% of women had deficient concentrations of folate.[15]

This study suggested that having higher intakes of both micronutrients are required to receive maximum protection against infections with HR-HPVs. Biological mechanisms by which folate and vitamin B12 could modify the risk of being infected with HR-HPVs are highly plausible. Reduced immunocompetence associated with deficiencies of folate and vitamin B12[4,5,16] could increase the risk of infection and persistence with multiple types or higher viral loads of HR-HPVs. Vitamin B12 is an important micronutrient in the folate metabolic pathway which provides nucleic acids for DNA synthesis. Above average intakes of folate and vitamin B12 are shown to be associated with reduced genome damage rate as measured by using the micronucleus assay.[17] Reduced DNA damage associated with higher folate and vitamin B12 status is likely to reduce the integration of HPV into the human genome and allow HPVs to stay as episomal. A common chromosomal fragile site that is sensitive to folate deficiency has been shown to coincide with a site of HPV-16 integration in the tissues of primary cervical carcinomas[18] and three of the four sites at which HPV-18 integrates its DNA into the host,[19] suggesting a plausible mechanism through which suboptimal folate levels could increase the likelihood of integration with these HPV genotypes.[20] Therefore, control of HR-HPV infections by improvements

in folate and vitamin B12 status is likely to be an important nonvaccine-based approach for preventing HR-HPV associated risk of developing CC.

However, implementation of supplementation programs with folate and vitamin B12 for control of HPV infections in Indian women based on our results may be premature for the following reasons. This study was not designed to measure other important cancer protective micronutrients such as antioxidants (eg, vitamins C, A, E, carotenes) and this could be a limitation of the study. Even though these other micronutrients were not shown to play an important role for HPV natural history in our US study populations, they may play important roles in Indian women where the intake of these micronutrients could be lower than in US. In fact, the report of National Nutrition Monitoring Bureau, India. (technical report 21:2002) "Diet and Nutritional Status of Rural Population", revealed that the median and mean intakes of all micronutrients were well below the RDA for Indians. Oxidative stress induced by deficiencies in antioxidant micronutrients is likely to perturb the normal redox balance and transform the HPV-infected cells toward a carcinogenic process in the cervix.[21] Our study has also limited information on some important risk factors for CC such as lifetime number of sexual partners, use of oral contraceptives or vitamin supplements. Additionally because of the cross sectional nature of our data, the role of micronutrients on HPV acquisition vs persistence (or both) could not be determined. Therefore future studies that are carefully designed to address these limitations are needed to confirm that folate and vitamin B12 have independent or combined effects on controlling HPV infections in these women.

In summary, we demonstrated that Indian women with higher concentrations of both folate and vitamin B12 are 74% less likely to test positive for HR-HPVs. Comprehensive studies conducted in the US demonstrated that women with higher folate and sufficient vitamin B12 were 70% less likely to be diagnosed with HR-HPV associated higher grades of CIN 2+, which are precursor lesions for the development of invasive CC.[5] If similar results can be demonstrated for Indian women, supplementation with folate and vitamin B12 could be a viable nonvaccine approach for prevention of CC in India.

Acknowledgments

Supported by the INDO-US collaborative program of Department of Biotechnology, Ministry of Science and Technology, Government of India and the NIH, USA (BT/IN/US/CRHR/PP/2002), and the NIH Specialized Programs of Research Excellence P50 CA98252 and the UAB CCC.

Disclosures

The authors have no conflicts of interest that are directly relevant to the content of this study.

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