Research Report

Clinical appearance and vaginal cytology of atrophic vaginitis in postmenopausal women

Gambaran vaginitis atrofi pada perempuan pascamenopause berdasarkan tampilan klinis dan sitologi vagina

Agnes Imelda Immanuel[1], John Wantania[1], Eddy Suparman[1], Poppy Lintong[2]

[1]Department of Obstetrics and Gynecology Medical Faculty
[2]Department of Pathology Anatomy Medical Faculty
University of Sam Ratulangi/Prof. Kandou Hospital
Manado

Abstract

Objective: To find out atrophic vaginitis pattern in postmenopausal women based on clinical appearance and vaginal cytology.

Method: Descriptive retrospective study with hypothesis cohort to find out clinical appearance and vaginal cytology of atrophic vaginitis based on distribution of estrogen effect (maturation value/total maturation index) in post menopausal women in outpatient clinic of tertiary hospital in Manado, North Sulawesi.

Result: There were 50 subjects met the inclusion criteria. Most of them are 55 to 59 years old, with length of menopausal period 6 to 20 years, and onset of menopause in 45 to 49 years old. Cytology study showed that atrophic vaginitis present in 66% patients. Clinical symptoms are ranging from vaginal dryness (10%), vaginal discharge (6%), and dyspareunia (20%). From clinical appearance we found 44% women had atrophic vaginitis based on vaginal secretion, 42% based on macroscopic surface/integrity of vaginal epithelium, 54% based on vaginal rugae and elasticity, and 42% based on vaginal color. However, 80% of all participants showed vaginal pH more than 7 and most of them categorized as having atrophic vaginitis.

Conclusion: By using clinical appearance, we found 42% to 54% of menopausal women had atrophic vaginitis, while cytology study confirmed 66% having it.

[Indones J Obstet Gynecol 2010; 34-2: 92-6]

Keywords: clinical appearance, cytology, menopause, atrophic vaginitis

Abstrak

***Tujuan**: Untuk mengetahui gambaran Vaginitis Atrofi pada perempuan pascamenopause berdasarkan tampilan klinis dan sitologi vagina.*

***Metode**: Merupakan penelitian deskriptif-retrospektif dengan hypothesis cohort untuk mendapatkan tampilan klinis dan sitologi vagina dari vaginitis atrofi berdasarkan distribusi efek estrogen (Maturation Value/Total Indeks Maturasi) pada perempuan pascamenopause yang kontrol di Poliklinik Rumah Sakit di Manado, Sulawesi Utara.*

***Hasil**: Didapatkan 50 sampel sesuai kriteria inklusi, terbanyak usia 55 - 59 tahun (42%), lama menopause antara 6 - 20 tahun (50%), onset menopause antara 45 - 49 tahun (46%), Secara sitologi, prosentase vaginitis atrofi sebanyak 66%, lebih tinggi dari hipotesis penelitian ($p < 0,0001$). Keluhan pada vaginitis atrofi yaitu rasa kering sebanyak 10%, keputihan 6% dan dispareunia 20%. Berdasarkan tampilan klinis didapatkan sekresi vagina normal sebanyak 56%, sedangkan 44% atrofi. Integritas epitel vagina atrofi 46%. Berdasarkan ketebalan epitel vagina 42% atrofi. Warna vagina normal 58% sedangkan 42% atrofi. Delapan puluh persen sampel mempunyai pH vagina basa, dengan kejadian vaginitis atrofi 82%, sedangkan pada pH vagina asam tidak ditemukan kejadian vaginitis atrofi. Didapatkan kejadian infeksi pada vaginitis atrofi sebesar 21%.*

***Kesimpulan**: Dengan menggunakan tampilan klinis, kami menemukan 42% sampai 54% dari wanita menopause telah atrophic vaginitis, sementara studi sitologi dikonfirmasi 66% memilikinya.*

[Maj Obstet Ginekol Indones 2010; 34-2: 92-6]

***Kata kunci**: Tampilan klinis, sitologi vagina, pasca menopause, vaginitis atrofi*

Correspondence: Agnes Imelda Immanuel, Department of Obstetrics and Gynecology Medical Faculty University of Sam Ratulangi/ Prof. Kandou Hospital, Manado. Telp. 0431 - 859178. Email: obsgyn_manado@yahoo.com.sg

INTRODUCTION

Estrogen plays an important role in the function of the urogenital tissue in adults, in which receptors for estrogen and progesterone are found in the tissues of the vagina, urethra, urinary bladder and the muscles of the pelvic floor. Post menopausal estrogen deficiency may cause atrophic changes of the urogenital tract and cause symptoms of vaginal atrophy, such as, dyspareunia, itching, rawness, and feelings of dryness. Changes also are found in the urinary tract with symptoms of frequency, urgency, nocturia, incontinence, and recurrent infections.[1,2]

Estrogen deficiency causes atrophy of connective tissues and epithellium of the urogenital tract including the vaginal mucousa. Estrogen receptor α and β distribution along the urogenital tract are found on the vaginal side walls and uterosacral ligaments in the premenopausal women, but are not seen in the postmenopausal women. Estrogen influences the synthesis and metabolism of collagen in the lower genital tract. Atrophic vaginitis is a term used to explain a condition of inflammation of the vagina due to decreased estrogen that causes vaginal epithelial atrophy, in which all of the vaginal epithelial layers are absent, except for the basal cell layer. The tissue that under-

goes atrophy is the vaginal mucosa, cervix, endocervix, endometrium, and uroepithellium. Along with the decrease in estrogen production, the vaginal mucosa becomes thin, vaginal secretions reduced, in a way that gives way to symptoms of sexual dysfunction and discomfort of the vagina and vulva, in which these symptoms in the end will result in disturbance of daily life of the postmenopausal women.

Post menopausal women with atrophic vaginitis are also susceptible to vaginal infections. Susceptibility to infection, in addition to be caused by the thinning of the vaginal epithelium, is also due to an increase in vaginal pH and alterations of the normal vaginal flora in such a way that its function as a barrier to infections by microorganism becomes less effective. Alteration of the normal vaginal flora cause colonilization by gram negative bacilli that may cause symptoms of local imitation of the vaginal mucosa.[1,3] Brizzolara et al.[4] in the United States found that a vaginal pH > 4 is associated with the increase in number of parabasal cells (≥ 20%) in smears from the proximal to mid vagina. According cytologic definitions, in atrophic vaginitis there is an increase in parabasal and intermediate cells in comparison with superficial cells that illustrates low estrogen effect.[5-8] Nuryanto K.H[5] reported the incidence of atrophic vaginitis of 63.2%. Atrophic vaginitis are found clinically found in around 75 - 85% of postmenopausal women, even so only around 40% of postmenopausal women complain symptoms of atrophic vaginitis.[2,3]

METHOD

This is a descriptive study. The sample population are post menopausal women, who has not received any estrogen or hormone replacement therapy, aged 46 - 65 years old that comes regularly to the gynecology clinic of the Prof. Dr. R. D. Kandou Hospital, Manado - Department of Obstetrics and Gynecology, Faculty of Medicine Sam Ratulangi University and the Obstetrics and Gynecology clinic of the Robert Wolter Monginsidi Hospital, Manado. Sample collection was conducted from December 2008 to January 2009.

History taking of the study participants were conducted by the authors with a guide to the presence of atrophic vaginitis, which covers the presence of a feeling of dryness, friction on the perineum during sexual intercourse, a feeling of fullness, pressure or swelling of the vagina, pain or irritation of the perineum when in contact with clothing, pain during sexual intercourse and presence of vaginal secretion. Clinical vaginal examinations elucidate vaginal secretions, vaginal epithelial cell integrity, vaginal surface thickness, color and texture of the vaginal surface. Vaginal examination were conducted using a graves speculum, and when required was lubricated with water to aid speculum insertion.

Examination of the vaginal pH were conducted using a pH dipstick indicator by applying it to the fluid found on the surface of the vaginal wall or in cases where secretion were not visible, the dipstick was applied to the vaginal wall for a duration of 1 minute. Quantitative interpretation was done based on color contrast parameters from pH indicator container. A pH of 5 or less was considered acidic and a vaginal pH of 7 or more was considered to be basic.

Vaginal smears were taken for evaluation of cell maturity from the lateral wall of the one-third proximal vagina using blunt Ayre spatula and was then smeared on an object glass that has already been labeled with the participants' identity. The sample was then immersed in 95% alcohol for approximately 30 minutes, then dried off using an air dryer, packed in a safe container and was then sent to the cytology laboratory of the Anatomic Pathology Department, Faculty of Medicine UNSRAT. The smears were then stained using papanicolau staining.

Evaluation of cell maturation was conducted by counting the number of parabasal cells (PB), Intermediate cells (I), and superficial cells (S) and expressed by the ratio PB: I: S. Evaluation of cell maturity are done under the microscope by counting the number of parabasal, intermediate, and superficial cells within 100 cells. The number of parabasal cells are then multiplied by 0, the number of intermediate cells are multiplied by 0.5 and the number of superficial cells multiplied by 1. From the sum of all three cell calculations, the value of cell maturity is acquired. A maturity index/value between 0 - 49 reflects low estrogen effect, a value of 50 - 64 shows intermediate estrogen effect, and a maturation value of 65 - 100 shows high estrogen effect.

Comparisons of parabasal, intermediate, and superficial cells were conducted. If by microscopic evaluation superficial cells are found fewer than 30% or not at all, it would mean that the effect of estrogen is low. Evaluation and quantification were conducted by the author(s)/(research team), and were also conducted by the cytopathologist for confirmation to increase the objectivity of the results. In the event that the maturation index is found to show low estrogen effect, the samples were considered as atrophic vaginitis and would then be analyzed further and treated.

In this study we conducted data collection, evaluation, and tabulation and presented in the form of tables, then further analyzed descriptively by applying the Z test for hypothesis testing of the study.

RESULT

A study was conducted on 49 post menopausal women that came to the Obstetrics and Gynecology clinic of Prof. Dr. R.D. Kandou General Hospital and Robert W. Monginsidi Hospital, Manado between December 2008 to January 2009 and 50 samples of postmenopausal women fulfilled the study inclusion criteria.

In accordance with cytology definition, in atrophic vaginitis, the number of parabasal and intermediate cells will increase in comparison with the number of superficial cells, which illustrates a low estrogen effect.

Atrophic vaginitis are found in 33 (66%) of the study participants. This result was higher than was stated in the study hypothesis of > 40% of postmenopausal women experiencing atrophic vaginitis ($p < 0.0001$).

The occurrence of infection were also found along with atrophic vaginitis in 7 participants, and as much as 26 participants without infection among the total

study participants. From this entire study we found a total of 15 participants having infections, specifically:

- Gardnerella Vaginalis : 4 participants
- Coccen vaginalis : 7 participants
- Trichomonas vaginalis : 1 participants
- HPV infection : 2 participants

Table 1. Distribution of estrogen effect on vaginal cytology in postmenopausal women

Estrogen effect	Infection (+)	Infection (-)	N	%
Low	7	26	33	66
Moderate	4	1	5	10
High	4	8	12	24
Total	15	35	50	100

Information:
Atrophy vaginitis (+) : low estrogen effect
Atrophy vaginitis (-) : moderate or high estrogen effect

Five (10%) participants complains about dryness feeling, and there are no participants complaining about vaginal compresion and iritation based on anamnesis, however there are only three (6%) participants complaining leukorhea and 10 (20%) participants complaining about dispareunia (Table 2).

Table 2. Distribution based of vaginal symptoms

Symptoms	Atrophy vaginitis (+)	Atrophy vaginitis (-)	N	%
Dryness				
(+)	2	3	5	10
(-)	31	14	45	90
Compression				
(+)	0	0	0	0
(-)	0	50	50	100
Iritation				
(+)	8	2	10	20
(-)	19	21	40	80
Dispareunia				
(+)	3	0	3	6
(-)	30	17	47	94
Leukorhea				
(+)	0	0	0	0
(-)	0	50	50	100

- **Vaginal secretions**
  Classification of vaginal secrete are normal (positive secretes) and atrophy (no secretes or need lubrication for painless speculum examination). From vaginal secretions, there are 28 (56%) participants have normal secretes and 22 (44%) participants have atrophy (Table 3).
- **Macroscopic surface/integrity of vaginal epithelium**
  The classification of the vaginal epithelium are normal (pink vaginal wall, humid and un-shinny) and atrophy (pale vaginal wall, dry, shiny and there are no petechiae on vaginal surface or bleed easily on vaginal examination). Normal vagina epithelium integrity found in 27 (54%) participants, however there are 21 (42%) were atrophy (Table 3).
- **Rugae/vaginal wall elasticity**
  The classification of vaginal wall elasticity are normal (elastic vaginal wall and proper number of rugae) and atrophy (less number of rugae or very smooth vaginal wall with only upper third elasticity of vaginal wall or there is no elasticity at all). we found 29 (58%) participants have normal thickness of vaginal epithelium and 21 (42%) participants are atrophy (Table 3).
- **Vaginal colour**
  The classification of the vaginal colour are normal (pink vaginal wall) and atrophy (pink to pale white colour vaginal wall). We found 29 (58%) participants have normal vaginal wall colour, however we found atrophy on 21 (42%) participants (Table 3).

Table 3. Distribution of frequency based on vaginal examination

Examination	Normal	Atrophy
Vaginal secretion	28 (56%)	22 (44%)
Vaginal wall epithelium integrity	27 (54%)	23 (46%)
Vaginal wall surface	29 (58%)	21 (42%)
Vaginal colour	29 (58%)	21 (42%)

From table 4, we got base vaginal pH on 40 (80%) participants and the incidence of atrophy vaginitis is 33 (82%) participants. Thus, acid vaginal pH on 10 (20%) participants and there is no atrophy vaginitis.

Table 4. Frequency distribution on vaginal pH

Vaginal pH	Atrophy vaginitis (+)	Atrophy vaginitis (-)	N	%
Acid (pH ≤ 7)	0	10	10	20
Base (pH ≥ 7)	33	7	40	80
Total	33	17	50	100

DISCUSSION

In this research, from 59 women screened for post menopause, we got 50 women who include in the criteria for this research. The research based on the characteristic (age, duration of menopause, onset of menopause, education status, occupation, obstetric history taking, coital history taking), vaginal complains, vaginal examination, vaginal pH examination and vaginal cytology examination.

The highest range of age in post menopausal women in this research is between 55 - 59 years old for 21 (42%) participants. Followed by 50 - 54 years old for 16 (32%) participants, > 59 years old for 9 (18%) participants and 45 - 49 years old for 4 (8%) participants. The youngest participant in this research is on age 45 years old and the oldest is on 65 years old.

Based on other cross sectional research, they found mean of age of menopausal women between 44 - 56 years old. They report only on cigarete smoking participants have an early menopausal incidence with deviation of 1.5 years more early. A corelation between mother and female daughter have a high incidence of early menopause (< 40 years), but only in sporadic manner.[9]

Based on the duration of post menopause, the longest duration is 6 - 20 years for 25 (50%) participants, followed by 4 - 5 years for 13 (26%) participants, one year for 7 (14%) participants and 2 - 3 years for 5 (10%) participants. The shortest duration of post menopause is one year and the longest is 20 years. This time of duration wil be correlated to symptoms and clinical findings.

We predict in four to five year of menopause, there will be one of women not using estrogen replacement therapy experiencing an atrophy. The symptoms of it are dryness feeling, dispareunia and recurent vaginal infection. But, these symptoms are reversible with the therapy of estrogen. In research with placebo control, they found estrogen administration will reduce the symptoms of vaginal atrophy i.e vaginal dryness, which have the effect on increasing libido and sexual function because it has the effect on vaginal lubrication.[10]

In this research, the most frequent complains dispareunia for 10 participants (20%), followed by leukorhea for 3 participants (6%) and dryness feeling for 5 participants (10%). However feeling of compression and iritation have not been elucidated.

Although menopause is a nature process, actually it can produce many symptoms in daily life. A symptoms like vaginal atrophy count for 40% of post menopausal women, but majority of them does not seek for therapy.

Clinical diagnosis of vaginal atrophy was established based on symptom of reduced vaginal discharge to 22 people (44%), the reduced integrity of the vaginal epithelium by 23 women (46%), reduced thickness of the vaginal epithelium and pale vaginal color each consisted of 21 women (42%).

Based on clinical examination, vaginal pH is a more objective examination compared to others. Where from overall clinical variables, except the pH of the vagina, athropical vaginitis did not reach half of the number of study participants. But when related with vaginal pH and the effects of estrogen on the results of cytology, the examination should be more accurate where the occurrence of atrophical vaginitis was found in more than half of study participants. This is coherent with the theory results are consistent with the theory that atrophica vaginitis 40% in postmenopausal women.

According to the examination of vaginal hormonal cytology, it was stated found that low estrogen effect is occuringthis 66% samples, indicating the occurrence of atrophical vaginitis in postmenopausal women examined. Meanwhile the distribution of the moderate-estrogen effects is 5 people (10%) and distribution of the high-estrogen effects as many as was 24% of 50 samples, indicating the absence of atrophical vaginitis among women in the postmenopausal women examined. Comparison between clinical appereance and cytology study reveals that cytology study give a better sensitifity diagnois athropic vaginitis. Through the vaginal cytology examination, find Gardnerella vaginalis, Coccen vaginalis and HPV Trichomonas vaginalis infection manifestations.

Bacterial vaginosis (BV) is a polimicrobial disorder, characterized by the increase of vaginal pH above 4.5, reduction or absence of Lactobasillus colonization and the growth of certain anaerobic bacteria. Few literatures evaluated the prevalence of BV in menopausal women there was not enough data describing the effects of hormone replacement therapy on the prevalence of BV. Sabina, et al. assessing BV prevalence in women over 40 years with Gram stain vaginal smears of BV to italiana-caucásias women, They found up to 5.4% incidence in the use of HRT and 6% obtained HRT-free positive results with VB.

According to Nuryanto's research, that dryness complaints of 16.6% was found in women who experience atrophical vaginitis while Silalahi, et al. found 3.6% of menopausal women with symptoms of vaginal dryness who consulted to physician. Ziegler, et al. found more than 43% of vaginal dryness complaints in women above 60 years. There are 17% vaginal complaints dryness in menopausal women using infant detergent formula.

Hormonal vaginal cytology relationship with the distribution of the incidence of atrophical vaginitis in postmenopausal women without dyspareunia complaints showed atrophical vaginitis incidence was 19 people (59%) of 32 samples compared to the distribution of dyspareunia complaints in accordance with the incidence of atrophical vaginitis that showed 14 people (78%) of 18 samples.

Dyspareunea complaint and other sexual dysfunctional patterns usually occur in postmenopausal women. According to Laumann, et al. (1999) in a research study on the prevalence of sexual dysfunction in postmenopausal women. It was found 25% of women complained dyspareunia in which pain during sexual activity is correlated with the presence of sexual problems including decreased of libido, loss of sexual desire and anorgasmia. This may be related to the reduction of the number of male sexual partners with advanced age in a population. Menopausal vaginal changes in women can cause decreased sexual desire. This inconveniency is caused by the loss of vaginal lubrication effect that causes the underlying dyspareunia.

Based on the relationship between cytological hormonal vaginitis with the distribution of the atrophical vaginal incidence in postmenopausal women without leukorrheae complaint showed atrophy vaginitis incidence of as many as 30 people (64%) of 47 samples compared to the distribution according to the leukorrheae complaint showed atrophical vaginitis incident of 3 (100%) from 3 samples. In postmenopausal women, there was a decrease of Lactobacilus as a normal flora colonization, increased vaginal pH and coccengram-positive bacterial levels and coliform; prevalence of Bacterial Vaginosis (BV) is significantly lower compared to the reproductive age and perimenopausal women. Decrease of Lactobacilus colonization is the main cause of ecological development to become anaerobic vagina, which provide a complaint of inflammation and infection of the vaginal ecosystem damaging impacts on the complaints such as leukorrheae.

Distribution of atrophical vaginitis incidence based on clinical study in postmenopausal women showed that in the group of vaginal pH with the results of the alkaline pH as many as 33 people (82%) of 40 samples while in a group with acidic pH found no cases of atrophical vaginitis. In the study sample showed the lowest vaginal pH is 7, while the highest is 12.

Because this study is a diagnostic test thus there are 50 samples of only 33 in cytologic samples expressed as atrophic vaginitis. Since vaginal pH result is the most significant finding in the clinical examination thus we can get a significant relationship between vaginal pH with atrophical vaginitis.

Alkaline pH condition causes vaginal ambient less susceptible to the Lactobacillus and vulnerable to pathogenic bacterial of urogenital and fecal tract infections. Vaginal pH 6-7, the absence of aerobic pathogenic bacterial becomes marker of estradiol status on most of menopausal women.

Gebhart, et al. found atrophical vaginitis in 50% of 75 post menopausal women samples.

On the other hand, Elghany found the incidence of atrophy vaginitis was 15% in premenopausal women and 40% in postmenopausal women. Nuryanto K.H found 63.2% atrophy vaginitis from 38 samples studied.

Research in Manado, North of Sulawesi found the incidence of atrophy vaginitis was 33 subject (66%) from vaginal cytologic examination in 50 postmenopausal women. The incidence of atrophy vaginitis in postmenopausal women here was higher than the results of study conducted by other researchers.

CONCLUSION

Incidence of atrophy vaginitis in this study was 66% obtained from vaginal cytologic examination, based on distribution of low estrogen effect (Maturation value/Total Maturation index between 0 - 49). The most common complain in postmenopausal women who participated in this study was dyspareunia (20%) where 6% had atrophy vaginitis.

In the vaginal pH examination, mostly vaginal pH was alkaline where 80% had atrophy vaginitis.

Further study with larger samples is needed in order to know the relationship between the other complaints with clinical examination including vaginal pH and vaginal cytology should be evaluated either clinically or based on vaginal cytology, so that it could be the basis of management guideline in postmenopausal women.

REFERENCES

1. Brergman A, Brenner PF. Alteration in the urogenital system In: Mishell DR. Menopause: physiology and pharmacology 1st Ed. USA: Year book medical publisher Inc, 1987: 67-75.
2. Hall JE, Nieman LK. Menstrual dysfunction in postmenopausal women. In: Tortoriello, Hall J Handbook of diagnostic endocrynology. New Jersey: Humana Press, Inc. 2003: 295-319.
3. Culligan PJ, Goldberg RP. Urogynecology in primary care. 1st Ed. USA: Springer Science Publisher Inc, 2007: 11-14; 124-34.
4. Brizzolara S, Killeen J, Everino R. Vaginal pH and parabasal cells in post menopausal women. American College of Obstetrician and Gynecologists, 1999; 94: 700-3.
5. Nuryanto KH, Indarti J. Hubungan antara keluhan, gejala klinis dan sitologi vagina pada Vaginitis Atrofi perempuan pascamenopause. Disampaikan pada KOGI XII di Yogyakarta, 2003.
6. Neef JC. Cytology of the menopause. In: Clinical endocrine cytology. 1st Ed. New York: Hoeber Medical Div. Harper and Row Publish, 1975: 87-91.
7. Naib ZM. Cytology of the female genital tractus. In: Exfoliative cytopatology. 2nd Ed. Little Brown and Company, USA. 1976: 17-50.
8. Shefrin DK. Utilization of the maturation index from a pap smear. Nurse Pract, 1999; 24: 1-3.
9. Ziegler D, Girardet G. Menopause. Reading in: 10th Posgraduate Course for Training in Reproductive Medicine and Reproductive Biology, Geneva. Available at: http://www.gfmer.ch/Endo/Lectures-10/Menopause.htm.
10. Hurt WW. Menopause. In: Berek JS. Novak'S Gynecology.Thirteenth Ed. Lipincott Williams & Wilkins. Philadelphia. 2002: 1109-33.
11. Henriksson LA, Stjernquist M. A one-year multicenter study of efficacy and safety of a continuous, low dose, oestradiol-releasing vaginal ring (Estring) in postmenopausal women with symptoms and signs of urogenital aging. Am J Obstet Gynecol, 1996; 174(1): 85-92.
12. Schorge JO, Cunningham FG, Schaffer JI. Menopausal Transition. In: Williams Gynecology 1st Ed. McGraw Hill Companies, Inc. USA. 2008: 468-91.
13. Suparman E. Kontroversi Hormon Estrogen Alamiah atau Fitoestrogen Sebagai Regimen Terapi Sulih Hormon. Bagian Obstetri Ginekologi RS Prof. R.D. Kandou Manado: 1-32.
14. Silalahi EV, Masengi RJA, Taniowas LM. Profil perempuan menopause di kota Manado. Maj Obstet Ginekol Indones Juli, 2003: 27.
15. Clinical Aplication of Vaginal Maturation Index. ProQuest Company
16. Available at: http://adimg.cgnet.com/mac-ad/SP-16. RGRC - 2008.
17. Cailloute JC, Sharp CF. Vaginal pH as a marker for bacterial pathogens and menopausal status Am J Obstet Gynecol. 2000; 176(6): 1270-5.
18. Cauci S, Driusi S. Prevalence of Bacterial Vaginosis and Vaginal Flora Changes in Peri and Postmenopausal Women. J Clin Microbiol. 2002; 40(6): 2147-52.
19. Baziad A. Gambaran klinis, terapi dan pencegahan. FKUI, Yayasan Bina Pustaka Sarwono, Jakarta. Juni 2003: 7-66.
20. Ozkinay E, Terek MC, Yayci M. The Effectivenes of Live Lactobacilli in Combination with Low Dose Oestriol (Gynoflor) to Restore the Vaginal Flora after Treatment of Vaginal Infections. BJOG. 2005; 112: 234-40.