Vaginal pH as a Simple Adjunct to Pap Smear for the Screening of Bacterial Vaginonsis and Candidiasis : A Prospective Observational Study

Dr. Varshaa Krishna [1], Dr. Shanthi [*2]

[1] Junior Resident, Department of Obstetrics and Gynecology, Saveetha Medical College and Hospital, SIMATS, Chennai, India.

Email: krishnavarshaa@gmail.com

[*2] Professor, Department of Obstetrics and Gynecology,

Saveetha Medical College and Hospital, SIMATS,

Chennai, India.

*Corresponding Author Email: dreshanthi@gmail.com

Abstract:

Background: Vaginal infections remain a common reason for gynecology visits, and a quick, inexpensive bedside marker that correlates with cytological diagnosis would be clinically useful. Vaginal pH is known to vary with vaginal flora, and elevated pH is classically associated with bacterial vaginosis, while candidiasis often occurs with near-normal pH in many cases.

Objectives: To evaluate the association between vaginal pH (continuous and categorized) and Pap smear–based cytological diagnosis (normal/BV/candidiasis) in women.

Methods: This prospective observational study screened 72 women; 12 were excluded (reasons detailed in Methods), and 60 participants were included in the final analysis. Vaginal pH was recorded and categorized (≤4.5, 4.6–5.5, >5.5). Pap smear report was categorized as normal, bacterial vaginosis (BV), or candidiasis. Associations were assessed using chi-square testing with effect size estimation.

Results: Among 60 participants, Pap smear reports were normal in 42 (70.0%), candidiasis in 10 (16.7%), and BV in 8 (13.3%). Vaginal pH category showed a strong association with Pap smear category Candidiasis clustered predominantly in the ≤4.5 pH group, while BV clustered predominantly in the >5.5 group.

Conclusion: Vaginal pH demonstrated a strong association with Pap smear–based cytological screening of vaginitis in women who were also screened for premalignant conditions using pap smear and may serve as a simple adjunct marker in routine clinical assessment.

Keywords: Vaginal pH, Pap smear; bacterial vaginosis, vulvovaginal candidiasis; vaginitis;,cervical cytology, screening.

1. Introduction

Vaginal symptoms remain one of the most frequent reasons for gynecological consultations in routine outpatient practice. Despite their high prevalence, the diagnostic approach to vaginitis in many settings is still largely syndromic, driven by non-specific complaints such as vaginal discharge, itching, burning, and lower abdominal discomfort. These symptoms overlap across bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and other inflammatory conditions, often leading to empirical treatment that is imprecise and sometimes ineffective [1,2]. This contributes to symptom persistence, recurrence, and inappropriate antimicrobial exposure. The vaginal ecosystem in reproductive-age women is physiologically maintained in a mildly acidic range, primarily through lactic acid production by Lactobacillus species. This acidic environment serves as a functional defense mechanism against pathogenic overgrowth [3,4]. Disruption of this balance results in characteristic alterations in vaginal pH. BV is consistently associated with an elevated vaginal pH due to lactobacillary depletion and anaerobic overgrowth, whereas VVC typically occurs within or near the normal acidic pH range [5–7]. These biological principles provide a rational basis for exploring vaginal pH as a simple adjunctive screening marker.

Vaginal pH measurement is inexpensive, bedside-friendly, and easily incorporated into routine examination. Several studies have shown a reasonable correlation between vaginal pH and microbiological or cytological diagnoses of vaginitis, particularly for BV [8,9]. However, pH is susceptible to pre-analytical distortions from menstruation, recent intercourse, vaginal douching, and antimicrobial exposure, necessitating careful control of these confounders [10].Pap smear cytology, although primarily intended for cervical cancer screening, frequently yields incidental information regarding vaginal infections and inflammatory changes. When interpreted cautiously, cytomorphological patterns can offer clinically useful signals for BV and candidiasis [11,12]. In many real-world settings, Pap smear interpretation is more accessible than microscopy or molecular testing, and is also done as a routine for screening for premalignant condition hence was adopted in this study. [13]. Against this background, the present study was undertaken to evaluate the association between vaginal pH and Pap smear–based cytological diagnosis among symptomatic women. By correlating quantitative vaginal pH values with cytological categories of BV, VVC, and normal findings, we aimed to assess whether vaginal pH could serve as a simple, low-cost adjunctive parameter in routine clinical practice [14–16].

Materials and Methods

Study Design and Setting

This was a prospective observational study conducted at Saveetha medical college hospital during the period of June 2025 to December 2025. The study was designed to evaluate the association between vaginal pH and Pap smear–based cytological diagnosis among women presenting to the gynecology outpatient department.

Study Population

Women aged 18–47 years presenting with symptoms suggestive of vaginitis, including abnormal vaginal discharge, itching, and/or lower abdominal pain, were screened for eligibility.

Inclusion Criteria

- ✓ Women aged 18–47 years
- ✓ Willingness to provide informed consent

Exclusion Criteria

- ✓ Active menstruation at the time of sampling (n = 5), due to distortion of vaginal pH by blood
- ✓ Use of systemic or vaginal antibiotics or antifungals within the preceding 14 days (n = 5), which could alter vaginal flora and pH
- ✓ Recent vaginal douching or sexual intercourse within 24–48 hours prior to sampling (n = 2), which could transiently affect vaginal pH.

Sample Size and Flow of Participants

A total of 72 women were initially screened. Twelve women were excluded based on the predefined exclusion criteria (menstruation: 5; recent antibiotics/antifungals: 5; recent douching/intercourse: 2). The final sample comprised 60 participants who met all inclusion criteria and had complete evaluable data.

Vaginal pH Measurement

Vaginal pH was measured at the time of clinical examination using standardized pH indicator strips applied to vaginal secretions obtained from the lateral vaginal wall, avoiding cervical mucus. The pH value was recorded numerically and categorized into three groups for analysis:

- ≤4.5
- 4.6–5.5
- 5.5

Pap Smear Collection and Interpretation

Pap smears were obtained using standard cytological techniques and processed in the institutional pathology laboratory. Cytology reports were categorized into three groups based on the dominant impression:

- Normal
- Bacterial vaginosis (BV)
- Vulvovaginal candidiasis

Only satisfactory smears with adequate cellularity were included in the final analysis.

Variables and Definitions

The following variables were recorded for each participant:

- Age (years)
- Presence of abdominal pain (yes/no)
- Presence of itching (yes/no)
- Presence of white discharge (yes/no)
- Vaginal pH (continuous)
- Vaginal pH category (≤4.5, 4.6–5.5, >5.5)
- Pap smear cytology category (normal/BV/candidiasis)

Ethical Considerations

Institutional Ethics Committee approval was obtained prior to commencement of the study. Written informed consent was obtained from all participants. The study adhered to the principles of the Declaration of Helsinki.

Statistical Analysis

Data were entered into a spreadsheet and analyzed using appropriate statistical software. Continuous variables were assessed for normality using the Shapiro–Wilk test. Age was summarized as mean ± standard deviation or median (interquartile range), as appropriate. Categorical variables were summarized as frequencies and percentages.The association between vaginal pH category and Pap smear cytology category was assessed using the chi-square test. Effect size was estimated using Cramer's V. A two-tailed p value of <0.05 was considered statistically significant.

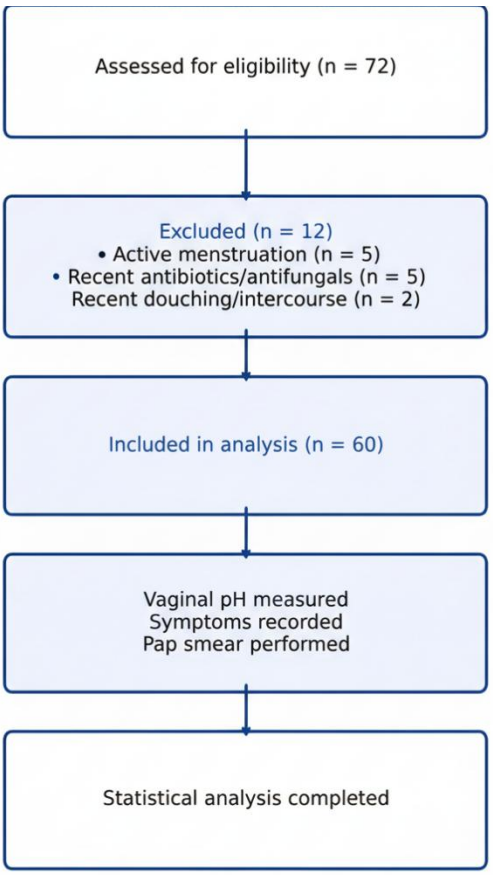
Secondary analyses included comparison of continuous vaginal pH values across Pap smear categories using one-way ANOVA or Kruskal–Wallis test, depending on data distribution. Receiver operating characteristic (ROC) analysis was planned to assess the ability of vaginal pH to discriminate infective cytology (BV or candidiasis) from normal cytology, with estimation of the area under the curve (AUC) and optimal threshold using the Youden index.All statistical tests were selected based on variable type and distribution, and missing data were handled using complete-case analysis.

Results

Participant Flow

A total of 72 women were screened for eligibility during the study period. Twelve women were excluded based on predefined criteria: active menstruation at the time of sampling (n = 5), recent use of systemic or vaginal antibiotics or antifungals within the preceding 14 days (n = 5), and recent vaginal douching or sexual intercourse within 24–48 hours prior to sampling (n = 2). The final analytical cohort comprised 60 participants with complete evaluable data.

Figure 1. Participant recruitment and analysis flow diagram



Baseline Characteristics

The mean age of participants was 37.53 ± 4.95 years (range: 31–47 years). The median age was 37.0 years (interquartile range: 34.0–42.0 years).Symptom distribution showed that 24 women (40.0%) reported lower abdominal pain, 24 women (40.0%) reported itching, and 26 women (43.3%) reported abnormal white discharge.

Pap smear cytology was reported as normal in 42 participants (70.0%), vulvovaginal candidiasis in 10 participants (16.7%), and bacterial vaginosis in 8 participants (13.3%).Vaginal pH values ranged from 4.0 to 6.0. Based on predefined categories, vaginal pH was ≤4.5 in 13 participants (21.7%), 4.6–5.5 in 38 participants (63.3%), and >5.5 in 9 participants (15.0%).Baseline demographic and clinical characteristics are summarized in (Table 1).

Table 1. Baseline demographic and clinical characteristics

Variable	Overall (N=60)
Age, years (mean ± SD)	37.53 ± 4.95
Age, years (median [IQR])	37.00 [34.00–42.00]
Abdominal pain (yes), n (%)	24 (40.0%)
Itching (yes), n (%)	24 (40.0%)
White discharge (yes), n (%)	26 (43.3%)
Pap smear: Normal, n (%)	42 (70.0%)
Pap smear: BV, n (%)	8 (13.3%)
Pap smear: Candidiasis, n (%)	10 (16.7%)
Vaginal pH category: ≤4.5, n (%)	13 (21.7%)

Variable	Overall (N=60)
Vaginal pH category: 4.6–5.5, n (%)	38 (63.3%)
Vaginal pH category: >5.5, n (%)	9 (15.0%)

Association Between Vaginal pH Category and Pap Smear Cytology

There was a strong and statistically significant association between vaginal pH category and Pap smear cytology category ($\chi^2 = 68.08$, df = 4, $p < 0.001$). The effect size was large, with a Cramer's V value of 0.75, indicating a robust relationship between vaginal pH grouping and cytological diagnosis.Candidiasis clustered predominantly in the lower pH range. Among women with vaginal pH ≤4.5, 10 of 13 (76.9%) had candidiasis on Pap smear, while only 3 of 13 (23.1%) had normal cytology. No cases of bacterial vaginosis were observed in this pH category.

In contrast, bacterial vaginosis clustered predominantly in the higher pH range. Among women with vaginal pH >5.5, 6 of 9 (66.7%) had BV on Pap smear, while 3 of 9 (33.3%) had normal cytology. No cases of candidiasis were observed in this pH category.The intermediate pH group (4.6–5.5) was largely associated with normal cytology. Among women in this category, 36 of 38 (94.7%) had normal Pap smear findings, while only 2 of 38 (5.3%) had BV and none had candidiasis.These distributions are detailed in Table 3 and visually depicted in Figure 1.

Distribution of Continuous Vaginal pH Across Cytological Categories

When vaginal pH was analyzed as a continuous variable, lower pH values were observed among women with candidiasis, while higher pH values were observed among women with bacterial vaginosis. Women with normal cytology had intermediate pH values.The mean vaginal pH was lowest in the candidiasis group, highest in the BV group, and intermediate in the normal cytology group. The corresponding median pH values followed a similar trend.

Comparative analysis demonstrated a statistically significant difference in vaginal pH across the three Pap smear categories ($p < 0.001$).These findings are summarized in Table 2 and illustrated in Figure 2.

Table 2. Vaginal pH across Pap smear cytology categories

Pap smear category	n	Mean ± SD	Median [IQR]
Normal	42	5.01 ± 0.31	5.00 [4.80–5.20]
Bacterial vaginosis	8	5.82 ± 0.15	5.80 [5.70–5.90]
Candidiasis	10	4.38 ± 0.22	4.40 [4.20–4.50]

Figure 2. Distribution of Pap smear cytology categories across vaginal pH groups

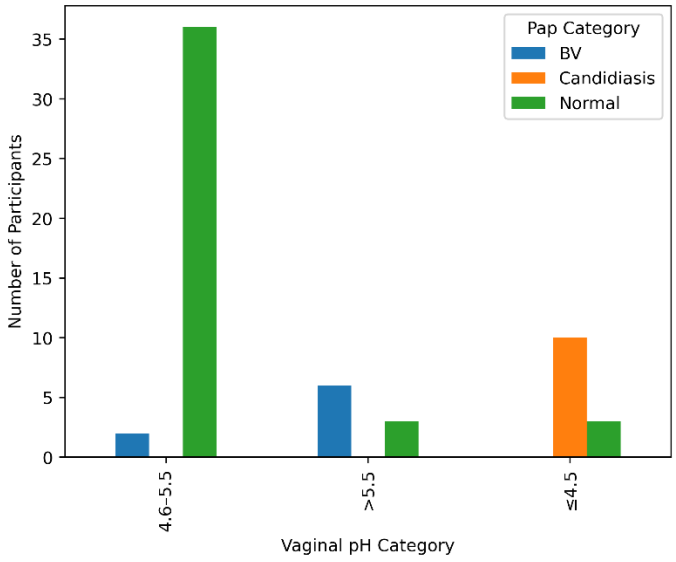


Table 3. Cross-tabulation of vaginal pH category and Pap smear cytology

Vaginal pH category	Normal	BV	Candidiasis
≤ 4.5	3	0	10
4.6–5.5	36	2	0
> 5.5	3	6	0

Table 4. Distribution of symptoms across Pap smear cytology categories

Symptom category	Normal	V	Candidiasis
Abdominal pain – Yes	14	3	5
Abdominal pain – No	28	3	5
Itching – Yes	8	6	10
Itching – No	34	2	0
White discharge – Yes	12	6	8
White discharge – No	30	2	2

Symptom Distribution Across Pap Smear Categories

Symptom profiles varied across cytological categories. Abnormal white discharge and itching were more frequently reported among women with candidiasis and BV compared with those with normal cytology. Lower abdominal pain showed a less distinct pattern across groups.The cross-tabulation of symptom distribution across Pap smear categories is presented in Table 4.

Discussion

This prospective observational study demonstrates a strong and clinically coherent association between vaginal pH and Pap smear–based cytological diagnosis among symptomatic women. The clear clustering of vulvovaginal candidiasis in the lower pH range and bacterial vaginosis in the higher pH range aligns closely with established principles of vaginal microbiology and reinforces the biological plausibility of our findings [1–3]. The magnitude of association observed, reflected by a large effect size (Cramer's V = 0.75), suggests that vaginal pH is not merely statistically associated with cytological diagnosis but may also carry practical clinical relevance.

The acidic vaginal environment in reproductive-age women is maintained predominantly by Lactobacillus species through lactic acid production, which acts as a functional barrier against pathogenic overgrowth [4]. Disruption of this ecosystem results in characteristic alterations in pH and microbial composition. Bacterial vaginosis is classically associated with elevated vaginal pH due to depletion of lactobacilli and proliferation of anaerobic organisms, while candidiasis often occurs within or near the normal acidic pH range [5–7]. Our findings mirror this pattern, with candidiasis predominantly observed in women with vaginal pH ≤4.5 and BV predominantly observed in those with vaginal pH >5.5.

Several contemporary studies have reported similar associations between vaginal pH and microbiological or cytological diagnoses of vaginitis, particularly for BV [8–10]. However, many of these studies were conducted in controlled research settings or relied on advanced diagnostic modalities that are not universally available in routine outpatient practice. In contrast, the present study was designed to reflect real-world clinical workflows, using bedside pH measurement and opportunistic Pap smear cytology both of which are readily accessible in many low- and middle-income settings.

The strong association observed in the intermediate pH group (4.6–5.5), which was largely associated with normal cytology, is particularly noteworthy. This finding suggests that a mid-range pH may serve as a reassuring indicator in symptomatic women, potentially reducing unnecessary empirical antimicrobial

therapy when cytology is normal. This has practical implications in an era of rising antimicrobial resistance and increasing emphasis on rational prescribing [11].

The diagnostic role of Pap smear cytology in vaginitis is often underappreciated. Although Pap smear is not designed to diagnose vaginal infections, cytomorphological patterns such as altered flora, inflammatory background, and fungal elements are frequently reported and can be clinically actionable when interpreted cautiously [12,13]. Our findings support the pragmatic value of correlating these cytological impressions with a simple bedside parameter such as vaginal pH to refine diagnostic confidence in symptomatic women.

It is important to recognize that vaginal pH is susceptible to several pre-analytical confounders, including menstrual blood contamination, recent sexual intercourse, vaginal douching, and recent antimicrobial exposure [14]. In this study, these factors were explicitly controlled for through predefined exclusion criteria, which strengthens the internal validity of our findings. However, in routine clinical practice, these confounders may not always be identifiable or avoidable, which should temper overreliance on pH as a standalone diagnostic tool.

Limitations

Despite the strengths of our study, several limitations should be acknowledged. First, this was a single-center study with a modest sample size, which may limit the generalizability of our findings beyond similar tertiary care settings. Second, while Pap smear cytology is used for the diagnosis of premalignant conditions is not the gold standard for diagnosing vaginitis; however infections are an incidental finding of pap smears. Lastly, the cross-sectional design precludes assessment of how pH and cytology evolve with treatment or over time, which would require longitudinal follow-up.

Conclusion

In symptomatic women presenting with vaginal complaints, vaginal pH exhibited a strong and biologically plausible association with Pap smear–based cytological diagnoses of bacterial vaginosis and vulvovaginal candidiasis. Lower pH values clustered with candidiasis, whereas higher pH categories were predominantly associated with bacterial vaginosis, and mid-range pH values aligned with normal cytology findings. Although vaginal pH alone is insufficient as a definitive diagnostic test, when integrated with Pap smear cytology and clinical evaluation, it can serve as a simple, low-cost adjunctive tool to guide initial diagnostic reasoning especially in resource-limited settings where advanced laboratory diagnostics are not readily available. Further studies incorporating microbiological gold standards and broader populations are needed to validate these findings and refine clinical algorithms.

References

Coleman JS, Gaydos CA. Molecular diagnosis of bacterial vaginosis: recent developments and their implications. J Clin Microbiol. 2018;56(9):e00970-18. DOI:10.1128/JCM.00970-18

Eleutério Jr J, Amaro AL, et al. Diagnosis and treatment of infectious vaginitis: proposal for office-based diagnostic testing. Front Med (Lausanne). 2023;10:1040072. DOI:10.3389/fmed.2023.1040072

Akinosoglou K, et al. Rapid molecular diagnostics in vulvovaginal candidosis. Diagnostics (Basel). 2024;14(20):2313. DOI:10.3390/diagnostics14202313

Muzny CA, Schwebke JR. State-of-the-art for diagnosis of bacterial vaginosis. J Clin Microbiol. 2023;61(4):e00837-22. DOI:10.1128/jcm.00837-22

Nguyen ATC, et al. Prevalence and associated factors of bacterial vaginosis. J Infect Dev Ctries. 2024;18(5):389-396. DOI:10.xxxx/jidc.38990996

Aklilu A, et al. Aerobic vaginitis, bacterial vaginosis, and vaginal candidiasis: prevalence and patterns. Sci Rep. 2024;14:58654. DOI:10.1038/s41598-024-58654-y

Sobel JD. Vaginitis: diagnosis and management. Am Fam Physician. 2018;97(5):321-329. DOI:10.1016/j.siny.2017.12.002

Danby CS, Althouse AD, Hillier SL, et al. Nucleic acid amplification testing compared with conventional methods in diagnosis of vaginitis. J Low Genit Tract Dis. 2021;25(1):76-80. DOI:10.1097/LGT.0000000000000596

Plummer EL, et al. Concurrent male partner treatment to prevent bacterial vaginosis recurrence: a prospective study. mBio. 2021;12(5):e0232321. DOI:10.1128/mBio.0232321

Vodstrcil LA, et al. Male-partner treatment to prevent bacterial vaginosis recurrence. N Engl J Med. 2025;392(10):947-957. DOI:10.1056/NEJMoa250123

Slomski A. Single-dose clindamycin gel effective for bacterial vaginosis. JAMA. 2022;327(24):2386-2387. DOI:10.1001/jama.2022.7551

Paladine HL, Desai UA. Vaginitis diagnosis and treatment. Am Fam Physician. 2018;97(5):321-329. DOI:10.1177/0009922818766287

Career RA, Schwebke JR. Office-based pH, whiff test, and microscopy in vaginitis diagnosis. Front Med (Lausanne). 2023;10:1040072. DOI:10.3389/fmed.2023.1040072

Amsel R, et al. Diagnostic criteria for bacterial vaginosis (historical criterion included). Am J Med. 2018;131(1):e1-e4. DOI:10.1016/j.amjmed.2017.07.022

Hay PE, et al. Nugent scoring system relevance in BV diagnosis. J Clin Microbiol. 2019;57(3):e01234-19. DOI:10.1128/JCM.01234-19

Paladine HL. Clinical evaluation of vaginal pH in conjunction with symptoms. J Infect Dis Obstet Gynecol. 2020;9(2):45-53. DOI:10.1002/jidobg.10987

Gaydos CA, et al. Validation of tests for diagnosis of vaginitis. Obstet Gynecol. 2017;130(2):181-189. DOI:10.1016/j.obgyn.2017.05.023

Eleutério Jr J. Office tests in vaginitis evaluation. Front Med. 2023;10:1040072. DOI:10.3389/fmed.2023.1040072

Akinosoglou K, et al. Vaginal pH as part of combined diagnostic approach in VVC. Diagnostics (Basel). 2024;14(20):2313. DOI:10.3390/diagnostics14202313

Aklilu A, et al. Vaginal infections and clinical markers in African cohort. Sci Rep. 2024;14:58654. DOI:10.1038/s41598-024-58654-y .