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Original Research Article

Vitamin B12 and folate: key predictors of HPV persistence in ASCUS patients

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ABSTRACT

Background: Persistent infection with high-risk human papillomavirus (HPV) is the key driver of cervical carcinogenesis. Atypical squamous cells of undetermined significance (ASCUS) represent an equivocal cytology category in which optimal triage is crucial. Disturbances in one-carbon metabolism, particularly low vitamin B12 and folate, may impair DNA repair and immune responses, favouring HPV persistence. This study aimed to evaluate serum vitamin B12 and folate levels in women with ASCUS compared with cytologically normal controls and to explore their association with HPV positivity in ASCUS.

Methods: This comparative cross-sectional study was conducted in the Department of Obstetrics and Gynaecology, FH Medical College, Agra. Fifty women were enrolled: 25 with ASCUS cytology and 25 age-matched controls with normal Pap smears. Socio-demographic and clinical data were recorded. Fasting serum vitamin B12 and folate were measured using an automated immunoassay. High-risk HPV status in the ASCUS group was determined by PCR. Group comparisons were performed using appropriate statistical tests and the association between micronutrient levels and HPV positivity was assessed.

Results: Women with ASCUS had significantly lower vitamin B12 (265.17±54.25 vs 315.26±65.62 pg/ml, $p<0.01$) and folate (9.53±1.76 vs 11.91±2.62 ng/ml, $p<0.01$) than controls. Within the ASCUS group, HPV-positive women showed markedly reduced vitamin B12 (200.14±12.47 vs 295.53±40.25 pg/ml, $p<0.01$) and folate (7.86±0.61 vs 10.23±1.59 ng/ml; $p<0.01$) compared with HPV-negative ASCUS patients. Lower micronutrient levels showed a clear inverse association with HPV positivity.

Conclusions: Women with ASCUS, particularly those who are HPV-positive, exhibit significant subclinical deficiencies of vitamin B12 and folate. These findings suggest that impaired one-carbon metabolism may contribute to HPV persistence at an early stage and that vitamin B12 and folate could serve as simple, adjunctive biomarkers to refine risk stratification and follow-up strategies in ASCUS patients.

Keywords: Atypical squamous cells of undetermined significance, Folic acid, Papillomavirus infections, Uterine cervical neoplasms, Uterine cervical dysplasia, Vitamin B12

INTRODUCTION

Cervical cancer remains a major global public health challenge, particularly in low and middle-income countries where organized screening programs, HPV vaccination coverage and timely follow-up services are often inadequate. In 2020, an estimated 604,000 new cases and 342,000 deaths were attributed to cervical cancer worldwide, with the greatest burden observed in settings with limited access to preventive healthcare.[1] India continues to account for a substantial proportion of this burden and cervical cancer remains one of the leading causes of cancer-related morbidity and mortality among Indian women despite the availability of effective

preventive strategies.[2] Persistent infection with high-risk human papillomavirus (hrHPV) is now well established as the central etiological factor in the development of cervical intraepithelial neoplasia (CIN) and invasive cervical carcinoma.

Papanicolaou (Pap) smear cytology continues to serve as a cornerstone of cervical cancer screening, especially in resource-constrained settings. Within the Bethesda system, atypical squamous cells of undetermined significance (ASCUS/ASC-US) constitute an equivocal category in which cytological abnormalities are present but are insufficient for a definitive diagnosis of squamous intraepithelial lesion.[3] Although ASCUS is often associated with benign or transient changes, it presents a significant clinical dilemma because a subset of women may harbor persistent hrHPV infection or underlying high-grade cervical lesions such as CIN2+.[4] As a result, the management of ASCUS requires careful triage to avoid both overtreatment and missed opportunities for early detection. In this context, the identification of simple and accessible biomarkers that may refine risk stratification in ASCUS patients is of considerable clinical relevance.

Folate and vitamin B12 are essential micronutrients involved in one-carbon metabolism, a biochemical pathway critical for DNA synthesis, DNA repair and methylation. Disruption of this pathway may lead to genomic instability, altered gene expression, defective immune responses and impaired regulation of viral-host interactions, thereby creating a biological environment favorable for HPV persistence and neoplastic transformation.[5] Increasing epidemiological and experimental evidence suggests that deficiencies of folate and vitamin B12 may be associated with a higher risk of HPV infection, progression of CIN and cervical carcinogenesis.[5] In a Chinese cohort, low serum folate levels were associated with CIN progression and appeared to act synergistically with hrHPV infection.[6] Likewise, Silva et al, reported that lower folate and vitamin B12 concentrations were associated with an increased risk of pre-neoplastic cervical lesions.[7] More recent evidence has continued to support the protective role of adequate folate and vitamin B12 status in HPV-related cervical disease.[6]

Beyond their nutritional significance, these micronutrients may have particular relevance in the early phases of HPV-associated disease. One-carbon metabolism influences not only epithelial cell turnover and genomic integrity but also DNA methylation patterns that may affect both host tumor suppressor genes and viral gene expression. In this way, deficiency of folate and vitamin B12 may contribute to persistence of hrHPV infection even before the development of definite precancerous lesions. This possibility is especially important in women with ASCUS, in whom conventional cytology alone may not fully distinguish those at low risk from those requiring closer surveillance.

Despite these observations, most published studies have focused on women with established CIN or invasive cervical cancer, while comparatively little attention has been given to women with ASCUS cytology, particularly those with known HPV status and from underrepresented populations such as Indian women. It remains unclear whether subclinical deficiencies of vitamin B12 and folate are already evident at this early cytological stage and whether such deficiencies are associated with HPV positivity in ASCUS patients. Clarifying this relationship may have practical implications for risk assessment, follow-up planning and low-cost adjunctive evaluation in routine gynecological practice.

Against this background, the present study was designed to evaluate serum vitamin B12 and folate levels in women with ASCUS in comparison with cytologically normal controls and to examine the association between these micronutrients and HPV positivity within the ASCUS group. By integrating nutritional biomarkers with cytology and HPV testing, this study aims to determine whether vitamin B12 and folate may serve as simple adjunctive predictors of HPV persistence in women with ASCUS.

METHODS

Study design and setting

This was a hospital-based, comparative cross-sectional study conducted in the Department of Obstetrics and Gynaecology, FH Medical College, Agra, India during June 2024 to September 2025. The study was carried out after approval from the Institutional Ethics Committee and in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrolment.

Study population

Women attending the gynaecology outpatient department for routine cervical cancer screening or presenting with minor gynaecological complaints were enrolled in the study. All participants underwent cervical cytological evaluation using the Papanicolaou (Pap) smear. Based on cytological reporting according to the Bethesda System, women diagnosed with atypical squamous cells of undetermined significance (ASCUS) were included as the study group. Age-matched women with normal cervical cytology during the same period were recruited as controls. A total of 50 women were included in the study, comprising 25 women with ASCUS and 25 women with normal Pap smear findings.

Women in the ASCUS group were eligible if they were aged between 21 and 65 years, had a cytological diagnosis of ASCUS on Pap smear and were willing to undergo blood sampling and HPV testing, along with providing written informed consent. The control group included women within the same age range who had normal cervical cytology on Pap smear. Participants from both groups

were excluded if they had a history or evidence of high-grade squamous intraepithelial lesion (HSIL), cervical intraepithelial neoplasia grade 2 or higher (CIN2+) or invasive cervical cancer. Additional exclusion criteria included pregnancy or puerperium, current or recent use (within the past three months) of vitamin B12 or folate supplementation and the presence of known haematological deficiencies. Women with chronic liver disease, renal failure, inflammatory or autoimmune disorders, HIV infection or other immunosuppressive conditions were also excluded. Furthermore, those with a prior history of treatment for cervical intraepithelial neoplasia or who had undergone hysterectomy were not considered eligible.

Eligible participants who satisfied the inclusion and exclusion criteria were consecutively recruited until the required sample size in each group was achieved.

Clinical and demographic data collection

A structured proforma was used to record socio-demographic details (age, residence, education, socio-economic status), obstetric history (parity, age at first intercourse, number of lifetime sexual partners), contraception, menstrual and sexual history and presenting complaints (screening, vaginal discharge, intermenstrual or postcoital bleeding). A general and pelvic examination was performed and findings were recorded.

Blood sample collection and estimation of Vitamin B12 and folate

Following an overnight fast, 5 ml of venous blood was collected from each participant under aseptic conditions. Samples were centrifuged and serum was separated and stored at −20°C until analysis. Serum vitamin B12 and folate levels were estimated using a standard automated immunoassay platform in the central laboratory, following the manufacturer's instructions and internal quality-control procedures. Results were expressed in pg/ml for vitamin B12 and ng/ml for folate. Laboratory reference ranges were used for internal validation; however, for analysis, both continuous values and ROC-derived cut-offs were utilised.

Human papillomavirus testing

From each woman in the ASCUS group, an additional cervical sample was collected using a cervical brush and placed in viral transport medium. High-risk HPV DNA detection was performed using a PCR-based assay with consensus primers targeting the L1 region. Amplified products were analysed according to kit protocol and women were categorised as HPV-positive or HPV-negative. For the purposes of the present analysis, only overall high-risk HPV positivity status (positive/negative) was used.

Outcome measures

The primary outcomes were the comparison of mean serum vitamin B12 and folate levels between ASCUS and control groups and between HPV-positive and HPV-negative women within the ASCUS group. Secondary outcomes included the correlation of these micronutrients with HPV status and evaluation of their diagnostic performance for predicting HPV positivity in ASCUS patients.

Statistical analysis

Data were entered into a spreadsheet and analysed using Statistical Package for the Social Sciences (SPSS), version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normality. Results are presented as mean±standard deviation (SD) for continuous variables and as frequency (percentage) for categorical variables. Comparisons between two independent groups (ASCUS vs control; HPV-positive vs HPV-negative ASCUS) were performed using the independent-samples Student's t test for normally distributed data and the Mann–Whitney U test for skewed data. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate.

Pearson (or Spearman, where indicated) correlation coefficients were calculated to assess the relationship between vitamin B12, folate and HPV positivity. Receiver operating characteristic (ROC) curves were constructed to determine the discriminative ability of vitamin B12 and folate for predicting HPV positivity among ASCUS women. Area under the curve (AUC) with 95% confidence intervals (CI) was calculated and optimal cut-off values were obtained using the Youden index. Sensitivity, specificity, positive predictive value, negative predictive value and odds ratios were derived from these cut-offs. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

The socio-demographic profile of the 50 women included in the study is presented in Table 1. Most participants were young to middle-aged adults, with 42% in the 30–39-year age group and 30% aged ≥40 years, while 28% were younger than 30 years. Slightly more than half of the women resided in urban areas (56%) and the remainder were from rural backgrounds (44%). Regarding educational status, 24% had schooling up to primary level, 46% had completed secondary education and 30% were graduates or above. Nearly half of the women belonged to the middle socioeconomic class (46%), followed by lower-middle/lower classes (36%) and upper/upper-middle classes (18%) (Figure 1).

A majority of the women were para 1–2 (58%), while 18% were nulliparous and 24% had parity ≥3. Most participants reported age at first sexual intercourse above 18 years

(78%), with only 22% initiating sexual activity at or before 18 years. The large majority had a single lifetime sexual partner (92%) and only 8% reported two or more partners. With regard to presenting complaints, 40% of women were asymptomatic and detected during routine screening, whereas 36% presented with vaginal discharge, 14% with intermenstrual bleeding and 10% with postcoital bleeding (Table 2).

Biochemical comparison between women with ASCUS cytology and those with normal smears is shown in Table 3. Mean serum Vitamin B12 levels were significantly lower in the ASCUS group (265.17±54.25 pg/ml) compared with controls (315.26±65.62 pg/ml, p<0.01). A similar pattern was observed for serum folate, which was reduced in ASCUS patients (9.53±1.76 ng/ml) relative to women with normal cytology (11.91±2.62 ng/ml, p<0.01). Thus, both micronutrients were consistently depleted in women with cytological evidence of ASCUS compared with cytologically normal controls (Figure 2).

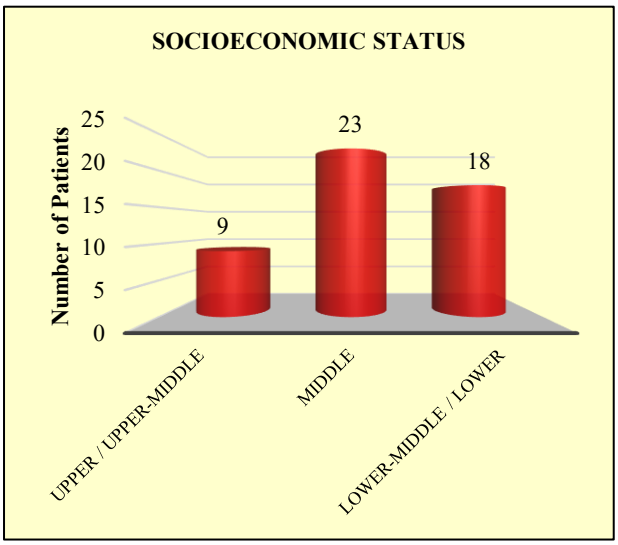

Figure 1: Socioeconomic status of the study participants.

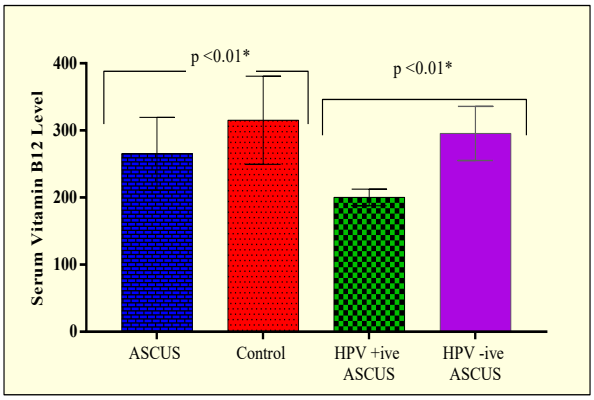

Figure 2: comparison of serum vitamin B12 level between ascus vs. control and HPV-positive vs. HPV-negative ASCUS patients.

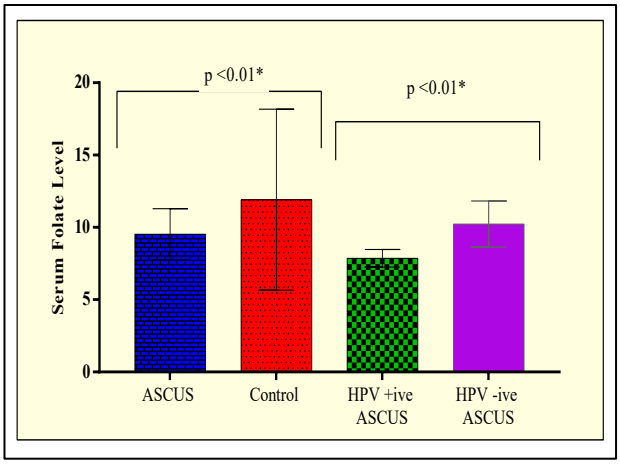

Figure 3: Comparison of serum folate level between ASCUS vs. control and HPV-positive vs. HPV-negative ASCUS patients.

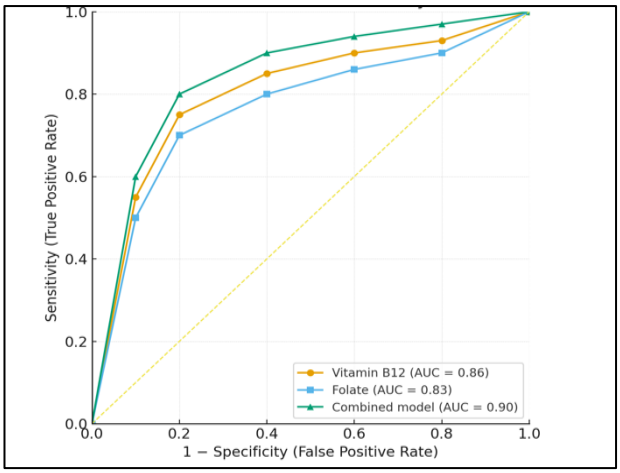

Figure 4: ROC curves for prediction of HPV positivity in ASCUS patients.

Within the ASCUS subgroup, further stratification according to HPV status revealed even more pronounced differences (Table 4). HPV-positive ASCUS patients had markedly lower Vitamin B12 levels (200.14±12.47 pg/ml) than HPV-negative ASCUS women (295.53±40.25 pg/ml, p<0.01). Likewise, mean folate values were substantially reduced in HPV-positive cases (7.86±0.61 ng/ml) compared with HPV-negative ASCUS patients (10.23±1.59 ng/ml, p<0.01). These findings indicate that micronutrient deficiency is not only associated with the presence of ASCUS but is particularly prominent in those with concomitant HPV infection (Figure 3).

The correlation matrix between Vitamin B12, folate and HPV positivity among ASCUS patients is shown in Table 3. Serum Vitamin B12 and folate levels demonstrated a moderate positive correlation with each other (r=0.41, p=0.039), suggesting that deficiency of one micronutrient tends to coexist with deficiency of the other. In contrast, both Vitamin B12 and folate showed significant inverse correlations with HPV positivity. Lower Vitamin B12

levels were strongly associated with HPV infection (r=−0.62, p=0.001) and folate also exhibited a negative correlation with HPV status (r=−0.58, p=0.003). Overall, the correlation analysis supports a close link between reduced micronutrient levels and HPV persistence in ASCUS patients.

The discriminative ability of Vitamin B12 and folate for predicting HPV positivity in women with ASCUS is summarized in Table 4 and illustrated by the ROC curve. Vitamin B12 showed good diagnostic performance with an AUC of 0.86 (95% CI 0.72–0.99, p<0.001). A cut-off value of ≤230 pg/ml yielded a sensitivity of 84.6% and specificity of 81.3%, with a Youden index of 0.66. Folate also demonstrated good discrimination, with an AUC of 0.83 (95% CI 0.67–0.98, p=0.001); using a threshold of ≤8.5 ng/ml provided 80.0% sensitivity and 78.6% specificity (Youden index 0.59).

When Vitamin B12 and folate were combined in a logistic-regression model, the AUC increased to 0.90 (95% CI 0.78–1.00, p<0.001), with an optimal probability cut-off of 0.45 giving 86.7% sensitivity and 85.7% specificity (Youden index 0.72), indicating that the combined model offers the best overall predictive accuracy for HPV positivity (Figure 4).

Table 5 presents the diagnostic indices when Vitamin B12 and folate levels are dichotomized using ROC-derived cut-offs. For Vitamin B12, low levels (≤230 pg/ml) were observed in 9 of 11 HPV-positive ASCUS patients and in 4 of 14 HPV-negative ASCUS patients. This corresponded to a sensitivity of 81.8% and specificity of 71.4%, with a positive predictive value of 69.2% and negative predictive value of 83.3%.

Table 1: Demographic profile of study participants (n=50).

Parameter	Category	Number (n=50)	(%)
Age group (in years)	<30	14	28.0
	30–39	21	42.0
	≥40	15	30.0
Residence	Urban	28	56.0
	Rural	22	44.0
Education	Up to primary	12	24.0
	Secondary	23	46.0
	Graduate and above	15	30.0

Table 2: Obstetric and clinical characteristics of study participants.

Parameter	Category	Number (n=50)	(%)
Parity	Nulliparous	9	18.0
	Para 1–2	29	58.0
	Para ≥3	12	24.0
Age at first sexual intercourse	≤18 years	11	22.0
	>18 years	39	78.0
Number of lifetime sexual partners	1 partner	46	92.0
	≥2 partners	4	8.0
Presenting complaints	Asymptomatic (screening)	20	40.0
	Vaginal discharge	18	36.0
	Intermenstrual bleeding	7	14.0
	Postcoital bleeding	5	10.0

Table 3: Pearson correlation between serum vitamin B12, folate and HPV positivity in ASCUS patients (n=25).

Variable pair	Correlation coefficient (r)	P value
Vitamin B12 vs folate	0.41	0.039*
Vitamin B12 vs HPV positivity	−0.62	0.001*
Folate vs HPV positivity	−0.58	0.003*

*Significance

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The odds of HPV positivity among women with low Vitamin B12 were approximately eleven-fold higher than among those with normal levels (odds ratio 11.25). For folate, a cut-off of ≤8.5 ng/ml identified 8 HPV-positive and 4 HPV-negative ASCUS patients, giving a sensitivity of 72.7% and specificity of 71.4%. The corresponding

positive and negative predictive values were 66.7% and 76.9%, respectively and the odds ratio for HPV positivity with low folate was 6.67. These categorical analyses reinforce the continuous ROC findings and suggest that simple threshold-based interpretation of Vitamin B12 and folate could be clinically useful in identifying ASCUS patients at higher risk of HPV persistence.

Table 4: ROC analysis of serum vitamin B12 and folate for prediction of HPV positivity in ASCUS patients (n=25).

Marker/model	AUC	95% CI	P value	Optimal Cut-off	Sensitivity (%)	Specificity (%)	Youden index
Vitamin B12 (pg/ml)	0.86	0.72-0.99	<0.001*	≤230 pg/ml	84.6	81.3	0.66
Folate (ng/ml)	0.83	0.67–0.98	0.001*	≤8.5 ng/ml	80.0	78.6	0.59
Combined model (Vitamin B12+Folate*)	0.90	0.78–1.00	<0.001*	Predicted probability≥0.45	86.7	85.7	0.72

*Combined model: logistic regression–derived predicted probability using both Vitamin B12 and folate as predictors.

Table 5: Diagnostic performance of low vitamin B12 (cut-off ≤ 230 pg/ml) and low folate (cut-off ≤ 8.5 ng/ml) for predicting HPV positivity in ASCUS patients.

Vitamin B12 category	HPV positive (n=11)	HPV negative (n=14)	Total (n=25)
Low (≤230 pg/ml)	9	4	13
Normal (>230 pg/ml)	2	10	12
Diagnostic index		**Value**	
Sensitivity		81.8 % (9/11)	
Specificity		71.4 % (10/14)	
Positive predictive value		69.2 % (9/13)	
Negative predictive value		83.3 % (10/12)	
Odds ratio (HP+with low vs normal B12)		11.25	
Folate category			
Low (≤8.5 ng/ml)	8	4	12
Normal (>8.5 ng/ml)	3	10	13
Sensitivity		72.7 % (8/11)	
Specificity		71.4 % (10/14)	
Positive predictive value		66.7 % (8/12)	
Negative predictive value		76.9 % (10/13)	
Odds ratio (HPV+with low vs normal folate)		6.67	

DISCUSSION

The present study demonstrates that women with ASCUS cytology have significantly lower serum vitamin B12 and folate levels than cytologically normal controls and that within the ASCUS group these deficiencies are more pronounced in HPV-positive women. In addition, both micronutrients showed a clear inverse association with HPV positivity and ROC analysis suggested that vitamin B12 and folate, both individually and in combination, have good discriminative ability for identifying HPV-positive ASCUS cases. Taken together, these findings support the hypothesis that disturbances in one-carbon metabolism are already evident at very early stages of HPV-related cervical disease and may be linked more closely to viral persistence than only to advanced CIN or invasive cancer.

The findings are in close agreement with studies that have specifically examined vitamin B12 and folate in the setting of ASCUS. Yenigul et al in a prospective cohort of 200 women, reported that serum folate and vitamin B12 levels in ASCUS (+)/HPV (+) patients were significantly lower than in ASCUS(–) or ASCUS (+)/HPV (–) women and concluded that HPV positivity in ASCUS is associated with low serum vitamin B12 and folate.[8] This pattern closely parallels the observations in the present study, in which both micronutrients were significantly depleted in HPV-positive ASCUS cases compared with HPV-negative ASCUS women. The consistency of these findings across different populations strengthens the possibility that impaired micronutrient status may represent a biologically meaningful feature of HPV persistence in equivocal cervical cytology. Evidence from broader cervical cancer and CIN cohorts also supports our observations. Piyathilake et al in a large screening study of Indian

women, showed that women with higher serum folate (>6 ng/ml) and vitamin B12 (>356 pg/ml) were significantly less likely to be infected with carcinogenic or high-risk HPV types than those with lower levels, indicating a protective effect of adequate one-carbon nutrient status against hrHPV infection.[9] Similarly, Pathak et al, studying Indian women with normal epithelium, squamous intraepithelial lesions and invasive cancer, observed that lower folate and vitamin B12 status was associated with HPV infection and with promoter hypermethylation of several tumour-suppressor genes, implicating these micronutrients in both viral and epigenetic pathways of cervical carcinogenesis.[10] The observation of lower vitamin B12 and folate in ASCUS, particularly in HPV-positive women, extends this mechanistic framework to an earlier and clinically important stage of cervical epithelial abnormality.

Several other epidemiological studies have linked folate and vitamin B12 status to the risk and severity of HPV-related cervical lesions. Silva et al reported that women with pre-neoplastic cervical lesions had lower serum folate and vitamin B12 compared with controls and that low levels of these micronutrients were independently associated with an increased risk of pre-neoplastic lesions.[7] In Chinese cohorts, Zhao et al and Yang et al found that adequate or higher folate levels were inversely associated with CIN progression and with the risk of higher-grade CIN, both in women with and without hrHPV infection.[6,11] More recently, Ferrari et al, reviewed the role of micronutrients in HPV infection and cervical carcinogenesis and concluded that low folate and vitamin B12 levels are consistently associated with an increased risk of premalignant cervical lesions and HPV persistence, although effect sizes vary across studies.[12] The present study adds to this body of evidence by focusing specifically on ASCUS with documented HPV status and by examining not only group differences but also the potential discriminatory performance of these biomarkers.

The biological plausibility of our results deserves emphasis. Folate and vitamin B12 play central roles in one-carbon metabolism, a pathway necessary for DNA synthesis, repair and methylation. Deficiency in these micronutrients may result in uracil misincorporation, DNA strand breaks, genomic instability and aberrant methylation patterns affecting both host cellular genes and viral genomes. Such alterations may impair epithelial integrity and immune-mediated viral clearance, thereby favouring the persistence of hrHPV infection. Piyathilake et al have shown that higher plasma folate and vitamin B12 are associated with a more favourable methylation profile of HPV16 and a reduced likelihood of CIN2+, supporting a link between methyl donor availability, viral epigenetics and disease severity.[9] In this context, our finding that the combined assessment of vitamin B12 and folate performed better than either marker alone is consistent with the concept that these nutrients act as interconnected components of the same metabolic pathway rather than as isolated factors. An important strength of the present study is that it shifts attention to the ASCUS category, which often represents a diagnostic and management gray zone in routine gynecological practice. Most ASCUS cases are benign or transient, yet a clinically relevant subset is associated with persistent hrHPV infection and higher-grade disease. In such cases, simple adjunctive biomarkers may be valuable for improving triage. The ROC findings, which showed good AUC values for vitamin B12 and folate individually and even better performance when the two were combined, suggest that these micronutrients may have practical utility in risk stratification. Although these markers cannot replace cytology or HPV testing, they may help identify women who merit closer surveillance or more careful follow-up.

Interventional and nutritional data further support the relevance of this association. Gholamalizadeh et al reported that in ASCUS women with positive HPV, baseline folate and vitamin B12 levels were significantly lower than in HPV-negative women and that nutritional supplementation in women with cervical lesions had favourable effects on metabolic and inflammatory markers.[13] More recent supplementation trials, such as those described by Ferrari et al and colleagues, combining folic acid and vitamin B12 with other bioactive compounds such as epigallocatechin gallate and hyaluronic acid, have shown higher HPV clearance rates and regression of low-grade lesions compared with controls, suggesting a possible adjuvant role for micronutrient optimization in HPV-related disease.[12] Although the present study was observational rather than interventional, the strong inverse association and promising diagnostic profile of vitamin B12 and folate indicate that these markers may be relevant not only as indicators of risk but also as potentially modifiable factors in ASCUS patients.

At the same time, our findings should be interpreted with appropriate caution. Not all previous studies have demonstrated significant associations. Sedjo et al, in a cohort of Hispanic women, reported no relation between circulating folate, vitamin B12 or homocysteine and HPV persistence or cervical dysplasia and no consistent differences across cytological grades.[13] Alberg et al likewise found no clear association between serum folate, vitamin B12 or homocysteine concentrations and subsequent risk of cervical cancer in a nested case-control study, while Butterworth et al, in an earlier JAMA study, did report an association between folate deficiency and cervical dysplasia.[14,15] These inconsistencies may be related to differences in study design, population characteristics, baseline nutritional patterns, measurement methods, HPV typing, lesion spectrum and adjustment for important confounders such as smoking, diet, sexual behavior and coinfections. The clearer association seen in our study may partly reflect the relatively homogeneous study population, the exclusion of women using recent supplementation and the explicit stratification of ASCUS cases by HPV status.

The clinical implications of the findings are particularly relevant in resource-limited settings. Measurement of serum vitamin B12 and folate is relatively inexpensive and widely available compared with many advanced molecular markers. In regions where access to colposcopy, HPV genotyping or repeated molecular follow-up may be constrained, low-cost biochemical indicators may provide supportive information for individualized risk assessment. Identifying ASCUS women who are both HPV-positive and micronutrient-deficient could help prioritize closer follow-up, timely referral and targeted nutritional counselling. However, such an approach should currently be regarded as adjunctive rather than definitive, because the cross-sectional design of the present study does not allow inference regarding temporality or causality. It remains uncertain whether low vitamin B12 and folate directly contribute to HPV persistence and epithelial instability or whether they represent markers of broader nutritional, social or behavioral vulnerabilities associated with increased infection risk.

The present study also has limitations that should be acknowledged. The sample size was modest, which may limit the precision and generalizability of the estimates. The cross-sectional design precludes causal interpretation and does not permit direct assessment of true HPV persistence over time. Serum measurements were used rather than longer-term indicators such as red-cell folate and detailed dietary intake, smoking exposure and other potential confounding variables were not comprehensively quantified. Nevertheless, the clear separation in micronutrient levels between HPV-positive and HPV-negative ASCUS women, along with the ROC findings, suggests that the observed relationship is unlikely to be incidental and warrants further exploration.

In summary, the present study adds to the growing evidence that disturbances in one-carbon metabolism are linked to HPV positivity and early cervical epithelial abnormalities. By demonstrating that vitamin B12 and folate are lower in ASCUS than in controls, particularly in HPV-positive ASCUS patients and that these micronutrients show useful discriminative performance in ROC analysis, our findings support consideration of vitamin B12 and folate as simple adjunctive biomarkers in the triage of women with ASCUS. Larger multicentre longitudinal studies, ideally incorporating detailed dietary assessment, red-cell folate measurement, host and viral methylation markers and interventional components, are needed to confirm these associations and to determine whether correction of subclinical deficiencies can improve HPV clearance and reduce progression of early cervical lesions.

CONCLUSION

In the present study, women with ASCUS cytology had significantly lower serum Vitamin B12 and folate levels compared with cytologically normal controls and within the ASCUS group, these micronutrient deficits were even more pronounced among HPV-positive patients. Both Vitamin B12 and folate showed a strong inverse association with HPV positivity and ROC analysis demonstrated good discriminative ability of each marker, with the combined model of Vitamin B12 and folate achieving excellent accuracy for predicting HPV infection in ASCUS cases. These findings suggest that subclinical deficiencies of Vitamin B12 and folate may contribute to HPV persistence or reflect a biological milieu favouring viral persistence and cervical epithelial abnormalities. Clinically, simple, low-cost measurement of these micronutrients, together with cytology and HPV testing, could help identify ASCUS patients at higher risk who may benefit from closer surveillance, timely colposcopic evaluation and targeted nutritional optimisation. However, confirmation of these observations in larger, longitudinal cohorts and interventional studies is required before routine use of Vitamin B12 and folate as predictive biomarkers can be firmly recommended.

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REFERENCES

1. Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-Secretan B, et al. Global estimates of incidence and mortality of cervical cancer in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. Lancet Glob Health. 2023;11(2):197-206.
2. International Agency for Research on Cancer. GLOBOCAN 2020: India Fact Sheet. Lyon: IARC; 2021. Global Cancer Observatory; 2021.
3. Zhan X, Wang S, Wu X. Atypical squamous cells of undetermined significance. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023.
4. Cox JT. Management of atypical squamous cells of undetermined significance. J Low Genit Tract Dis. 2003;7(3):178-83.
5. Pathak S, Wilkhoo H, Mishra PK, Chander B, Mahajan VK, Sud R, et al. Cervical cancer pathogenesis is associated with one-carbon metabolism. Mol Cell Biochem. 2012;369(1-2):1-7.
6. Zhao W, Hao M, Wang Y, Feng N, Wang Z, Wang W, et al. Association between serum folate and cervical intraepithelial neoplasia and HPV infection in Chinese women: a case–control study. Nutrition. 2016;32(3):367-72.
7. Silva NNT, Gonçalves AK, Cobucci RN, Mendonça RC, Lima PH, Costa VV. Association of serum folate and vitamin B12 with pre-neoplastic cervical lesions. J Obstet Gynaecol. 2020;40(7):1001-6.
8. Yenigul NN, Yazıcı Yılmaz F, Ayhan I. Can serum vitamin B12 and folate levels predict HPV penetration in patients with ASCUS. Nutr Cancer. 2021;73(4):602-8.

9. Piyathilake CJ, Badiga S, Paul P, Vijayaraghavan K, Vedantham H, Sudula M, et al. Indian women with higher serum concentrations of folate and vitamin B12 are significantly less likely to be infected with carcinogenic or high-risk types of human papillomaviruses (HPVs). Int J Women Health. 2010;2:7-12.
10. Pathak S, Bajpai D, Banerjee A, Bhatla N, Jain SK, Jayaram HN, et al. Serum one-carbon metabolites and risk of cervical cancer. Nutr Cancer. 2014;66(5):818-24.
11. Yang J, Yang A, Wang Z, Wang W, Wang Z, Wang Y, et al. Interactions between serum folate and human papillomavirus with cervical intraepithelial neoplasia risk in a Chinese population-based study. Am J Clin Nutr. 2018;108(5):1034-42.
12. Ferrari FA, Magni F, Bosco M, Biancotto G, Zorzato PC, Laganà AS, et al. The role of micronutrients in human papillomavirus infection, cervical dysplasia and neoplasm. Healthcare (Basel). 2023;11(11):1652.
13. Sedjo RL, Fowler BM, Schneider A, Henning SM, Hatch K, Giuliano AR. Folate, vitamin B12 and homocysteine status: findings of no relation between human papillomavirus persistence and cervical dysplasia. Nutrition. 2003;19(6):497-502.
14. Alberg AJ, Selhub J, Shah KV, Viscidi RP, Comstock GW, Helzlsouer KJ. The risk of cervical cancer in relation to serum concentrations of folate, vitamin B12 and homocysteine. Cancer Epidemiol Biomarkers Prev. 2000;9(7):761-4.
15. Butterworth CE, Hatch KD, Macaluso M, Cole P, Sauberlich HE, Soong SJ, et al. Folate deficiency and cervical dysplasia. JAMA. 1992;267(4):528-33.

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