ORIGINAL ARTICLE

Association of Serum Calcium and Vitamin D Levels with Genitourinary Microbiological Shifts in Post-Menopausal Women: A Tertiary Care Center Study

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ABSTRACT

Objective: To assess serum calcium and vitamin D levels and identify their relationship with genitourinary microbiological changes in post-menopausal women visiting a tertiary care center.

Study Design: Cross-sectional, observational.

Place and Duration of Study: This study was done at Sadaf Yahya Hospital, a tertiary care center in Daska, Pakistan, between 3rd March 2025 and 29th August 2025.

Material and Methods: There were 217 post-menopausal women (over the age of 45 years) enrolled through non-probability consecutive sampling. Serum vitamin D and Serum calcium levels were measured. The vaginal flora was assessed using high vaginal swabs, followed by Gram staining with Nugent scoring and aerobic and anaerobic culture techniques. Data were analyzed using SPSS version 29. The Spearman's rank test was used to assess the associations. The level of statistical significance is taken at p-value below 0.05.

Results: The mean age of the participants was 54.0 years. 52.1% of the women were found to be deficient in vitamin D, and 68.7% of the women had low calcium levels in their serum. Bacterial vaginosis (Nugent score 7-10) was identified in 34.1% of participants. A significant association was found between vitamin D status and Nugent score categories ($p < 0.001$). Both vitamin D deficiency ($p<0.001$) and low calcium levels ($p=0.001$) were significantly associated with alterations in vaginal flora (as indicated by Nugent score). Serum vitamin D ($r=-0.509$) and calcium levels ($r=-0.199$) showed significant negative correlations with Nugent score ($p<0.001$).

Conclusion: Low serum vitamin D and calcium levels are significantly associated with altered vaginal flora consistent with bacterial vaginosis in post-menopausal women. However, further longitudinal and interventional studies may be done before clinical screening recommendations are made.

Key Words: *Bacterial Vaginosis, Calcium, Menopause, Vaginal Microbiota, Vitamin D.*

Introduction

Post-menopausal phase is characterized by a series of different psychological, physical, and emotional changes in a woman. Females undergoing menopause experience physiological changes that not only affect the genitourinary anatomy but also influence mucosal immunity and microbial balance of the genitourinary area.[1] Altered hormonal balance, especially a lack of estrogen, leads to mucosal thinning and an altered epithelial barrier function. This eventually leads to dryness in the vaginal area, painful intercourse, and repeated urinary tract infections (UTI). The constellation of these symptoms is also referred to as the genitourinary syndrome of menopause (GSM).[2]

Along with hormonal changes, some minerals and vitamins may also influence the female genitourinary environment. Notably to mention here are calcium metabolism and vitamin D status, which are believed to influence epithelial integrity. The role of Vitamin D is believed to be due to receptors that are located across urogenital tissues. These receptors play a role in regulating antimicrobial peptides and tight-junction proteins.[3] Serum calcium is believed to have a role in epithelial cell signaling and muscular activity. Deficiency of these factors can lead to shifts in vaginal and urinary microbial flora. These shifts make an individual

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susceptible to genitourinary infections and inflammation.[4]

With a global rise in life expectancy globally, the burden of genitourinary morbidity in older women is substantial and growing as life expectancy rises. Recurrent UTIs and GSM impair quality of life, increase healthcare utilization, and drive antibiotic exposure, with important public-health consequences including antimicrobial resistance. Previous microbiological studies demonstrate that post-menopausal vaginal microbiological composition shows increased diversity and reduced Lactobacillus dominance compared with premenopausal states, patterns associated with symptomatic disease and recurrent cystitis.[5] In parallel, epidemiological and meta-analytic data suggest associations between low serum vitamin D and increased risk of UTIs and lower urinary tract symptoms, mediated via immune modulation and mucosal barrier effects; professional societies note high prevalence of hypovitaminosis D in older women and call for targeted evaluation.[6] These international results indicate that micronutrient status and the microbial flora patterns are modifiable factors that may help to decrease the risk of infection and enhance genitourinary prognosis.[7]

Regionally, particularly in South Asia, data on the knowledge and information about the interaction of vitamin D, calcium, and genitourinary microbial flora in post-menopausal women has not been limited.[8] Pakistan as well as other neighboring countries' population studies report high rates of vitamin D deficiency in all age groups, including middle-aged and elderly women, but regular tests of vitamin D or calcium in women with recurring urinary symptoms are not a common practice.[9] Local microbiological practice often focuses on culture-based pathogen identification and antibiotic susceptibility without integrating culture-independent investigations or host nutritional status. This gap in regional evidence and clinical practice creates missed opportunities to understand context-specific drivers of recurrent UTIs and GSM.[10]

Given these gaps, our study evaluated serum calcium and 25-hydroxyvitamin D concentrations, along with genitourinary microbiological assessment using Nugent scoring and culture in postmenopausal women at a tertiary care center. We aimed to clarify whether low vitamin D levels or altered calcium levels are associated with vaginal microbiological changes and/or a higher infection burden. We hoped that the findings could inform targeted screening, non-antibiotic preventative strategies (nutritional optimization, topical/systemic vitamin D approaches, probiotic or estrogenic therapies), and stewardship-friendly care pathways that would reduce morbidity and antibiotic exposure.

Materials And Methods

This cross-sectional observational study was conducted at the Sadaf Yahya Hospital, a tertiary care center in Daska, Pakistan from 3rd March 2025 till 29th August 2025, having received approval from the Institutional Review Board (IRB) under reference number SYH-IRB/2025/125. Based on extensive literature search, a sample size of 217 participants was calculated using the world health organization (WHO) sample size calculator. It comprises 5% margin of error, confidence level of 95% and prevalence rate of bacterial vaginosis in post-menopausal women as 16.93%.[11] The sampling was performed through a non-probability convenience method of sampling.

All women aged 45 years or older with natural menopause for at least 12 months were eligible to participate in this study.

Any female with history of antibiotics, antifungals, probiotics, or hormone replacement therapy intake within the past four weeks of sampling was excluded from the study. Women with history of chronic illnesses such as kidney disease, endocrine diseases, malabsorption syndromes, immunosuppressive therapy and those who were not willing to give informed consent were also excluded.

All patients were enrolled with prior written permission. Confidentiality of patients was ensured at all levels. Data were collected in the outpatient clinics on pre-approved forms via direct consultation. Demographic details (age, BMI, parity), lifestyle (dietary calcium consumption, sun exposure, supplementation), and medical history (duration of menopause, comorbid conditions such as diabetes, genital symptoms such as vaginal dryness, itching, dysuria, discharge), and a history of recurring UTI were recorded in the interview questionnaire.

After the interview, all participants were sampled by

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venipuncture following strict aseptic technique. 5 mL of blood was collected in EDTA sampling tube. After separating out the serum by centrifugation, both serum 25-hydroxyvitamin D (25(OH)D levels) and serum calcium levels were measured. Serum Vitamin D levels were analyzed on Roche Cobas® 6000 Series by Electrochemiluminescence immunoassay technique using the Elecsys® Vitamin D total III reagent kit. Roche-provided reagent inserts indicate interpretation of 25(OH)D levels for vitamin D status assessment as: Deficient: < 20 ng/mL (< 50 nmol/L), Insufficient: 20–30 ng/mL (50–75 nmol/L), Sufficient: > 30 ng/mL (> 75 nmol/L), Potential High/Excess: > 100 ng/mL (> 250 nmol/L).[12]

Serum Calcium levels were also measured on the same analyzer by Photometric colorimetric assay using Calcium Gen.2 (CA2) reagent Kit. The typical Machine-Associated Reference Interval for serum calcium levels followed in our lab is as follows: Low (hypocalcemia): < 8.6 mg/dL (< 2.15 mmol/L), Normal: 8.6–10.2 mg/dL (2.15–2.55 mmol/L), and High (hypercalcemia): > 10.2 mg/dL (> 2.55 mmol/L).[13]

Vaginal microbiota was assessed using the Nugent scoring system. High vaginal swabs were collected from the posterior fornix, and a thin smear was prepared on a clean glass slide, air-dried, and heat fixed. Gram staining was performed using crystal violet (1 minute), Gram's iodine (1 minute), decolorization with acetone–alcohol (10–15 seconds), followed by counterstaining with safranin (30–60 seconds). Slides were examined under oil immersion at 1000× magnification, and 10–20 representative high-power fields were evaluated. Bacterial morphotypes were quantified semi-quantitatively, including large Gram-positive rods (Lactobacillus morphotypes), small Gram-variable rods (Gardnerella/Bacteroides morphotypes), and curved Gram-negative or Gram-variable rods (Mobiluncus morphotypes). A composite score ranging from 0 to 10 was calculated based on the relative abundance of these morphotypes. Interpretation was as follows: scores 0–3 were considered normal flora, 4–6 intermediate flora, and 7–10 consistent with bacterial vaginosis, according to CDC-referenced standards.[14]

Data was analyzed using SPSS version 29. The Shapiro-Wilk test was applied to check the normality of continuous data. Categorical data were presented as frequencies and percentages, while continuous data were presented as mean and standard deviation if normally distributed and median and IQR for non-normally distributed data. Chi-square test was applied to check the association between categorical variables. Pearson correlation was applied to check the correlation between continuous variables. Statistical significance was considered as a p-value less than 0.05.

Demographic and clinical variables, including BMI, diabetes status, sun exposure, and calcium supplementation, were recorded; however, these variables were not included as covariates in multivariable modeling and were analyzed descriptively.

Results

A total of 217 postmenopausal women were included in this study. The median age of the participants was 54.00 (50.00–59.00) years. The baseline demographic, clinical and microbiological characteristics of study participants are shown in Table-I.

The association between Nugent score and Vitamin D is shown in Table-II. A significant association was found between vaginal microbiota composition and Vitamin D status (p<0.001). This indicates that the prevalence of bacterial vaginosis decreased with improving vitamin D status, indicating a strong inverse association between serum vitamin D levels and Nugent score categories.

Table-III presents the association of vitamin D and calcium status with genitourinary microbiological shift among postmenopausal women. A statistically significant association was observed between vitamin D status and microbiological shift (p < 0.001). Among vitamin D deficient women, 51.3% exhibited microbiological shift, compared to 12.9% in insufficient and 18.2% in sufficient groups. Similarly, serum calcium status showed a significant association with microbiological shift (p = 0.001), with 40.9% of women with low calcium levels demonstrating microbiological shift compared to 16.2% among those with normal calcium levels.

The correlation of vitamin D and calcium with Nugent score is shown in Table-IV. Spearman´s rank correlation analysis showed a statistically significant negative correlation between serum vitamin D levels

and Nugent score (r = −0.509, 95% CI: −0.60 to −0.40, p < 0.001), indicating that lower vitamin D levels were moderately associated with higher Nugent scores and greater vaginal microbiological imbalance. Similarly, serum calcium levels showed a significant inverse correlation with Nugent score (r = −0.199, 95% CI: −0.32 to −0.07, p < 0.001), suggesting that low calcium levels were associated with increased severity of vaginal microbiological imbalance.

Table I: Baseline Demographic, Clinical and Microbiological Characteristics of Postmenopausal Women (n = 217)

Variables		Median, IQR
Age (years)		54.00 (50.00–59.00)
Menopausal Duration (years)		5.00 (1.00–9.00)
Parity		3.00 (2.00–5.00)
Nugent Score		5.00 (4.00–7.00)
		Mean ± SD
BMI (kg/m²)		26.62 ± 3.80
Vitamin D (ng/mL)		19.36 ± 6.40
Calcium		8.27 ± 0.39
		n (%)
Diabetes	No	150 (69.1%)
	Yes	67 (30.9%)
Vitamin D status	Deficient (<20 ng/mL)	113 (52.1%)
	Insufficient (20–29 ng/mL)	93 (42.9%)
	Sufficient (≥30 ng/mL)	11 (5.1%)
Calcium Status	Low	149 (68.7%)
	Normal	68 (31.3%)
Nugent Category	Normal (0–3)	32 (14.7%)
	Intermediate (4–6)	111 (51.2%)
	BV (7–10)	74 (34.1%)
Microbiological shift	No	143 (65.9%)
	Yes	74 (34.1%)
Lactobacillus Dominance (Lactobacillus spp.)	No	185 (85.3%)
	Yes	32 (14.7%)
BV Associated Anaerobes *(Gardnerella, Prevotella, Mobiluncus)*	No	140 (64.5%)
	Yes	77 (35.5%)
Uropathogens *(E. coli, Klebsiella, Enterococcus, Proteus)*	No	152 (70.0%)
	Yes	65 (30.0%)

Table II: Association between Vitamin D Status and Nugent Score Categories (n=217)

Vitamin D Status	Nugent Score Category			Total	p Value
	Normal	Intermediate	BV		
Deficient	4 (3.5%)	51 (45.1%)	58 (51.3%)	113 (52.1%)	**<0.001**
Insufficient	29 (31.2%)	52 (55.9%)	12 (12.9%)	93 (42.9%)	
Sufficient	3 (27.3%)	6 (54.5%)	2 (18.2%)	11 (5.1%)	
Total	**36 (16.6%)**	**109 (50.2%)**	**72 (33.2%)**	**217 (100%)**	

Table III: Association of Vitamin D and Calcium Status with Genitourinary Microbiological Shift (n=217)

Variables		Microbiological Shift		*p*-value
		No n (%)	Yes n (%)	
Vitamin D Status	**Deficient**	55 (48.7%)	58 (51.3%)	**<0.001**
	Insufficient	81 (87.1%)	12 (12.9%)	
	Sufficient	9 (81.9%)	2 (18.2%)	
Calcium Status	**Low**	88 (59.1%)	61 (40.9%)	**0.001**
	Normal	55 (80.9%)	13 (19.1%)	

Table IV: Correlation of Serum Vitamin D and Calcium Levels with Nugent Score (n=217)

Variable	Nugent score		
	r	CI	p Value
Vitamin D	−0.509	−0.60 to −0.40	**<0.001**
Calcium	−0.199	−0.32 to −0.07	**<0.001**

Discussion

This cross-sectional study demonstrates a significant association between serum vitamin D and calcium levels with genitourinary microbiological imbalance among postmenopausal women. Our findings indicate that lower vitamin D and calcium levels are associated with higher Nugent scores, increased prevalence of bacterial vaginosis (BV), and loss of Lactobacillus dominance. The existence of such associations is becoming biologically plausible, as supported by international literature.[15] It has been demonstrated by several studies that vitamin D is very important in supporting mucosal immunity via the regulation of antimicrobial peptides like cathelicidin and defensins, which are needed to maintain Lactobacillus-dominant vaginal ecosystems.[16] Calcium may also contribute to epithelial barrier integrity and cellular signaling pathways that influence mucosal defense mechanisms within the genitourinary tract.[17]

Neugent et al. proved that a postmenopausal estrogen deficiency with a change in host immunity predisposes to BV-related anaerobes, which further predispose to genitourinary infection.[18] Similarly,

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Hugenholtz et al. established that the abundance of Lactobacillus is lower in postmenopausal women with or without symptomatic infection, highlighting the importance of systemic host factors including micronutrient status.[19] Our results of high negative correlation between the vitamin D level and the Nugent score (r = -0.509) are consistent with meta-analytical findings of vitamin D deficiency with a higher risk of BV and UTIs.[20,21] Although calcium has received comparatively less research attention in relation to genitourinary microbiota, emerging evidence suggests that it plays an important role in epithelial cell signaling, tight junction stability, and mucosal barrier integrity.[22] These mechanisms may influence the maintenance of a healthy vaginal microbiological environment. The relatively high proportion of low serum calcium observed in this study may reflect regional nutritional patterns and laboratory-specific reference ranges and therefore should be interpreted cautiously.

Regionally, there are limited data treating micronutrient status and genitourinary microbiota in postmenopausal women. Pakistani and South Asian studies continue to indicate that vitamin D deficiency is commonly found among women, in some cases reaching over 50% and this is similar to our results, where 52.1% of the participants were vitamin D deficient.[23] Nevertheless, the majority of regional research concentrates on bone health or metabolic health, and in most cases, it pays little attention to urogenital health.[24] Local microbiological research remains mostly based on culture-based detection of pathogens without the addition of Nugent scoring and host nutritional parameters. The gap that our research fills is the inclusion of biochemical, microbiological, and clinical evidence to provide region-specific data showing that micronutrient deficiencies can contribute to genitourinary dysbiosis in postmenopausal women.

Overall, this study demonstrates that lower serum vitamin D and calcium levels were associated with higher Nugent scores and increased prevalence of microbiological imbalance among postmenopausal women. Evaluation of micronutrient status, including both vitamin D and calcium, may be considered in future research investigating genitourinary health in postmenopausal women reporting with recurring genitourinary symptoms by determining modifiable systemic variables associated with the presence of microbiological imbalance. These findings highlight the importance of considering host nutritional status alongside microbiological findings when evaluating postmenopausal women presenting with recurrent genitourinary symptoms. On the level of public health, these interventions might minimize frequent infections, exposure to antibiotics, and the burden of antimicrobial resistance.

Limitations

There are some limitations to this study. As a cross-sectional, single-center study, causal associations cannot be drawn, and the results may not be applicable to all groups. Potential confounders such as dietary calcium intake, sunlight exposure, and metabolic comorbidities were not controlled through multivariable analysis, which may influence micronutrient levels and microbiological composition. Also, no molecular microbiome sequencing was carried out, which would have given more profound insights into microbial diversity and functional modification.

Conclusion

In conclusion, low serum vitamin D and calcium levels are potentially associated with genitourinary microbiological dysbiosis in post-menopausal women. These findings highlight a potential association between micronutrient status and vaginal microbiological dysbiosis and warrant further multicenter and interventional studies.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.