RESEARCH ARTICLE

Vaginal Acidity Affects Vaginal Microbiota in Postmenopausal Women

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Abstract

BACKGROUND: The changes in vaginal acidity impact the composition of the vaginal microbiota, either commensal or pathogenic. After menopause, the vaginal tract is more susceptible to infection. Current study was conducted to analyze the effect of vaginal acidity changes on the vaginal microbiota composition in menopausal women.

METHODS: A cross-sectional study was conducted on 32 subjects with vulvovaginal atrophy (VVA). Vaginal pH was measured using a strip with colorimetric examination. The detection of *Candida sp.* was done by using 10% potassium hydroxide. Meanwhile for detection of *Trichomonas vaginalis*, *Gardnerella vaginalis*, *Lactobacillus iners*, and *Lactobacillus crispatus*, polymerase chain reaction was performed. The data were statistically analyzed.

RESULTS: *G. vaginalis* was the mostly found pathogenic microorganism in current study (40.63%), followed by *Candida sp.* (25%). Further analysis showed that *G. vaginalis* were found in *L. crispatus* positive samples for 9 cases and *L. iners* positive samples for 9 cases. *Candida sp.* had an increased risk at vaginal pH ≥6 (OR=8.273), *T. vaginalis* had a reduced risk at vaginal pH ≥6 (OR=0.765), *G. vaginalis* had an increased risk at vaginal pH ≥6 (OR=1.440), *L. crispatus* had an reduced risk at vaginal pH ≥6 (OR=0.077), while *L. crispatus* had an increased risk at vaginal pH ≥6 (OR=1.111).

CONCLUSION: Vaginal acidity alterations in postmenopausal women affect either commensal or pathogenic microorganism composition. A decrease in the number of *L. crispatus* and an increase in the number of *L. iners* and pathogenic microorganisms is in line with the increase of pH.

KEYWORDS: *Lactobacillus*, microbiota, menopause, pathogenic microorganisms, vaginal acidity

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Introduction

Lactobacillus sp. is one of the mostly found species in vagina. Living bacteria or yeast could give positive benefits to the host.(1,2) Microorganisms in the vagina require glycogen as the nutrient for metabolism. Glycogen is converted into lactic acid by *Lactobacillus*, inducing an acidic pH in vagina. The vaginal acidity inhibits the growth of many potential pathogens.(3,4)

Several studies have shown that the ratio of *Lactobacillus species*, *Lactobacillus crispatus* and *Lactobacillus iners*, can predict normal or abnormal microflora in the vagina. A decrease in the proportion of *Lactobacilli* and lactic acid production causes an increase of vaginal pH, causing the vagina more susceptible to infection and exacerbating the symptoms associated with vulvovaginal atrophy (VVA).(5-10) VVA occurs around 75-85% of postmenopausal women. Some menopausal women complain about the burning sensation in the vulva, and at

least 50% of postmenopausal women experience urogenital tract changes associated with VVA.(11-16)

Decrease in estrogen levels during menopause affect the female urogenital tract.(17) During menopause, the proliferation of epithelial cells is reduced, causing the thinning of vaginal mucosal epithelium, atopy of vaginal wall, and parabasal cell dominance in the epithelial layer. These changes trigger reduction of glycogen.(16,18) Decreased glycogen in the vaginal epithelium causes the vaginal pH to be more alkaline. Thus, it increases the risk of vaginal infection or atrophic vaginitis in menopausal women. (12,19) The most common vaginal infections are bacterial vaginosis, vulvovaginal candidiasis, and trichomonal vaginitis.(20-23) *Candida albicans*, *Trichomonas vaginalis*, and *Gardnerella vaginalis* are common microorganisms causing VVA.(5,6) The main cause of genital tract infection in women is a bacterial infection.(4) Common bacterial species associated with bacterial vaginitis are *G. vaginalis* and *T. vaginalis*. *L. crispatus* and *L. iners* were detected in normal and disturbed vaginal microbiota.(6,10,20,21)

After menopause, the vaginal tract has a neutral pH and consists of mixed flora, which makes it susceptible to infection. The abnormal microbial flora of the vagina causes various infectious diseases, which can lead to moderate to severe infectious conditions. It can affect the physical, psychological, and quality of life.(24,25) Therefore, current study was conducted to analyze the effect of vaginal acidity changes on the vaginal microbiota composition in menopausal women.

Methods

Study Design and Subject Recruitment
A cross-sectional study was conducted on subjects with VVA. Subjects were recruited from Gynecology Clinic in Kendari, from September to December 2020. The inclusion criteria were postmenopause, menopausal syndrome and intact uterus. Exclusion criteria were vulvar infection, ongoing antibiotics treatment and gynaecological malignancy. Purposive sampling method were conducted.

The protocol of current study was approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Halu Oleo (No. 044/UN29.17.1.3/ETIK/2020). All subjects signed written informed consent.

Subject Information and Sample Collection
The subject information was collected with a questionnaire. Vaginal pH was measured using a strip with colorimetric examination. Vaginal swab using ESwab (COPAN Diagnostics, Murrieta, CA, USA) was performed at the vaginal wall with approximately 2 inches depth. The swab was sent to Microbiology Research Laboratory, Faculty of Medicine, Universitas Halu Oleo, for the detection of microbiota.

Detection of Microbiota
Conventional method was carried out for detection of *Candida sp.* by using 10% potassium hydroxide. Detection was conducted under a microscope with 10x and 40x magnification. Meanwhile for detection of *T. vaginalis*, *G. vaginalis*, *L. iners*, and *L. crispatus*, polymerase chain reaction (PCR) was performed. Briefly, DNA extraction was carried out with High Pure PCR Template Preparation Kit (Merck, Darmstadt, Germany). KAPA2G Fast HotStart ReadyMix (Sigma-Aldrich, Darmstadt, Germany) was used in PCR reaction. PCR was run in 40 cycles with the setting of 55-62°C annealing temperature for 30-60 seconds. A pre-denaturation at 94-95°C for 5-10 minutes and a final extension at 72°C for 10 minutes were included. Primers used in current study were listed in Table 1. The PCR results were electrophoresed and stained with ethidium bromide.

Statistical Analysis
Data analysis was performed with SPSS Statistics for Windows version 23 (IBM, Armonk, NY, USA). The relationship between variables was analyzed by the chi-square test, with a significance value of $p<0.05$. The risk of changes in the number of vaginal microbiota on vaginal pH was analyzed by Odds Ratio, with 95% CI and significance value $p<0.05$.

Table 1. List of primers used in this study.(8,24)

Microbiota	Forward Primer	Reverse Primer	Product Length (bp)
T. vaginalis	ATTGTCGAACATTGGTCTTACCCTC	TCTGTGCCGTCTTCAAGTATGC	300
G. vaginalis	GCGGGCTAGAGTGCA	ACCCGTGGAATGGGCC	206
L. crispatus	AACTAACAGATTTACTTCGGTAATGA	AGCTGATCATGCGATCTGC	145
L. iners	AGTCTGCCTTGAAGATCGG	CTTTTAAACAGTTGATAGGCATCATC	166

Results

During the three months period, 32 postmenopausal female subjects were recruited (Table 2). Most subjects were in the age of 50-55 years (56.25%), ≤5 years duration of menopause (68.75%) and ≥6 vaginal pH (56.25%). *Candida sp.* was detected by the appearance of the hyphae of *Candida sp.* (Figure 1) and there were 8 cases of *Candida sp.* found (25%) (Table 2). For other microbiota, clear and correct length of DNA bands were seen by PCR (Figure 2). There were 2 cases of *T. vaginalis* (6.25%), 13 cases of *G. vaginalis* (40.63%), 22 cases of *L. crispatus* (68.75%) and 21 cases of *L. iners* (65.63%) (Table 2).

Further analysis showed that *G. vaginalis* were found in *L. crispatus* positive samples for 9 cases and *L. iners* positive samples for 9 cases. In addition, for vaginal ph ≥6, *G. vaginalis* cases were found positive for 8 cases (Table 3). For vaginal pH <6, there were *Candida sp.* (12.5%), *T. vaginalis* (50%), *G. vaginalis* (38.46%), *L. crispatus* (59.09%), and *L. iners* (42.86%) (Table 4).

Figure 1. Detection of *Candida sp.* with potassium hydroxide staining. Red circle: hyphae of *Candida sp.*

Candida sp. had an increased risk at vaginal pH ≥6 (OR=8.273; 95% CI: 0.877-78.010; *p*=0.065). *T. vaginalis* had a reduced risk at vaginal pH ≥6 (OR=0.765; 95% CI: 0.044-13.411; *p*=0.854). *G. vaginalis* had an increased risk at vaginal pH ≥6 (OR=1.440; 95% CI: 0.343-6.048; *p*=0.618). *L. crispatus* had an reduced risk at vaginal pH ≥6 (OR=0.077; 95% CI: 0.008-0.718; *p*=0.024). *L. crispatus* had an increased risk at vaginal pH ≥6 (OR=1.111; 95% CI: 0.256–4824; *p*=0.888) (Table 4).

Discussion

G. vaginalis was the mostly found pathogenic microorganism in current study, followed by *Candida sp.* In line with previous study, the common VVA causing bacteria are *G. vaginalis*, *Candida sp.*, and *T. vaginalis*. *G. vaginalis* was reported to be associated with bacterial vaginosis.(5-10,23)

Current study showed that *L. crispatus* and *L. iners* were found in almost equal numbers. Both *Lactobacilli* were found in healthy and altered vaginal conditions. *Lactobacillus sp.* is believed to play a role in maintaining
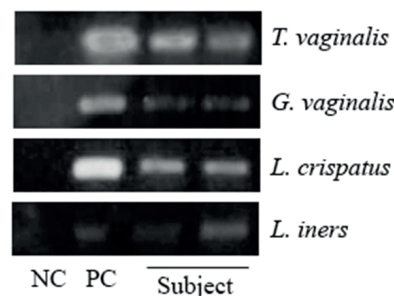
Table 2. Subject characteristic and vaginal microbiota.

Variable	n	%
Subject Characteristic		
Age (year)		
≤49	5	15.63
50-55	18	56.25
≥56	9	28.12
Duration of menopause (year)		
≤5	22	68.75
>5	10	31.25
Vaginal pH		
<6	14	43.75
≥6	18	56.25
Vaginal Microbiota		
Candida sp.		
Negative	24	75
Positive	8	25
T. vaginalis		
Negative	30	93.75
Positive	2	6.25
G. vaginalis		
Negative	19	59.37
Positive	13	40.63
L. crispatus		
Negative	10	31.25
Positive	22	68.75
L. iners		
Negative	11	34.37
Positive	21	65.63

Figure 2. Detection of *T. vaginalis*, *G. vaginalis*, *L. crispatus*, *L. iners* (166-bp) with PCR. NC: negative control; PC: positive control.

Table 3. Distribution of *G. vaginalis* according to *Lactobacillus sp.* positive and vaginal pH ≥6 subjects.

Variable	G. vaginalis				p-value
	Negative		Positive		
	n	%	n	%	
L. crispatus (positive)	13	59.09	9	40.91	0.961
L. iners (positive)	12	57.14	9	42.86	0.722
Vaginal pH (≥6)	10	55.56	8	44.44	0.725

*Significancy value $p<0.05$, was tested with the chi-square test.

a healthy vaginal environment.(5-10) *L. iners* were found together with *G. vaginalis*. This result is in line with other studies which found that *L. iners* increase along with the vaginal microbiota marker of bacterial vaginosis. Difference in the number of *Lactobacillus sp.* indicates a shift in the vaginal microbiota.(26)

Number of pathogenic microorganisms increased in line with the increase of vaginal pH (pH ≥6). There was no particular type of pathogenic microorganisms that dominated in each group. Changes in the normal flora in the vagina lead to overgrowth of anaerobic species.(23) Number of subjects with *L. iners* increased in line with increasing pH but was not statistically significant. In line with the previous study, *L. iners* could be dominant in the disturbed vagina.(5-10)

Current study showed a significant decrease in the number of subjects with *L. crispatus* in line with the increase in pH. Epithelial thinning as well as decrease of cell proliferation and vaginal secretions during menopause, affect glycogen as the nutrient for *Lactobacilli.* This condition is not favorable for the growth of vaginal microbiota, especially vaginal *Lactobacilli*.(16,27) This finding is in line with a study that found *L. crispatus* was dominant in healthy vaginal conditions.(10) This finding is also similar to a previous study that found a low proportion of *Lactobacillus sp.* in the vagina of menopausal women. (28,29) Current study are in line with a study conducted on postmenopausal women using hormone replacement therapy, which showed that the number of *L. crispatus* increased. Among vaginal *Lactobacilli*, *L. crispatus* is the most related with the reduction of sexually transmitted infection risk.(26,30-32).

Conclusion

Based on this study, we conclude that vaginal acidity changes in postmenopausal women affect either commensal or pathogenic microorganism composition. A decrease in the number of *L. crispatus* and an increase in the number of *L. iners* and pathogenic microorganisms is in line with the increase in pH.

Acknowledgements

Table 4. Distribution of vaginal pH based on microbiota positive subjects.

Microbiota	Vaginal pH				OR	CI 95%	p-value
	<6		≥6				
	n	%	n	%			
Candida sp. (positive)	1	12.5	7	87.5	8.273	0.877 - 78.010	0.065
T. vaginalis (positive)	1	50	1	50	0.765	0.044 - 13.411	0.854
G. vaginalis (positive)	5	38.46	8	61.54	1.44	0.343 - 6.048	0.618
L. crispatus (positive)	13	59.09	9	40.91	0.077	0.008 - 0.718	0.024
L. iners (positive)	9	42.86	12	57.14	1.111	0.256 - 4.824	0.888

*Significancy value $p<0.05$, was tested with the odds ratio.

Authors Contribution

All authors contributed equally in all stages of the study and agreed with the final version of the article to be published and accountable in all phases of the work.

References

1. Ranuh RG, Athiyyah AF, Darma A, Riawan W, Surono IS, Sandra F, *et al.* Lactobacillus plantarum IS-20506 probiotic restores galectin-4 and myosin-1a expressions in duodenum, jejunum and ileum of lipopolysaccharide-induced rats. Indones Biomed J. 2020; 12(3): 283-7.

2. Valentina I, Achadiyani, Adi SS, Lesmana R, Farenia R. Effect of Lactobacillus reuteri administration on wrinkle formation and type I procollagen levels in UVB-exposed male balb/c mice (Mus musculus). Mol Cell Biomed Sci. 2020; 4(3): 113-20.

3. Amin M, Goodarzi H, Orang Z, Farsi S, Jorfi M. Isolation and identification of lactobacillus species from the vagina and their antimicrobial properties. Afr J Microbiol Res. 2011; 5(20): 3300-4.

4. Dasari S, Karanam S, Anandan, Rajendra W, Valluru L. Role of microbial flora in female genital tract; A comprehensive review. Asian Pac J Trop Dis. 2016; 6(11): 909-7.

5. De Backer E, Verhelst R, Verstraelen H, Alqumber MA, Burton JP, Tagg JR, *et al.* Quantitative determination by real-time PCR of four vaginal Lactobacillus species, Gardnerella vaginalis and Atopobium vaginae indicates an inverse relationship between L. gasseri and L. iners. BMC Microbiol. 2007; 7: 115.

6. Dumonceaux TJ, Schellenberg J, Goleski V, Hill JE, Jaoko W, Kimani J, *et al.* Multiplex detection of bacteria associated with normal microbiota and with bacterial vaginosis in vaginal swabs by use of oligonucleotide-coupled fluorescent microspheres. J Clin Microbiol. 2009; 47(12): 4067-77.

7. Zozaya-Hinchliffe M, Lillis R, Martin DH, Ferris MJ. Quantitative PCR assessments of bacterial species in women with and without bacterial vaginosis. J Clin Microbiol. 2010; 48(5): 1812-9.

8. Fredricks DN. Molecular methods to describe the spectrum and dynamics of the vaginal microbiota. Anaerobe. 2011; 17(4): 191-5.

9. Srinivasan S, Hoffman NG, Morgan MT, Matsen FA, Fiedler TL, Hall RW, *et al.* Bacterial communities in women with bacterial vaginosis: High-resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. PLoS One. 2012; 7(6): e37818. doi: 10.1371/journal.pone.0037818.

10. Kusters JG, Reuland EA, Bouter S, Koenig PS, Dorigo-Zetsma JW. A multiplex real-time PCR assay for routine diagnosis of bacterial vaginosis. Eur J Clin Microbiol Infect Dis. 2015; 34: 1779-85.

11. Santoro N, Komi J. Prevalence and impact of vaginal symptoms among postmenopausal women. J Sex Med. 2009; 6: 2133-42.

12. Immanuel AI, Wantania J, Suparman E, Lintong P. Clinical appearance and vaginal cytology of atrophic vaginitis in postmenopausal women. Indones J Obstet Gynecol. 2010; 34(2): 92-6.

13. Nappi RE, Kokot-Kierepa M. Women's voices in menopause result from an international survey on vaginal atrophy. Maturitas. 2010; 67(3): 233-8.

14. Simon JA, Kokot-Kierepa M, Goldstein J, Nappi RE. Vaginal health in the United States, results from the vaginal health, insights, views, and attitudes survey. Menopause. 2013; 20(10): 1043-8.

15. Minkin MJ, Maamari R, Reiter S. Postmenopausal vaginal atrophy: Evaluation of treatment with local estrogen therapy. Int J Women's Health. 2014; 6: 281-8.

16. Saimin J, Hendarto H, Soetjipto. The effect of tomato juice in increasing Ki-67 expression and epithelial thickness on the vaginal wall of menopausal ratstomato juice effects on menopausal rats. Indones Biomed J. 2019; 11(2): 152-8.

17. Sturdee DW, Panay N. International Menopause Society Writing Group Recommendations for the management of postmenopausal vaginal atrophy. Climacteric. 2010; 13(6): 509-22.

18. Amran R. Menentukan menopause berdasarkan indeks maturasi dan pH vagina. JKK. 2010; 42(3): 2981-6.

19. Grady D. Management of menopausal symptoms. N Engl J Med. 2006; 355(22): 2338-47.

20. Bachmann GA, Nevadunsky NS. Diagnosis and treatment of atrophic vaginitis. Am Fam Physician. 2000; 61(10): 3090-6.

21. WHO. Sexually Transmitted and Other Reproductive Tract Infections: A Guide to Essential Practice. Geneva: World Health Organization; 2005.

22. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015; 64(No. RR–3): 1-137.

23. Saimin J, Ridwan S, Purnamasari NI, Irawati, Purnamasari Y, Mulyawati SA. Clinical symptoms of vulvovaginitis among premenopausal women: A retrospective study in Southeast Sulawesi. The International Congress of Asia-Oceania Research Organisation in Genital Infection and Neoplasia (AOGIN). 2021 April 9-11.

24. Takahashi TA, Johnson KM. Menopause. Med Clin N Am. 2015; 99(3): 521-34.

25. Matthes ACS, ZuccaMatthes G, Oliveira MA. The genito urinary syndrome of menopause presents sexual symptoms that can be best explained by the relative short vagina syndrome. Gynecol Obstet (Sunnyvale). 2016; 6(1): 382.

26. Gliniewicz K, Schneider GM, Ridenhour BJ, Williams CJ, Song Y, Farage MA, *et al.* Comparison of the vaginal microbiomes of premenopausal and postmenopausal women. Front Microbiol. 2019; 10: 193. doi: 10.3389/fmicb.2019.00193.

27. Mirmonsef P, Modur S, Burgad D, Gilbert D, Golub ET, French AL, *et al*. Exploratory comparison of vaginal glycogen and Lactobacillus levels in premenopausal and postmenopausal women. Menopause. 2015; 22(7): 702-9.

28. Hummelen R, Macklaim JM, Bisanz JE, Hammond JA, McMillan A, Vongsa R, *et al.* Vaginal microbiome and epithelial gene array in post-menopausal women with moderate to severe dryness. PLoS One. 2011; 6(11): e26602. doi: 10.1371/journal.pone.0026602.

29. Brotman RM, Shardell MD, Gajer P, Fadrosh D, Chang K, Silver MI, *et al.* Association between the vaginal microbiota, menopause status, and signs of vulvovaginal atrophy. Menopause. 2014; 21(5): 450-8.

30. Barrons R, Tassone D. Use of Lactobacillus probiotics for genitourinary infections in women: A review. Clin Ther. 2008; 30(3): 453-68.

31. Verstraelen H, Verhelst R, Claeys G, De Backer E, Temmerman M, Vaneechoutte M. Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. BMC Microbiol. 2009; 9: 116. doi: 10.1186/1471-2180-9-116.

32. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, *et al.* Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis. 2011; 52(10): 1212-7.