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Association of Lactobacillus and Vaginal pH with Precancerous and Cancerous Lesions of the Cervix

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KEYWORDS

Cervical cancer, Lactobacillus, vaginal pH, vaginal microbiota, HPV.

ABSTRACT:

Background: Cervical cancer is a significant global health issue, particularly in developing countries. Emerging evidence highlights the role of vaginal microbiota, especially Lactobacillus species and vaginal pH, in modulating susceptibility to human papillomavirus (HPV) infection and cervical carcinogenesis. This study aimed to investigate the association of Lactobacillus presence and vaginal pH with precancerous and cancerous cervical lesions.

Methods: A case-control study was conducted at the Department of Gynaecological Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, involving 120 women aged 20 to 85 years. Participants were categorized into three groups: Healthy (n=40), pre-cancerous (n=40), and Cancerous (n=40). Vaginal swabs were cultured to identify Lactobacillus, and vaginal pH was assessed using pH paper. Statistical analysis was performed using Chi-square and parametric tests.

Results: Lactobacillus was present in 93.1% of the Healthy group, 25.0% of the pre-cancerous group, and only 6.9% of the Cancerous group (p=0.001). Acidic vaginal pH was observed in 88.4% of Healthy women compared to 52.8% in the pre-cancerous and 11.6% in the Cancerous groups (p=0.001). A significant association was found between higher vaginal pH, reduced Lactobacillus, and presence of cervical lesions.

Conclusion: The findings demonstrate that diminished Lactobacillus presence and elevated vaginal pH are significantly associated with precancerous and cancerous cervical lesions. These

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biomarkers may serve as early indicators for cervical cancer risk, suggesting their potential inclusion in routine gynecological assessments.

Introduction

Cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women, with an estimated 604,000 new cases and 342,000 deaths worldwide in 2020 [1]. In Bangladesh the incidence of cervical cancer is 8268(5.3%) per year, while the mortality rate is 4971 (4.1%) each year [2]. Cervical cancer and its precancerous lesions are caused by human papilloma virus (HPV).

It is well established that persistent infection with high-risk HPV genotypes causes high-grade cervical intraepithelial neoplasia and invasive cervical cancer. The peak age of HPV infections is around 20 years among women; by the age of 50, about 80% of sexually active women will have been infected at some point in life [3]. HPV clearance rate is 43% within 6 months and a median duration of clearance rate is 224 days. Up to 90% of HPV, infections are believed to resolve within 2 years. In contrast, abnormal cytological findings are associated with high-risk HPV persistence and 2-year cumulative regression rates between 35% and 53% are reported [4,5].

Some co-factors are responsible for persistence of HPV infection likes sexual intercourse, vaginal irrigation, inflammation of the vagina or sexually transmitted infection can also alter the vaginal microbiota [6,7]. Sometimes HPV infections are not successfully controlled by immune system. When a high-risk HPV infection persists for many years, it can lead to cell changes that, if untreated, may get worse over time and become cancer [8].

Normal vaginal microbes known as vaginal microbiota are gaining more and more attention, in recent years, from medical researchers [9,10]. Lactobacillus is the prevalent vaginal microbiota in healthy women with no genital infection [11,12]. Although more than 50 different species of bacteria have been identified in the vaginal tract, lactobacillus species, mainly L. crispatus, L. gasseri, L. icersin, L. jensen, make up about 70% of the vaginal microbiota. The structure and function of the vaginal microbiota can vary with genetic disposition, ethnicity, diet, hygiene, infections, antibiotics use, sexual activity, physiologic status of the vagina and specially estrogen levels [13,14].

Vaginal microbiota plays a major role in regulating vaginal pH. Lactobacillus produce lactic acid, thus maintain relatively low pH in the female genital tract. Low pH inhibits the growth of pathogenic bacteria. Lactobacilli also produces antimicrobial compounds which promote vaginal health [15]. The lactate produced by Lactobacillus increase the viscosity of cervical mucus that traps the viral particles and inhibits the access of Papillomavirus to basal keratinocytes, assuring an essential role in the maintenance of the cervical barrier. It has appeared that normal vaginal microbes are involved not only in maintaining female normal reproductive tract health but also in reproductive tract disease [16]. Therefore, the purpose of the study is to find out the association of Lactobacillus and vaginal pH with precancerous and cancerous lesion of cervix.

Objective

The objective of this study was to investigate the association of Lactobacillus and vaginal pH with precancerous and cancerous lesions of the cervix.

Methodology & Materials

This was a case-control study conducted at the Department of Gynaecological Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from November 2023 to October 2024. One year from the date of Institutional Review Board (IRB) approval. A total of 120 women aged between 20 and 85 years were recruited and divided into three groups: Healthy (n = 40), pre-cancerous (n = 40), and Cancerous (n = 40). The study population included women with healthy cervices or those with biopsy-proven cervical intraepithelial neoplasia (CIN) or cervical cancer, attending the colposcopy clinic, outpatient department (OPD), or inpatient department (IPD) of BSMMU.

Inclusion Criteria

For control group (Healthy):

1. Women aged 20 to 85 years
2. Clinically healthy cervix

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For case group (Pre-cancerous and Cancerous):

1. Women aged 20 to 85 years
2. Histologically confirmed cervical cancer
3. Colposcopy-directed biopsy-proven precancerous lesion
4. Colposcopically normal cervix

Exclusion Criteria

1. History of prior treatment for precancerous lesions
2. Treated and recurrent cervical cancer cases
3. Use of vaginal douche, cream, or tablet within the last 7 days
4. Current pregnancy
5. Use of oral antibiotics within the last 30 days
6. Diagnosed with immunosuppressive diseases (e.g., HIV)
7. Diagnosed with autoimmune diseases (e.g., SLE)

Data Collection and Study Procedure: Following IRB approval, eligible participants were identified and recruited after obtaining written informed consent. Data were collected from patients attending the colposcopy clinic, OPD, and IPD using a structured data collection sheet. Participants were assigned to either the case or control group based on clinical and histological findings. High vaginal swabs were collected using standard procedures. Vaginal pH was measured by applying the sample to pH paper and comparing it to a color chart. Swabs were also cultured in blood agar, chocolate agar, and MacConkey agar to detect the presence of Lactobacillus and other bacteria. Data were checked, cleaned, and coded to ensure consistency and reliability before analysis.

Ethical Considerations: Ethical approval was obtained from the Institutional Review Board (IRB) of BSMMU. Written informed consent was secured from all participants in accordance with the Declaration of Helsinki. Confidentiality was maintained by anonymizing data using special ID numbers, and all records were securely stored in locked cabinets. Patients were informed of their right to withdraw from the study at any time. No additional drugs, interventions, or placebos were used, and the study posed minimal risk to participants. Only the researchers had access to the data, ensuring privacy and ethical integrity throughout the research process.

Statistical Analysis: Data analysis was performed using IBM SPSS Statistics for Windows, Version 26.0. Descriptive statistics were used for demographic and clinical variables. Categorical variables were compared using the Chi-square test, while continuous variables were analyzed using appropriate parametric tests. A p-value <0.05 was considered statistically significant. The sample size was calculated based on prior data on vaginal pH with a confidence level of 95% and power of 80%, resulting in 40 participants per group to accommodate possible dropouts and lab errors.

Results

This observational study was conducted by the department of Gynaecological Oncology, BSMMU. Total 120 women who fulfilled the inclusion and exclusion criteria were included in this study. Result and observation of this study are given below in tables and figures.

Table I: Distribution of age of study subjects (n=120)

Characteristics		**Frequency**	**Percentage**
Age (in yeas)	25-39	53	43.3
	40-54	45	37.5
	55-69	19	15.8
	70-84	4	3.3
Mean±SD		43.43±11.77	
Educational status	Illiterate	29	24.2
	Primary	39	32.5
	Secondary	37	30.8
	Higher Secondary	9	7.5
	Graduate	5	4.2
	Post-graduate	1	0.8

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Occupation	Housewife	98	81.7
	Day labour	15	12.5
	Private service	7	5.8
Types of residence	Urban	34	28.3
	Rural	86	71.7
Personal habit	Non-smoker	80	66.7
	Betel nut chewer	40	33.3
Marital status	Married	98	81.7
	Divorced	1	0.8
	Widow	20	16.7
	Separated	1	0.8
Age of marriage	**Mean±SD**	15.58±2.56	

The table summarizes data from 120 study subjects. Most (43.3%) are aged 25–39, followed by 40–54 (37.5%), 55–69 (15.8%), and 70–84 (3.3%). The mean age is 43.43 years (SD = 11.77). Educationally, 32.5% completed primary school, 30.8% secondary, 24.2% are illiterate, and few have higher education. Most (81.7%) are housewives; others are day laborers (12.5%) or in private service (5.8%). Rural residents dominate (71.7%). Most are non-smokers (66.7%), and 33.3% chew betel nut. The majority (76.7%) are in the upper-income class. Most (81.7%) are married. The mean age of marriage is 15.58 years (SD = 2.56).

Table II: Menstrual, reproductive and contraceptive characteristics of study subjects (n=120)

Clinical characteristics		**Frequency**	**Percentage**
Menstrual history	Regular cycle	46	38.3
	Abnormal uterine bleeding	35	29.2
Menopause	Yes	39	32.5
	No	81	67.5
Parity	Nulliparous	7	5.8
	Primiparous	108	90
	Multiparous	5	4.2
Contraceptive history	OCP	65	54.2
	Barrier	5	4.2
	Injectable	27	22.5
	Norplanon	1	0.8
	Tubal ligation	1	0.8
	No	21	17.5
Duration of contraceptive use (Mean±SD)		7.74±3.63	
Age at first childbirth	**(Mean±SD)**	17.81±3.14	

The table presents menstrual, reproductive, and contraceptive data for 120 study subjects. Regular cycles were reported by 38.3%, and 29.2% had abnormal uterine bleeding. Menopause was observed in 32.5%. Most subjects (90%) were primiparous, while 5.8% were nulliparous and 4.2% multiparous. Oral contraceptive pills were most used (54.2%), followed by injectables (22.5%), barrier methods (4.2%), Norplanon and tubal ligation (each 0.8%). Notably, 17.5% used no contraception. The mean age at first childbirth was 17.81 years (SD = 3.14).

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Table III: Clinical presentations of study subjects (n=120)

Clinical finding		Frequency	Percentage
Symptoms	Post coital bleeding	34	19.9
	Leukorrhea	73	42.7
	Excessive foul-smelling discharge	49	28.7
	No symptom	15	8.8
Duration of symptoms	<6 Months	65	54.2
	<12 Months	26	21.7
	>12 Months	17	14.2

The table presents the clinical and histological findings of the 120 study subjects. The most common symptom reported by the subjects is leukorrhea, affecting 42.7% (73). This is followed by excessive foul-smelling discharge in 28.7% (49) and post-coital bleeding in 19.9% (34) subjects. 8.8% (15) subjects present with no symptom. Duration of Symptoms: The duration of symptoms varies, with most subjects 54.2% (65) experiencing symptoms for less than 6 months. Additionally, 21.7% (26) reported symptoms lasting less than 12 months, and 14.2% (17) had symptoms persisting for more than 12 months.

Table IV: Distribution of study participants according to histological type (n=120)

Histological finding	Frequency	Percentage
Histological type		
Squamous cell carcinoma	31	25.8
Adenocarcinoma	9	7.5
CIN1	20	16.7
CIN2	12	10.0
CIN3	8	6.7
Normal	40	33.3

In terms of histological type, squamous cell carcinoma (SCC) was the most common, found in 25.8% (31) Other histological types include adenocarcinoma (AD) in 7.5% (9), CIN1 in 16.7% (20), CIN2 in 10.0% (12), and CIN3 in 6.7% (8). Notably, 33.3% (40) had normal histological findings.

Table V: Distribution of study participants according to high vaginal swab (n=120)

High vaginal swab	Frequency	Percentage
Lactobacillus colony	38	31.7
Others	13	10.8
No colony	69	57.5

The results of high vaginal swabs show that 31.7% (38) had lactobacillus colonies, 10.8% (13) had other types of colonies, and 57.5% (69) had no colony growth.

Table VI: Distribution of study participants according to pH (n=120)

Vaginal pH	Frequency	Percentage
Acidic	77	64.2
Basic	43	35.8

The vaginal pH was found to be acidic in 64.2% (77) and basic in 35.8% (43).

Table- VII: Distribution of presence of Lactobacillus and vaginal pH among healthy and precancerous lesion group (n=80)

Variables	Types of participants		P value
	Healthy Group (40)	Pre-cancerous Group (40)	
High vaginal swab			
Lactobacillus colony	27(75.0%)	9(25.0%)	**0.001**
Others	1(20.0%)	4(80.0%)	
Absent	12(30.8%)	27(69.2%)	

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Vaginal pH			
Acidic	38(52.8%)	34(47.2%)	0.13
Basic	2(25.0%)	6(75.0%)	

The table compares the Healthy and Pre-cancerous Groups based on Lactobacillus colony presence and vaginal pH. Lactobacillus was detected in 75% (27/40) of the Healthy Group, but only 25% (9/40) of the Pre-cancerous Group—a statistically significant difference (P = 0.001). Other bacteria were found in 20% of the Healthy Group and 80% of the Pre-cancerous Group. Absence of Lactobacillus was more common in the Pre-cancerous Group (69.2%) than in the Healthy Group (30.8%). Acidic vaginal pH was observed in 52.8% of the Healthy Group and 47.2% of the Pre-cancerous Group (P = 0.13), while basic pH was more frequent in the Pre-cancerous Group (75% vs. 25%).

Table VIII: Distribution of presence of Lactobacillus and vaginal pH among healthy and cancerous group (n=80)

Variables	**Types of participants**		**P value**
	Healthy Group (40)	**Cancerous Group (40)**	
High vaginal swab			
Lactobacillus colony	27(93.1%)	2(6.9%)	**0.001**
Others	1(11.1%)	8(88.9%)	
Absent	12(28.6%)	30(71.4%)	
Vaginal pH			
Acidic	38(88.4%)	5(11.6%)	**0.001**
Basic	2(5.4%)	35(94.6%)	

The table compares the Healthy and Cancerous Groups based on Lactobacillus presence and vaginal pH levels. Lactobacillus colonies were found in 93.1% of the Healthy Group but only 6.9% of the Cancerous Group, showing a significant difference (P = 0.001). Other bacteria were more common in the Cancerous Group (88.9%) than in the Healthy Group (11.1%). Absence of Lactobacillus was higher in the Cancerous Group (71.4%). Regarding pH, 88.4% of the Healthy Group had an acidic pH, compared to 11.6% of the Cancerous Group (P = 0.001). Basic pH was significantly more frequent in the Cancerous Group (94.6%) than in the Healthy Group (5.4%).

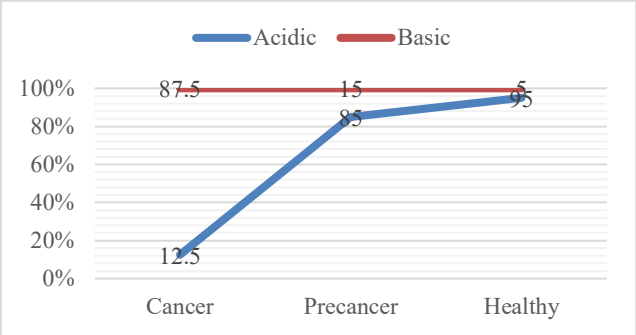

Figure 1: Distribution of the participants according to vaginal pH

Figure 1 shows the distribution of participants according to vaginal pH across healthy, pre-cancerous, and cancerous groups. The proportion of individuals with acidic versus basic vaginal pH is illustrated, showing a significant trend toward elevated pH in the cancerous group compared to the healthy group.

Discussion

This study demonstrated a significant association between reduced Lactobacillus colonization and elevated vaginal pH, particularly in the presence of both precancerous and cancerous cervical lesions. These findings support the hypothesis that a healthy vaginal microbiota, dominated by Lactobacillus species, contributes to cervical health by maintaining an acidic environment that inhibits the growth of pathogenic microbes and potentially interferes with HPV persistence.

In our study, we examined a cohort of 120 women with a mean age of 43.43 years. This demographic aligns with studies showing cervical lesion prevalence in middle-aged women aged 30-50 years. Getinet et al. reported that women aged 46-55 and 56-65 were 3.8 times and 12.2 times more likely to develop these lesions compared to women aged 25-35 [17]. Our participants' educational background showed that many had only primary or secondary education, with 24.2% being illiterate. This aligns with Getinet et al.'s finding that lower educational

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levels can hinder health-seeking behavior and cancer screening awareness [17]. The high proportion of housewives (81.7%) may reflect socio-economic factors limiting healthcare access, as studies show poor socio-economic conditions can increase cervical lesion likelihood [18]. Regarding marital status, 81.7% were married, 16.7% were widows, and 0.8% were divorced or separated. This contrasts with research showing separated or widowed women may have more lifetime sexual partners, increasing HPV infection and precancerous lesion risk [19]. In Bangladesh, cultural factors might influence sexual behavior and partner dynamics differently.

Notably, in our study, women from rural regions (71.7%) were more likely to develop precancerous cervical lesions compared to their urban counterparts (28.3%). This finding aligns with similar research conducted in India, indicating that financial and transportation challenges in rural areas may limit access to cervical cancer screening while women in these regions often have lower access to information about cervical cancer and available screening services, contributing to their increased vulnerability [17].

Our results also indicated that the majority of participants were non-smokers (66.7%), with a notable percentage (33.3%) engaging in betel nut chewing. This contrasts with findings from studies that link smoking to an increased risk of cervical precancerous lesions. This contrasts with the knowledge that smoking is known to elevate the likelihood of developing precancerous lesions of the cervix and significantly increases the risk of cervical [20]. The absence of smoking as a significant risk factor in our cohort may suggest cultural differences in lifestyle choices or the impact of local health education initiatives.

Early marriage is associated with increased risk factors for cervical cancer, as studies have shown that a significant proportion of patients were married before the age of 16 [21]. In our study, the mean age of marriage among the subjects was 15.58 years, highlighting the proportion of early marriage in this population. This early age of marriage is concerning, as it often correlates with increased exposure to sexual activity at a young age, which can heighten the risk of human papillomavirus (HPV) infection—a primary etiological factor in the development of cervical cancer. However, the study by Yuliawati et al. found no direct correlation between age at first marriage and cervical precancer, suggesting that other factors may play a more critical role [22].

The investigation into the association of Lactobacillus and vaginal pH with precancerous and cancerous cervical lesions reveals significant insights into vaginal microbiota's role in cervical health. Our study indicated significant differences ($p = 0.001$) in Lactobacillus colonies and vaginal pH levels between the Healthy Group and Pre-cancerous Group. This underscores Lactobacillus as a crucial component of healthy vaginal microbiota, providing protective benefits against pathogenic organisms and cervical lesions [23]. The absence of Lactobacillus colonies in 69.2% of the Pre-cancerous Group and 71.4% of the Cancerous Group suggests a disruption in vaginal microbiota, associated with increased cervical cancer risk. Studies have shown that a healthy vaginal microbiome, predominantly composed of Lactobacillus species, is essential for preventing infections and maintaining an acidic environment that inhibits pathogenic organisms [7,24]. Łaniewski et al. found that abnormal vaginal pH levels indicate gynecologic cancers, suggesting Lactobacillus is crucial for maintaining a healthy vaginal environment [7].

Our study revealed that 52.8% of the Healthy Group had an acidic pH, compared to 47.2% in the Pre-cancerous Group and 11.6% in the Cancerous Group. The significant P-value of 0.001 indicates a strong association between acidic vaginal pH and cancer absence, reinforcing that lower pH is protective against cervical cancer [25]. This aligns with research showing cervical lesions are linked to abnormal vaginal microbiota and elevated pH levels [25]. The correlation analysis revealed a moderately positive correlation between vaginal pH and precancerous and cancerous lesions. Research found that women with vaginal pH ≥5.0 had higher HPV infection rates, a significant risk factor for cervical cancer [26], indicating that higher pH levels facilitate HPV adherence to vaginal epithelial cells.

The presence of bacteria in Pre-cancerous and Cancerous Groups shows a shift towards dysbiosis, marked by decreased Lactobacillus and increased pathogenic bacteria. This microbial imbalance has been linked to inflammation and higher HPV persistence risk, a

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significant factor in cervical lesion progression. Our findings aligned with Mitra et al., who found increased vaginal microbiome diversity was associated with cervical intraepithelial neoplasia progression [27]. Other studies showed reduced Lactobacillus species in cervical cancer patients, with increased pathogenic bacteria like Prevotella, Fusobacterium, and Anaerococcus [28,29].

In conclusion, our study emphasizes Lactobacillus's importance in maintaining vaginal health and its potential role in preventing cervical precancerous lesions and cancer. The differences in Lactobacillus presence and vaginal pH levels across Healthy, pre-cancerous, and Cancerous Groups highlight the need for research into how vaginal microbiota influence cervical health. Understanding these relationships could inform cervical cancer prevention strategies, including potential probiotic use to restore a healthy vaginal microbiome.

Limitations of the study

- The study was conducted among a cross-section of people. Purposive sampling technique was adopted, that may cause bias.
- We performed the study in a small population of a single center, which may not reflect the actual scenario of the whole country.

Conclusion

This study shows a strong link between decreased Lactobacillus species and elevated vaginal pH with the development of precancerous and cancerous cervical lesions. The microbial imbalance and pH alteration may contribute to cervical carcinogenesis by disrupting vaginal defence mechanisms. These findings emphasize vaginal microbiota and pH as potential biomarkers for early detection of cervical neoplastic changes. Incorporating vaginal pH assessment and Lactobacillus screening into routine gynecological evaluations could enhance cervical cancer screening by identifying at-risk women earlier, particularly where cytological testing is limited. Further studies are needed to evaluate whether probiotic interventions or microbiota modulation could prevent cervical cancer progression.

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Conflicts of interest

There are no conflicts of interest.

Ethical approval

The study was approved by the Institutional Ethics Committee.

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