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Prevalence and hematological characteristics of bacterial vaginosis in postmenopausal women

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ABSTRACT

Background: Bacterial vaginosis (BV) is a common vaginal dysbiosis associated with systemic inflammatory responses. However, its hematological impact in postmenopausal women remains unclear. This study investigates hematological parameters in postmenopausal women with BV to assess potential systemic inflammatory alterations.

Objectives: This study aimed to investigate various hematological parameters across different BV conditions to better understand their potential role in BV diagnosis and pathophysiology.

Materials and methods: A total of twenty-five postmenopausal women were recruited and categorized into three groups: bacterial vaginosis (BV), intermediate vaginal microbiota, and healthy vaginal microbiota based on Nugent scoring. Vaginal samples were collected aseptically with the participants in the lithotomy position by swabbing the vaginal walls circumferentially near the cervical fornix. Nugent scoring was performed on Gram-stained smears to classify the subjects. Subsequently, Verify® urinalysis reagent strips were directly applied to the vaginal wall to measure pH, protein, and glucose levels. Additionally, hematological parameters including leukocyte count, lymphocyte, eosinophil, neutrophil, monocyte, basophil, platelet counts, and platelet-to-lymphocyte ratio (PLR) were assessed from peripheral blood samples. All data were statistically analysed and compared among the three groups using ANOVA and Kruskal–Wallis tests, with a significance level set at $p<0.05$.

Results: The prevalence of BV in postmenopausal women was 72%. Hematology parameters did not show significant differences across BV, intermediate, and healthy groups ($p>0.05$). Leukocyte, neutrophil, and PLR values were slightly higher in BV cases, but not to a statistically significant degree. These findings suggest that BV in postmenopausal women may not elicit strong systemic inflammatory responses compared to premenopausal populations. Additionally, pH, glucose, and protein levels did not differ significantly among the groups, highlighting the need to reconsider standard vaginal health biomarkers in postmenopausal women due to physiological changes induced by menopause.

Conclusion: The prevalence of bacterial vaginosis (BV) in this study was higher than that reported in previous studies; however, it was not associated with significant hematological alterations. Further research is needed to identify reliable systemic and vaginal biomarkers for BV diagnosis in this population, considering the potential influence of hormonal and immunological factors.

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Introduction

In postmenopausal women, the reduction in estrogen levels contributes to vaginal atrophy, decreased lactobacilli colonization, and a higher susceptibility to bacterial vaginosis (BV). It is characterized by an imbalance in the vaginal microbiota, where the dominance of *Lactobacillus* species is replaced by anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Mobiluncus* species. This dysbiosis leads to an increased vaginal pH and a reduction in protective lactic acid production, which predisposes women to secondary infections and inflammatory conditions.[1,2] Although we understand the burden of BV in women of reproductive age, much less is known about the burden of BV in postmenopausal women.

The prevalence of BV varies globally, with estimates ranging from 2% to 57% among postmenopausal women, depending on the diagnostic criteria used.[3,4] While BV is often asymptomatic, it has been associated with serious health consequences, including an increased risk of sexually transmitted infections (STIs), pelvic inflammatory disease, and adverse pregnancy outcomes. Despite its clinical relevance, BV remains underdiagnosed in menopausal women due to the overlap of symptoms with other vaginal conditions such as atrophic vaginitis.[4]

Gold standard diagnostic methods for BV, such as Amsel's criteria and Nugent scoring, rely on clinical symptoms and microscopic evaluation.[5] However, these methods may be challenging to apply in postmenopausal women due to the discomfort associated with swab collection, which is exacerbated by vaginal atrophy.[6,7] Additionally, some postmenopausal women may find it difficult to assume the lithotomy position required for swab collection due to age-related musculoskeletal limitations. BV not only disrupts the vaginal microbiota and compromises vaginal health but may also contribute to systemic inflammatory responses, as indicated by alterations in hematological parameter. Although BV is primarily considered a localized condition of the lower genital tract, emerging evidence suggests that it may also elicit systemic inflammatory responses. Several studies have reported elevated serum levels of inflammatory markers such as C-reactive protein (CRP) and pro-inflammatory cytokines (e.g., IL-6, TNF-α) in women with BV, indicating a potential systemic immune activation beyond the vaginal environment.[8] However, studies explain hematological parameters in BV remain limited and inconclusive. In this study, we aim to investigate various hematological parameters across different BV conditions to better understand their potential role in BV diagnosis and pathophysiology.

Materials and methods

This cross-sectional study aims to evaluate the hematological profile as an indicator of BV status. The study population recruited menopausal women who attended the obstetrics and gynecology polyclinic of an independent clinic in Kendari City, Southeast Sulawesi, Indonesia, between April and June 2024 and consented to participate as research subjects. During the study period, 78 postmenopausal women attended the sampling site. Of these, 25 met the inclusion and exclusion criteria and were defined as the accessible population. All eligible subjects were recruited using a total sampling. We collected 25 samples with inclusion criteria consisted of menopausal women without a history of systemic disease and without symptoms indicative of reproductive tract infection, with menopause defined as the absence of menstruation for a minimum of 12 consecutive months.

Subject information and sample collection

Vaginal fluid specimens were obtained from participants using sterile cotton-tipped swabs using ESwab (COPAN Diagnostics, Murrieta, CA, USA) during a pelvic examination. Participants were placed in the lithotomy position to facilitate sample collection and direct application Vaginal swab at the vaginal wall with approximately 2 inches depth. The Verify® Urinalysis Reagent Strips (Verify®, Indonesia) were employed to evaluate vaginal secretions for pH, glucose, and protein levels.

BV assessment

Obstetricians and gynaecologists collected vaginal swabs according to established protocols. We performed Gram staining on all vaginal swabs to determine the Nugent score. The gram-stained smears were heat-fixed, and sequentially stained with crystal violet, iodine solution, decolorized with alcohol, and counterstained with safranin. The stained slides were examined under a microscope at 1000x magnification to assess the presence of *Lactobacillus* morphotypes and other which were then scored based on the Nugent criteria.[9]

pH, glucose and protein levels

A single reagent strip was directly applied to the lateral vaginal wall, ensuring contact between the reagent pads and the vaginal secretions for approximately 1-2 seconds. Care was taken to fully moisten the pads corresponding to pH, glucose, and protein parameters. The reagent strip was then withdrawn and held horizontally to prevent mixing of the reagents. Colour changes on the pads were compared to the manufacturer's reference colour chart at 60 seconds. The resulting colour intensities were recorded and categorized based on the manufacturer's semi-quantitative scale.

Hematological assessment

We collected venous blood samples to evaluate hematological parameters, including leukocytes, eosinophils, basophils, neutrophils, lymphocytes, monocytes, and platelets. Blood samples were drawn

from the antecubital vein using a sterile technique and collected into ethylenediaminetetraacetic acid (EDTA) tubes to prevent coagulation. Routine hematological analysis was performed using an automated haematology analyser to determine complete blood counts (CBC). The PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count. We need no specific patient preparation, such as fasting, was required prior to blood collection. All samples were processed promptly to ensure accuracy and reliability of the results.

Data analysis

Statistical analysis was performed to compare biochemical and hematological parameters among the BV, intermediate, and healthy groups. The associations between BV status and pH, protein, and glucose levels were analysed using the Chi-square test or Fisher's exact test, as appropriate. Hematological variables were compared using one-way ANOVA or the Kruskal–Wallis test, depending on data distribution. A $p<0.05$ was considered statistically significant.

Results

This study involved 25 postmenopausal women who consented to participate. The baseline characteristics of the participants are summarized in Table 1. We performed the BV diagnosis using the Nugent score, a well-established scoring system for classifying vaginal microbiota status based on Gram-stain microscopy. Bacterial vaginosis assessment according to Nugent score microscopy.[9] Health (score 0-3) defined as Gram-positive rods (lactobacilli) predominate representing normal vaginal flora; Intermediate (score 4-6) when decreased lactobacilli with increased presence of small Gram-variable rods (*Gardnerella vaginalis*) and Gram-negative curved rods; BV (score 7-10) when significant depletion of lactobacilli with predominance of Gram-variable coccobacilli (*Gardnerella vaginalis*) and curved Gram-negative rods (*Mobiluncus* species), characteristic of bacterial vaginosis (Figure 1).

This microscopic assessment serves as the gold standard for BV diagnosis according to Nugent criteria, which quantifies bacterial morphotypes to determine vaginal flora status. The shift from lactobacilli dominance to mixed anaerobic flora represents the hallmark dysbiosis of bacterial vaginosis. The results indicated that we categorized 18 samples (72%) as BV-positive, 5 samples (20%) as intermediate, and 2 samples (8%) as health (Table 2).

Table 1. Characteristics of the participants.

Characteristic	Total (N)	Percentage (%)
Age (year)		
≤50	5	23.07
>50	20	76.92
Menopause age (year)		
≤50	13	53.85
>50	12	46.15
Menopause duration (year)		
≤2	5	23.07
>2	20	76.92
Estradiol level (pg/mL)		
≤20	23	84.61
>20	4	15.38
Occupation		
Housewife	10	38.46
Employee	15	61.53
Education		
Middle	10	38.46
Hight	15	61.53
Parity		
<4	15	61.53
≥4	10	38.46

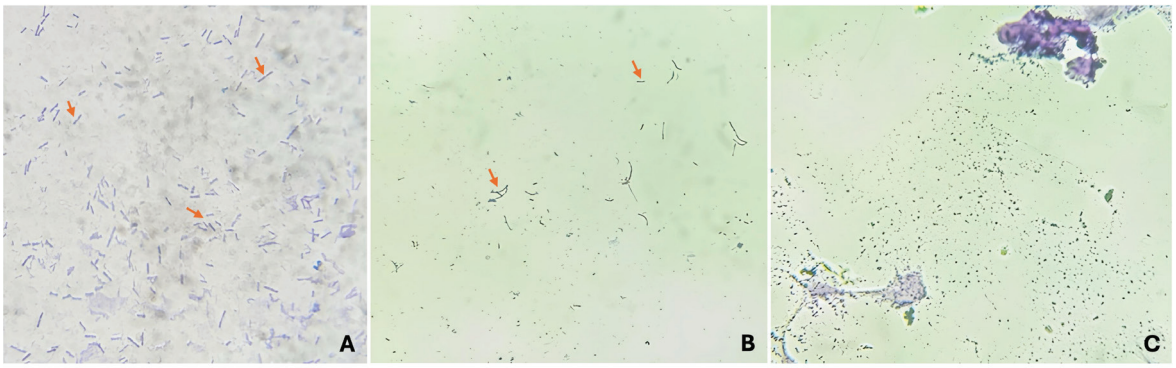

Figure 1. *Bacterial vaginosis assessment based on Nugent scoring. Arrows indicate of Lactobacillus sp (100x magnification), A: non BV (Lactobacillus-dominant), B: intermediate (Lactobacillus with others), C: BV (Lactobacillus-absent).*

Table 2. BV State and vaginal charge examination.

BV state	N (%)	pH		Glucose		Protein	
		<5	>5	Negative	Positive	≤2	>2
BV	18 (72%)	12 (66.7%)	6 (33.3%)	17 (94.4%)	1 (5.6%)	9 (50%)	9 (50%)
Intermediate	5 (20%)	4 (80%)	1 (20%)	5 (100%)	0 (0%)	4 (80%)	1 (20%)
Health	2 (8%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)	1 (50%)	1 (50%)
	p value	0.551		0.817		0.482	

Table 3. Variable analysis test with Anova.

Variable	BV (N=18)	Intermediate (N=5)	Health (N=2)	*p* value
Leucocyte	7.67±1.60	7.14±2.15	6.48±1.05	0.576
Lymphocyte	34.89±6.94	38.90±3.88	37.55±6.29	0.453
Eosinophil	3.50±1.94	3.66±1.24	3.30±1.13	0.970
Neutrophil	56.07±6.37	52.32±4.62	53.75±7.85	0.472
Monocyte	5.53±0.94	5.06±0.63	5.35±0.35	0.565
Basophil*	0.07±0.12	0.06±0.08	0.05±0.05	0.999
Platelet	281.28±61.39	231.20±14.62	231.50±14.85	0.143
PLR	8.66±3.60	5.96±0.26	6.22±0.46	0.156

**Kruskal wallis*

Discussion

The elevation of vaginal pH associated with the depletion of Lactobacillus in postmenopausal women creates a favourable environment for the proliferation of anaerobic pathogens. In postmenopausal women, decreased estrogen levels lead to a significant reduction in glycogen content within the vaginal epithelium. As a result, the growth of Lactobacillus species-key producers of lactic acid that help maintain an acidic vaginal environment-is impaired. The subsequent decline in Lactobacillus populations causes an elevation in vaginal pH, creating a more alkaline environment. This shift favours the proliferation of anaerobic and facultative anaerobic pathogens that are normally suppressed by the acidic milieu, thereby increasing the risk of bacterial vaginosis and other vaginal infections. The combined assessment of vaginal pH, glucose, and protein profiles offers valuable diagnostic insight and contributes to a deeper understanding of the complex pathophysiological mechanisms underlying bacterial vaginosis.[10-12]

In our study, we observed a higher prevalence of bacterial vaginosis (BV) among postmenopausal women compared to several previous reports.[4] Notably, our analysis did not reveal significant differences in vaginal pH, glucose, or protein levels among the BV, intermediate, and healthy groups. This finding may be attributed to the physiological changes associated with menopause, particularly the decline in estrogen levels, which leads to a naturally elevated vaginal pH and a reduction in Lactobacillus

species. These alterations can obscure the typical pH distinctions observed between BV and non-BV cases in premenopausal women.

The differential white blood cell (WBC) count reflects various components of the immune response. Neutrophils and lymphocytes, in particular, are key indicators of systemic inflammation and immune status. An increased neutrophil count often reflects acute inflammatory responses, while lymphocyte levels are associated with adaptive immunity. Changes in the ratio between these cell types-such as the neutrophil-to-lymphocyte ratio (NLR) or PLR-have been proposed as accessible biomarkers of systemic inflammation, including in gynaecologic and infectious conditions.[13]

A notable study published in *Scientific Reports* found that women with BV exhibited higher systemic inflammation markers, characterized by increased total WBC and lymphocyte counts suggest a link between BV and systemic inflammatory responses.[14] The analysis of hematological parameters in this study reveals no statistically significant differences among the BV, intermediate, and healthy groups (Table 3). Leukocyte counts were slightly higher in the BV group (7.67±1.60) compared to the intermediate (7.14±2.15) and healthy groups (6.48±1.05), but the difference was not significant (p=0.576). This finding suggests that systemic inflammatory responses in BV may not always be reflected in routine leukocyte counts. However, some studies have reported elevated leukocytes in BV cases, particularly in younger, premenopausal populations, possibly due to a more active immune response in the presence of bacterial dysbiosis.[13] Similarly, lymphocyte, eosinophil, neutrophil, monocyte, and basophil counts showed no significant variations among the groups, suggesting that BV-related inflammation may not elicit a marked systemic hematological response in postmenopausal women.

Notably, platelet levels were slightly higher in the BV group (281.28±61.39) compared to the intermediate (231.20±14.62) and healthy groups (231.50±14.85), though the difference was not statistically significant (p=0.143). The PLR, an emerging inflammatory marker, was also higher in the BV group (8.66±3.60) but did not reach statistical significance (p=0.156). PLR may reflect systemic inflammatory responses in various infectious and chronic conditions.[14,15] Its cut-off values vary depending on the condition, typically ranging from 121 to 232 cells/μL.[16] In our study, PLR values did not exceed the inflammatory thresholds reported in previous studies. Nevertheless, the noticeable differences observed between groups highlight the need for further investigation into its relevance in bacterial vaginosis, particularly in postmenopausal women.

Estrogen acts as an important immunomodulatory hormone, promoting anti-inflammatory responses and maintaining immune homeostasis. Its reduction leads to an increase in proinflammatory cytokines such as TNF-α, IL-1β, and IL-6, while decreasing anti-inflammatory mediators like IL-10. Additionally, menopause is characterized by immunosenescence, marked by a decline in naïve T cells, accumulation of memory T cells, and dysregulation of T-cell subsets, including increased effector T cells and decreased regulatory T cells. These changes contribute to a heightened risk of inflammatory and autoimmune conditions and may alter systemic and mucosal immune responses to infections, including bacterial vaginosis. Furthermore, menopause affects B-cell function, antibody production, and natural killer (NK) cell activity, collectively impacting the overall immune competence of postmenopausal women.[17,18]

The absence of significant hematological changes across the groups suggests that BV in postmenopausal women may not trigger the same systemic inflammatory response observed in younger populations. This could be due to the hormonal and immunological changes associated with menopause, which may alter the host response to bacterial imbalances.

Limitations

This study is limited by its small sample size and the use of a non-probability sampling technique, which may restrict the generalizability of the findings. However, considering the challenges in recruiting postmenopausal women for invasive procedures, the current sample represents the maximum achievable cohort under ethical and practical constraints.

Conclusion

The prevalence of bacterial vaginosis (BV) in this study was higher than that reported in previous studies; however, it was not associated with significant hematological alterations. Further research is needed to identify reliable systemic and vaginal biomarkers for BV diagnosis in this population, considering the potential influence of hormonal and immunological factors.

Ethical Approval

This study was approved by the health ethics commission of Hasanuddin University's faculty (B3/UN4.6.4.5.31-1/PP36/2024) adhered to the principles of the Declaration of Helsinki. All participants consented to engage in the research and publishing, we provided that their anonymity was guaranteed.

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Conflict of Interest

The authors declare no conflict of interest

CRediT authorship contribution statement

Yenti Purnamasari: conceptualization, methodology, data curation, writing: original draft; **Firdaus Hamid**:

supervision, project administration, validation, writing; review and editing; **Juminten Saimin:** investigation, formal analysis, resources, writing: review and editing; **Agussalim Bukhari:** visualization, supervision, writing; review and editing.

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