Austin Journal of Obstetrics and Gynecology

Open Access

Austin Publishing Group

Research Article

Vaginal pH Measurement as a Strategy for the Early Diagnosis of Vulvovaginal Atrophy in the Development of Menopause

Silva TRC[1], Oliveira PR[1], Oliveira AB[2] and Batista ML[1]*

[1]Integrated Group of Biotechnology, University of Mogi das Cruzes, Brazil

[2]Santa Casa de Misericórdia of São Paulo, Brazil

***Corresponding author:** Miguel Luiz Batista, Integrated Group of Biotechnology, University of Mogi das Cruzes, Laboratory of Adipose Tissue Biology, Av. Dr. Cândido Xavier de Almeida Souza, 200, Vila Partênio, Mogi das Cruzes, SP, 08780-911, Brazil

Received: March 03, 2021; **Accepted:** April 14, 2021; **Published:** April 21, 2021

Abstract

Objective: Most studies use clinical examination and evaluation of symptoms as subjective forms for the diagnosis of vaginal atrophy. Vaginal hormonal cytology and vaginal pH are objective forms commonly used, however, mainly for treatment control purposes. Despite the relevance of the early diagnosis of vaginal atrophy, we are not aware of studies that objectively or subjectively evaluate the evolution of the parameters with this intention. The aim of the study was to assess if the instruments most used in scientific studies for indication and follow-up of AVV therapy could be used for the early diagnosis of AVV.

Methods: The sample consisted of 40 women, 21 in the menopausal/premenopausal transition (Group 1) and 19 in the postmenopausal (Group 2), between 40 and 65 years old.

Results: It was observed that the set of parameters evaluated, such as: symptoms, clinical examination, vaginal pH, cell maturation value and questionnaire, mainly when used in an associated way, suggest being efficient for the diagnosis of AVV. In general, the observed changes in pH measurement show a good association with cell cytology, which was very characteristic in the two experimental groups (pre and postmenopausal). In addition, pH also showed good association with clinical examination parameters and the main symptoms and signs of menopause. Conclusion: Finally, the results herein shown seems to be promising in relation to the use of vaginal pH measurement as a "tool" for the early diagnosis of AVV.

Keywords: Hypoestrogenism; Vaginal pH; Cell maturation value; FSFI; Early diagnosis

Abbreviations

VVA: Vulvovaginal Atrophy; GSM: Genitourinary Syndrome of Menopause; FSH: Follicle Stimulating Hormone; FSFI: Female Sexual Function Index; SPSS: Statistical Package for the Social Sciences; BMI: Body Mass Index; WHO: World Health Organization

Introduction

Vulvovaginal Atrophy (VVA) or Genitourinary Syndrome of Menopause (GSM) is defined as a set of signs and symptoms associated with decreased circulating levels of estrogen and other sex steroids, involving morphological changes in the large and small labia, clitoris, vaginal opening, vagina, urethra and bladder [1]. Its etiology is explained by the reduction in circulating estrogen levels associated with the natural aging process of the menopausal transition. Regarding the morphological aspects, there is breakdown of the collagen and elastin fibers of the vaginal epithelium, resulting in a series of events, such as overall reduction of tissue elasticity, loss of rugosity, and vaginal shortening and narrowing, making the epithelium thin and pale [2-6].

During menopause, hormonal changes resulting from reproductive aging, especially the decrease in estrogen, exert negative effects on the vaginal epithelium, whose integrity and functional aspects are essential for women's sexual health [7,8]. After menopause, the elasticity of the vagina is reduced and connective tissue increases. A decline in estrogen levels causes a decrease in vaginal blood flow and a decrease in vaginal lubrication, causing the main symptoms of VVA.

Symptoms associated with VVA affect 20% to 45% of postmenopausal women, which can progress and intensify if not treated [2,9]. Therefore, VVA is a common chronic condition that tends to progress with age and can lead to several unwanted symptoms such as vaginal dryness, itching, vaginal irritation, pain, dyspareunia, urinary urgency, dysuria, recurrent urinary infection and nocturia [2,8,10,11].

The subjective evaluation methods most used for the diagnosis of VVA are clinical examination and symptom assessment, while the objective ones include vaginal cytology (maturation value) and vaginal pH; however, when used individually, they do not present satisfactory and accurate results; thus, they are not used in routine practice. Therefore, most studies recommend that the subjective methods of diagnosis be combined with at least one objective and easy-to-use method, such as the pH measurement, which correlates

Citation: Silva TRC, Oliveira PR, Oliveira AB and Batista ML. Vaginal pH Measurement as a Strategy for the Early Diagnosis of Vulvovaginal Atrophy in the Development of Menopause. Austin J Obstet Gynecol. 2021; 8(4): 1179.

well with cytology, histology and various physical characteristics. Additionally, vaginal pH measurement is considered useful, effective and inexpensive [8].

In this scenario, considering that the prevalence of VVA is associated with the population aging, the early diagnosis of this syndrome would have a considerable impact on the possibilities of therapeutic interventions to ensure the health and well-being of the woman. Therefore, the present study proposed to evaluate the feasibility of the early diagnosis of vaginal atrophy, given the negative effects it has on the quality of life of a woman. In general, changes observed in pH measurement showed a good association with cell cytology and serum FSH and estradiol levels, which were very characteristic in the two experimental groups (premenopausal and postmenopausal). Additionally, pH also showed good association with both clinical parameters and the main symptoms and signs of menopause. Finally, the results found were promising regarding the use of vaginal pH measurement as a "tool" for the early diagnosis of VVA.

Methods

Study population

This was a descriptive cross-sectional case-control study. The sample consisted of 40 female subjects, aged between 40 and 65 years, among whom 21 patients were in the menopausal transition/premenopausal period (Group 1: control) and 19 in the postmenopausal period (Group 2: cases). The diagnosis of menopause was confirmed by clinical data (amenorrhea for at least 12 months), elevated gonadotrophin (FSH >35 mIU/mL) and low estrogen levels (estradiol <20 ng/dL) [12-17,18]. The cases were selected through clinical evaluation, physical, cytological, and laboratory tests and questionnaires of patients who underwent routine gynecological examination at a medical clinic in the municipality of Mogi Das Cruzes (São Paulo, Brazil), from October 2017 to February 2018. Group 1 included patients in menopausal transition/premenopausal for at least 3 months without the use of treatments for vaginal atrophy and without the use of hormone therapy for at least 6 months, as well as the presence of at least one sign or symptom of vaginal atrophy. Group 2 included postmenopausal patients i.e., those with amenorrhea for at least 12 months after the last menstrual period, with no pathologic cause of amenorrhea; plasma levels of FSH >35 mIU/ml and estradiol <20 ng/dL, indicating female climacteric stage; at least 3 months without the use of treatments for vaginal atrophy and without the use of hormone therapy; and the presence of at least one sign or symptom of vaginal atrophy. Patients who underwent treatments for vaginal atrophy in the last 3 months, as well as those who underwent hormone therapy in the last 6 months, with the presence of abnormal uterine bleeding, stage II or III genital prolapse, altered cervical cytology, vaginal surgeries in the last 6 months, psychiatric disorders and sexual activity in the last 3 days were excluded from the study. All patients were informed about the protocol to be adopted and signed an informed consent form. This study was submitted to and approved by the Research Ethics Committee of the University of Mogi das Cruzes under number CAAE: 76511517.8.0000.5497.

Operational procedures

Patients were instructed and questioned about sexual intercourse, creams, showers, and the use of any vaginal substance 72 hours before collection that would prevent examination. Disposable nonlubricated vaginal specula were used, and the first step was the clinical evaluation, where patients were examined for the presence or absence of the main signs of atrophy, namely pallor, petechiae, friability, dryness, urethral caruncle, narrowing of the vaginal opening, decreased elasticity and decreased vaginal rugosity. The plasma concentration of the hormones FSH and estradiol were measured using Labtest® commercial kits, and the patients who menstruated collected samples between the first and fifth day of the cycle.

For the oncotic cytology of the uterine cervix, smears were collected with a cytobrush and *Ayre* spatula, were fixed with absolute ethanol and then were sent for laboratory analysis by the Papanicolaou technique [19]. The smear was collected on the upper third of the left lateral wall of the vagina, scraped with the *Ayre* spatula and fixed on a glass slide with absolute alcohol, sent to the laboratory and stained by the Papanicolaou method [19]. The slides were examined by light microscopy by the same cytologist, blinded to the clinical findings, using a 10× eyepiece and a 10× objective lens for the initial evaluation, after which 100 cells were analyzed with the same eyepiece and 40× objective lens in randomly selected fields. The percentage count of each cell type i.e., Parabasal (P), Intermediate (I), and Superficial (S) cells was calculated to obtain the vaginal maturation index or Frost index [13,20]. To enable statistical analysis, the Meisels index or maturation value was subsequently calculated.

Collection of qualitative and quantitative variables

Vaginal pH: This parameter was measured in the upper third of the right lateral wall of the patient using vaginal pH strips, Macherey-Nagel® (MN®), REF 92130, pH-Fix 3.6-6.1 (LOT 30A4841/ISO 13485), and the strip color change was compared immediately with a colorimetric scale representative of vaginal pH, and the measurement was recorded. pH values lower than 5 suggest a normal hormonal pattern, those between suggest 5 and 5.49 mild atrophy, those between 5.5 and 6.49 suggest moderate atrophy, and those greater than or equal to 6.5 suggest severe atrophy [21].

Female sexual function index: The Female Sexual Function Index (FSFI) questionnaire [22] is a questionnaire validated in the Portuguese language [23,24], specific and multidimensional, that evaluates the female sexual response and its domains. The instrument was located after a search in databases because it is widely used in clinical research and has valid psychometric properties. The questionnaire consists of 19 questions grouped into six domains that measure desire (items 1 and 2), excitation (items 3-6), lubrication (items 7-10), orgasm (items 11-13), satisfaction (items 14-16) and pain or discomfort (items 17-19). Each domain receives a score on a scale of 0 to 6 with higher scores indicating better function. Individual scores are obtained by summing the scores of the items that comprise each domain (simple score), which are then multiplied by the factor of that domain and provide the weighted score. The final score (total score: minimum of 2 and maximum of 36) is obtained by the sum of the weighted scores of each domain. Values <26 indicate sexual dysfunction [22-25].

Statistical analysis

Initially, the data were analyzed descriptively. Absolute

and relative frequencies were used for the categorical variables and summary measures (mean, standard deviation, minimum, maximum, and quartiles for the numerical variables). Possible associations between two categorical variables were analyzed using the chi-squared test or, alternatively, Fisher's exact test in cases of small samples. When differences were observed in the distributions, the adjusted standardized residuals were used to identify the local differences cells with absolute values above 1.96 indicate evidence of (local) associations between the categories related to these cells. The comparisons of means between two groups were performed using Student's t-test for independent samples. Student's t-test assumes a normal distribution of the data that was tested using the Kolmogorov-Smirnov test. In the case of violation of this assumption, the nonparametric Mann-Whitney test was used. To evaluate the simultaneous effects of demographic and clinical characteristics (predictor variables) on VVA [2] (dependent variable), logistic regressions were fitted to the data. Due to the large number of predictor variables relative to the sample size, all variables significant at the 5% level were included in the initial models of the univariate analysis. To determine the best model, the forward selection procedure was used that consists of adding the other variables one by one to the most significant predictor variable, until the inclusion of one more variable is no longer significant. Additionally, the goodness-of-fit of the final model was assessed *via* the Hosmer and Lemeshow test. Sensitivity and specificity were calculated from the ROC curve that allowed the definition of a cut-off point for the probabilities of occurrence of vulvovaginal atrophy estimated from the fitted regression model. A significance level of 5% was used for all statistical tests. Statistical analyses were performed using the statistical software SPSS 20.0 and STATA 12.

Results

Forty women participated in this study: 21 premenopausal and 19 postmenopausal women. Table S1 shows the results according to sociodemographic and behavioral characteristics. The mean age was 49.8 years, 80.0% were white, 95% had a college degree, 56.4% did not use alcohol, 70.0% were physically active, and 92.5% did not smoke. The data between the premenopausal and postmenopausal groups are also presented.

Table S2 contains data regarding the distribution of patients according to Body Mass Index (BMI), pregnancies, parity, miscarriages and type of delivery. For the analysis of the physical characteristics, the weight and height were measured to calculate the body mass index (BMI = weight/height^2), classified according to the 2002 World Health Organization (WHO) criteria [26-28]. Among the participating patients, 55.0% had an adequate BMI, the mean number of pregnancies was 1.7, the parity was 1.4, miscarriages numbered 0.3, and 62.5% had a cesarean section. The data for the premenopausal and postmenopausal groups are also presented.

Regarding the symptoms reported, in the group of menopausal women, there were higher percentages of hot flashes (63.2% *vs.* 14.3%), sweating (31.6% *vs.* 4.8%), decreased sexual desire (89.5% *vs.* 19.0%), vulvovaginal dryness (84.2% *vs.* 14.3%), pain during intercourse (73.7% *vs.* 4.8%) and joint pain (47.4% *vs.* 9.5%). The associations between the groups and clinical findings showed that the group of menopausal women had higher percentages of pallor (94.7% *vs.* 14.3%), petechiae (31.6% *vs.* 4.8%), friability (68.4% *vs.* 9.5%), dryness (89.5% *vs.* 14.3%), urethral caruncle (31.6% *vs.* 4.8%), narrowing of the vaginal opening (94.7% *vs.* 23.8%%), decreased elasticity (100.0% *vs.* 23.8%), and decreased rugosity (100.0% *vs.* 33.3%). All of the data are provided in Table S3.

Table 1: Distribution of patients by atrophy and classification of CMV (n=40).

	n	%
Atrophy (pH)	40	100
Normal (< 5)	20	50
Mild atrophy (5 ≤ pH < 5,5)	5	12.5
Moderate atrophy (5.5 ≤ pH < 6.5)	15	37.5
MC (class form)	40	100
Low (< 50)	18	45
Moderate (50 ≤ VMC < 65)	18	45
High (≥ 65)	4	10

n: Number; %: Percentage; MC: Maturation Value

After the main signs and symptoms of the patients were characterized, the patient distribution by atrophy and maturation value was analyzed. It was observed that 12.5% of the women presented with mild atrophy and 37.5% with moderate atrophy; additionally, 45.0% of the women had low estrogen levels. Low pH correlated well with an elevated maturation value (Table 1).

Considering the results presented above, the next step of the analysis was to combine the normal and mild atrophy categories according to a criterion based on the pH (MN*). In this sense, there was an association between atrophy (pH) and symptoms, such as decreased sexual desire, vulvovaginal dryness and pain during intercourse (Table S4). There was also an association between atrophy (pH) and the clinical findings of pallor, petechiae, friability, dryness, narrowing of vaginal opening, decreased elasticity and decreased rugosity (Table S4).

Table 2 shows associations between atrophy (pH) and maturation value in in class form ($p<0.001$) and numerical form ($p<0.001$), number of symptoms ($p=0.001$) and number of abnormal clinical findings suggestive of atrophy ($p<0.001$). All of the data are provided in Table S4.

Considering the results presented above, it was evidenced that pH has a strong relationship with the maturation value, symptoms and clinical findings, and the next step was to evaluate the possible associations with the factors associated with atrophy. According to Table 3, there were associations among age, menopause, FSH, estradiol, FSFI, desire, excitation, lubrication, satisfaction, pain, time since menopause, FSFI classification, lubrication classification and pain classification.

Thus, in the group with moderate atrophy, higher mean age, FSH and time since menopause were observed than those in the normal/mild atrophy group. An inverse pattern was observed for estradiol, FSFI, desire, excitation, lubrication, satisfaction and pain. It was also observed that all the patients in the premenopausal group without lubrication dysfunction were classified as normal or with mild atrophy. This percentage was also higher in those without pain dysfunction (86.7% *vs.* 48.0%) and without female sexual dysfunction

Table 2: Changes in MV, number of symptoms and number of abnormal clinical findings per atrophy group according to pH (n=40).

	Atrophy (pH)		p
	Normal or mild atrophy n = 25	Moderate n = 15	
MC (class form) -			< 0.001[a]
Low (< 50)	4 (22.2)	14 (77.8)	
Moderate (50 ≤ VMC < 65)	18 (100.0)	0 (0.0)	
High (≥ 65)	3 (75.0)	1 (25.0)	
MC (numerical form)	55.3 ± 20.8	12.3 ± 19.3	< 0.001[b]
Number of symptoms	3.6 ± 3.3	7.3 ± 3.3	0.001[b]
Number of abnormal clinical findings	1.9 ± 2.5	6.4 ± 1.4	< 0.001[b]

n: Number; MC: Maturation Value

Table 3: Univariate analysis of factors associated with atrophy (pH).

	Atrophy (pH)		p
	Normal or mild n = 25	Moderate n = 15	
Age (yr)	47.4 ± 4.2	53.7 ± 2.7	< 0.001[b]
Menopause			< 0.001
Premenopausal	21 (100.0)	0 (0.0)	
Menopausal	4 (21.1)	15 (78.9)	
FSH	30.2 ± 43.8	64.0 ± 22.1	0.008[b]
Estradiol	71.6 ± 73.1	10.8 ± 1.8	<0.001[c]
FSFI	26.0 ± 5.5	16.9 ± 5.2	<0.001[b]
Desire	3.5 ± 1.0	2.4 ± 0.7	0.001[b]
Excitation	4.2 ± 1.0	3.2 ± 1,0	0.005[b]
Lubrication	4.7 ± 1.3	2.6 ± 1.4	<0.001[b]
Orgasm	4.2 ± 1.2	3.4 ± 1.4	0.093[b]
Satisfaction	4.5 ± 1.2	3.0 ± 1.6	0.001[b]
Pain	5.0 ± 1.2	2.3 ± 1.9	<0.001[b]
Time since menopause	0.6 ± 1.7	5.7 ± 3.0	<0.001[c]
FSFI - classification			0.002
Absence of risk for sexual dysfunction	14 (93.3)	1 (6.7)	
Risk for sexual dysfunction	11 (44.0)	14 (56.0)	
Lubrication - classification			0.006[a]
Absence of risk for dysfunction	10 (100.0)	0 (0.0)	
Risk for dysfunction	15 (50.0)	15 (50.0)	
Pain - classification			0.014
Absence of risk for dysfunction	13 (86.7)	2 (13.3)	
Risk for dysfunction	12 (48.0)	13 (52.0)	

p: descriptive level of the Chi-square test or Fisher's exact test ([a]); Student's t-test ([b]) or Mann-Whitney test ([c]); FSH: Follicle-Stimulating Hormone; FSFI: Female Sexual Function Index; n: Number.

(93.3% *vs.* 44.0%).

Next, logistic regressions were fitted using atrophy as the dependent variables and each of the variables in Table 3 as predictor variables (all of the data are provided in Tables S5 and S6), except for menopause, smoking, and FSFI classification desire and lubrication for which one of the levels had no cases of atrophy and was thus deterministic in this sample, preventing the calculation of the odds ratio for atrophy (Table 4). In the logistic regression model, the exponentiated coefficients were interpreted as odds ratios. In this study, the odds ratio is given by the ratio between the probability that a patient has atrophy and the probability of not presenting such condition.

Thus, as shown in Table 4, age, FSH, estradiol, FSFI, desire, excitation, lubrication, satisfaction and pain were significant. For more in-depth analyses, particularly of possible associations, a logistic regression model was fitted, using as predictor variables those that were significant at the 5% level in the univariate analysis. For the predictor variables present in numerical and categorical forms,

Table 4: Crude odds ratio (OR) of atrophy.

	OR (CI 95%)	p
Age (years old)	1.68 (1.21-2.33)	0.002
FSH	1.02 (1.004-1.046)	0.02
Estradiol	0.68 (0.47-0.96)	0.029
FSFI	0.74 (0.61-0.89)	0.001
Desire	0.26 (0.10-0.67)	0.006
Excitation	0.34 (0.15-0.81)	0.015
Lubrification	0.34 (0.18-0.64)	0.001
Satisfaction	0.45 (0.26-0.78)	0.004
Pain	0.38 (0.22-0.67)	0.001
Time since menopause	2.22 (1.33-3.68)	0.002

p: descriptive level of the Chi-square test or Fisher's exact test; FSH: Follicle-Stimulating Hormone; FSFI: Female Sexual Function Index; 95%: 95% Confidence Interval.

the form adopted was that with a more significant association with atrophy (Table 5). Thus, the following predictor variables were included in the initial model: age, FSH, estradiol, FSFI, desire, excitation, lubrication, satisfaction, pain and time since menopause. However, because FSFI corresponded to a combination of aspects of sexual dysfunction, two initial multivariate models were fitted: one with the total score and the other with the different aspects of sexual dysfunction.

According to Table 5, the model with the predictor variables age, FSH, estradiol, total FSFI and time since menopause resulted in the inclusion in the final model of only time since menopause (p=0.017) and FSFI (0.056); when excluding the later variable, the model generated is the univariate model with only time since menopause as the predictor variable. Thus, according to the results of the model with only time since menopause as predictor variable (Table 4), the odds of atrophy in a woman with one extra year of time since menopause is 2.2 times greater than in that a woman with one less year of time since menopause. The model considering the FSFI aspects resulted in a final model with age (p=0.016) and pain score (p=0.018) as predictor variables. Thus, with each increase of one unit in the FSFI pain score (less dysfunction), there is a 54% reduction in the odds of atrophy occurring. On the other hand, with each 1-year increase in age, there is a 54% increase in the odds of developing vaginal atrophy.

Discussion

VVA is a chronic, progressive, highly prevalent condition that will occur in most postmenopausal women at some point in their lives, and its early diagnosis can be made using tools reproducible in clinical practice such as clinical and laboratory examinations, vaginal pH measurement and specific questionnaires. Regarding the symptoms associated with menopause, this study demonstrated a higher prevalence of hot flashes, decreased sexual desire, vulvovaginal dryness and pain during intercourse in the postmenopausal group. In large cohort studies in western populations, 45% to 63% of postmenopausal women reported vaginal dryness as the most common symptom [9]. An *online* cohort survey conducted in the United States with 3, 046 postmenopausal women with VVA symptoms, in addition to vaginal dryness, reported pain during intercourse and irritation [29,30]. The most prevalent symptoms in the present study include decreased sexual desire, which differs from the studies cited because those reported mainly vulvovaginal dryness and pain during intercourse.

Pain during intercourse was more prevalent in the postmenopausal period, emphasizing the importance of the symptoms for diagnosis, especially when combined with other predictors of VVA. Although hot flashes are among the most prevalent symptoms associated with hypoestrogenism, they are more associated with the postmenopausal period in general and are less significant regarding VVA specifically. A literature review states that 75% of postmenopausal women report hot flashes [31]. The disparity between high prevalence and infrequent clinical diagnosis is observed in clinical practice, despite the great negative impact of this condition on quality of life.

Another set of variables studied was the distribution of patients according to abnormal clinical findings. In this study, it was observed that in the group of postmenopausal women, there is a significantly higher percentage of abnormal clinical findings. However, the most prevalent, further emphasizing the difference between the clinical findings of both groups, were vaginal dryness, pallor of the vaginal mucosa, narrowing of vaginal opening, decreased vaginal elasticity and decreased rugosity of the vaginal mucosa, which are more frequent in postmenopausal women due to the clinical state of hypoestrogenism with the onset of menopause. In this situation, differential diagnoses must be performed during the clinical examination to exclude other causes such as bacterial vaginosis, trichomoniasis, candidiasis, use of contact irritants, foreign body infections, sexual traumas, cancer, endocrine disorders, vaginal stenosis secondary to radiation, lichen sclerosus and lichen planus [32].

The main purpose of using pH categorization, already used in previous studies [21]. was to evaluate a possible relationship between this parameter and other predictors during the onset and evolution of VVA, which would be more restricted if only pH >5 was used. At this point, considering the results described above, the next step was to evaluate the presence of symptoms and atrophy group according to pH. Thus, it was observed that the subjective symptoms of VVA were highly related to the objective signs observed during the clinical examination, mainly in the moderate atrophy groups. Patients with

Table 5: Results of the multivariate logistic regression model (N=40).

	Model 1 (Total score of FSFI)		Model 2 (Different aspects of FSFI)	
	OR (CI 95%)	p	OR (CI 95%)	p
Age (years old)	-	-	1.54 (1.08-2.19)	0.016
Time since menopause	2.27 (1.16-4.44)	0.017	-	-
FSFI	0.62 (0.38-1.01)	0.056	-	-
Pain	-	-	0.46 (0.24-0.88)	0.018

p: descriptive level of the Chi-square test or Fisher's exact test; OR: Odds Ratio; CI: Confidence Interval; FSFI: Female Sexual Function Index.

moderate atrophy had a greater number of symptoms and abnormal clinical findings than did the normal/mild atrophy group. In fact, thinning of the epithelial lining can cause dryness and pain, especially during intercourse, which can have a significant impact on general quality of life, being associated in most cases with a high vaginal pH. Particularly, the sensation of vaginal dryness was highly related to the vaginal dryness observed by the gynecologist. Vaginal pH levels were significantly higher in women with atrophy symptoms than in asymptomatic women. These were significantly more prevalent in the moderate atrophy group, showing a good correlation between pH and abnormal clinical findings.

As described above, the maturation value is also considered a standard criterion for the confirmation of VVA but is generally not used in clinical practice. Thus, when analyzing the relationship between pH and the maturation value, it was observed that all women classified with moderate estrogen levels were normal or with mild atrophy (pH). On the other hand, in women classified with a low estrogen level, 77.8% presented moderate atrophy according to pH. This reinforces the association between hypoestrogenism or the presence of symptoms and changes in pH in patients who already present moderate atrophy i.e., with vaginal pH between $5.5 \leq pH < 6.5$ highlighting the higher percentages of symptoms in this range and importance of its possible application in routine practice. By contrast, all patients with a moderate maturation value had a normal pH or that indicative of mild atrophy. In this regard, a study of 132 postmenopausal women in Thailand [33] also used vaginal pH, the maturation value, lactobacilli, symptoms and clinical findings to demonstrate a greater correlation with tools and a diagnosis of VVA.

In this study, pH was the only parameter significantly correlated with the symptoms of VVA, explained by the decrease in lactobacilli and decrease in lactic acid resulting from hypoestrogenism, which was shown to be more efficient when associated with two symptoms vaginal dryness and pain during intercourse. In this sense, the present study was concordant with the literature, showing a higher prevalence of symptoms and abnormal clinical findings in patients with pH compatible with moderate atrophy and low maturation value.

After univariate analysis of factors associated with atrophy, age showed a strong relationship with the pH corresponding to moderate atrophy ($5.5 \leq pH < 6.5$). These data show similarity with a large cohort study conducted in the province of Guanacaste, Costa Rica, with 9,161 women who were categorized into two groups, one with vaginal pH between 4.0 and 4.5 (normal) and one with vaginal pH between 5.0 and 5.5 (atrophy). In that study, age was strongly associated with increased vaginal pH, and menopause was responsible for an additional 1.7-fold increase in the odds of having high pH [34]. This result confirms the correlation between age and pH during menopause.

In the present study, the increase in serum FSH levels and decrease in serum estradiol levels were more prevalent in the presence of moderate atrophy, which was also evident with the longer time since menopause. In this sense, the data are very similar to the Atrophy of the Vagina in Woman in Post-Menopause in Italy (AGATA) study, carried out with 913 postmenopausal women who underwent routine gynecological examination at 22 gynecological care centers distributed throughout Italy. The AGATA study demonstrated that the prevalence of VVA, when stratified for years since menopause, using as a cutoff pH >5 and the presence of a signal or symptom suggestive of VVA, was 64.7% at one year since menopause and reached almost a plateau prevalence of 84.2% at 6 years since menopause [29].

Regarding the set of signs and symptoms related to menopause, a questionnaire regarding female sexual function was selected as a differential to make an early diagnosis. It was observed that patients in the group with pH compatible with moderate atrophy had a total FSFI score close to 17, whereas those in the normal pH/mild atrophy group had a total FSFI score near the cut-off point (<26.5) for sexual dysfunction. The decrease in the score was also observed for the FSFI domains desire, excitation, lubrication, satisfaction and pain or discomfort, demonstrating that several aspects of sexual function were adversely affected by the presence of moderate atrophy. In this sense, regarding possible associations between atrophy (pH) and complaints of sweating, decreased sexual desire, vulvovaginal dryness, pain during intercourse and joint pain, as well as clinical findings of pallor, petechiae, friability, dryness, narrowing of vaginal opening, decreased elasticity and decreased rugosity, and maturation value in numerical form and in class form, were found to be indicative of the presence of atrophy. For these variables, there were higher percentages of complaints or abnormal clinical findings in the groups with moderate or medium atrophy than in the normal/mild atrophy group, a finding that coincides with that described in previous studies [29,35]. In this study, time since menopause presented an increase directly proportional to the increase in atrophy, a finding that is similar to that in a longitudinal, population-based study of 438 women born in Australia followed up for 7 years that observed that the incidence of atrophy increased from 3% in the premenopausal period to 21% in the late perimenopausal period and to 47% at three years postmenopause, due to the decrease in the level of estrogens, especially estradiol, after cessation of ovarian function [36].

In the present study, evaluation of the maturation index and value and measurement of the vaginal pH correlated with serum levels of FSH and estradiol, FSFI score and, consequently, VVA. When analyzing separately the various aspects of sexual dysfunction (FSFI), pain score and age were shown to be predictor variables. It was precisely because of the complexity and subjectivity of identifying the best predictor among the FSFI dysfunction aspects that the pain score was used because it becomes simpler and more uniform, being easier for the patient to define whether she has or does not have more pain than, for example, satisfaction. These findings are in agreement with that found in a multicenter study involving 7, 243 women in Latin America that found that the most important risk factor associated with sexual dysfunction was the decrease in sexual lubrication and pain during intercourse, which increases with the increase in woman's age [37].

However, some limitations should be noted. To carry out the evaluation at the time of a routine examination, the presence of bleeding, secretions, semen or vaginal substances used by the patient in the days before the visit was a limiting factor because it could change the measurement of pH and maturation value. This fact led to the exclusion of these patients (inclusion and exclusion criteria). Another challenge was the difficulty patients had in talking about their sex lives and assessing the quality of their sexual relations

because they often believe that symptoms such as vaginal dryness and pain may be a natural part of aging and are unaware of the possibility of treatment and do not even mention it if not asked specifically. The lack of standardization for the pH measurement strips and difficulty in finding specific strips for vaginal use was initially a great challenge but successfully addressed. For menstruating women, to obtain reliable results, collection of samples between the first and fifth day of the cycle did not prove to be a difficulty for the patients.

Conclusion

Finally, taken together, the results showed that the evaluated parameters, especially when used in conjunction, are seemingly effective for the early diagnosis of VVA and suggest that special attention should be given to the specific range of pH variation indicative of mild atrophy ($5 \leq pH < 5.5$) because most studies use $pH \leq 5$ as a cut-off for atrophy. This is based mainly on the negative effects observed on symptoms, clinical findings, maturation value and sexual function, significantly more prevalent in patients with pH indicative of moderate atrophy ($5.5 \leq pH < 6.5$). The results suggest that the combination of simple measures in routine gynecological practice, such as pH measurement and the specific evaluation of sexual dysfunction, with usual tools can increase the rate of an early diagnosis. This allows for early therapeutic intervention and better management of VVA and, consequently, a better response to treatment.

Acknowledgement

We thank Sancet Medical Laboratory for the pathological analysis assistance. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of FAPESP. Grant sponsor: FAPESP; Grant numbers: 15/19259-0 (Miguel L Batista)

Contribution

Silva, T.R.C. and Oliveira, P.R. participated in the design, the draft and in the revision of the study; Oliveira, A.B. participated in the revision of the manuscript; Batista, M.L. Jr. conceived the study and participated in its design, its coordination and in the drafting of the manuscript. All of the authors read and approved the final manuscript.

References

1. Portman DJ, Gass ML. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the international society for the study of women's sexual health and the North American menopause society. J Sex Med. 2014; 11: 2865-2872.
2. Bachmann GA, Nevadunsky NS. Diagnosis and treatment of atrophic vaginitis. Am Fam Physician. 2000; 61: 3090-3096.
3. Archer DF. Efficacy and tolerability of local estrogen therapy for urogenital atrophy. Menopause. 2010; 17: 194-203.
4. Stika CS. Atrophic vaginitis. Dermatol Ther. 2010; 23: 514-522.
5. North American Menopause Society. Management of symptomatic vulvovaginal atrophy: 2013 position statement of the North American menopause society. Menopause. 2013; 20: 888-902.
6. Lev-Sagie A. Vulvar and vaginal atrophy: physiology, clinical presentation, and treatment considerations. Clin Obstet Gynecol. 2015; 58: 476-491.
7. Mac Bride MB, Rhodes DJ, Shuster LT. Vulvovaginal atrophy. Mayo Clin Proc. 2010; 85: 87-94.
8. Weber MA, Limpens J, Roovers JPWR. Assessment of vaginal atrophy: a review. Int Urogynecol J. 2015; 26: 15-28.
9. Nappi RE, Kokot-Kierepa M. Women's voices in the menopause: results from an international survey on vaginal atrophy. Maturitas. 2010; 67: 233-238.
10. Portman DJ, Gass ML. Vulvovaginal Atrophy Terminology Consensus Conference Panel. Genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the international society for the study of women's sexual health and the North American menopause society. Maturitas. 2014; 79: 349-354.
11. Castelo-Branco C, Cancelo MJ, Villero J, Nohales F, Juliá MD. Management of post-menopausal vaginal atrophy and atrophic vaginitis. Maturitas. 2005; 52: 46-52.
12. Rodrigues D, Silva I, Medina J, Salvatore C, Bastos A. Colpocitologia funcional. Editors. In: Medina J, Salvatore CA, Bastos AC. Propedêutica Ginecológica. 3rd edition. Barueri: Manole. 1977: 159-174.
13. Lencioni LJ. Citologia Endócrina. Buenos Aires: Médica Pan Americana. 1987.
14. Wied GL, Bibbo M, Bibbo M. Comprehensive Cytopathology. Amsterdã: Elsevier. 1997.
15. Greendale GA, Lee NP, Arriola ER. The menopause. Lancet. 1999; 353: 571-580.
16. Van der Laak JAWM, Schijf CP, Kerstens HMJ, Heijnen-Wijnen THM, De Wilde PCM, Hanselaar AGJM. Development and validation of a computerized cytomorphometric method to assess the maturation of vaginal epithelial cells. Cytometry. 1999; 35: 196-202.
17. Lustosa ÁB, Girão MJBC, Sartori MGF, Baracat EC, Lima GRD. Citologia hormonal do trato urinário baixo e da vagina de mulheres na pós-menopausa, antes e durante estrogenioterapia oral e transdérmica. Rev Bras Ginecol Obstet. 2002; 24: 573-577.
18. Cabral PU, Canario AC, Spyrides MH. Influence of menopausal symptoms on sexual function in middle-aged women. Rev Bras Ginecol Obstet. 2012; 34: 329-334.
19. Papanicolaou GN, Traut HF. The diagnostic value of vaginal smears in carcinoma of the uterus. Am J Obstet Gynecol. 1941; 42: 193-206.
20. Frost J. Clinical cytology. Editors. In: Novak ER, Jones JS. Novak's Textbook of Gynecology. 6th ed. Baltimore: Williams & Wilkins Company. Cap. 36; 1961.
21. Yörük P, Uygur M, Erenus M, Eren F. The role of vaginal maturation value assessment in prediction of vaginal pH, serum FSH and E2 levels. Marmara Med J. 2006; 19: 52-57.
22. Rosen R, Brown C, Heiman J. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther. 2000; 26: 191-208.
23. Hentschel H, Alberton DL, Capp E, Goldim JR, Passos EP. Validação do Female Sexual Function Index (FSFI) para uso em língua portuguesa. Revista HCPA. 2007; 27: 10-14.
24. Thiel RDRC, Dambros M, Palma PCR, Thiel M, Riccetto CLZ, Ramos MDF. Tradução para português, adaptação cultural e validação do Female Sexual Function Index. Rev Bras Ginecol Obstet. 2008; 30: 504-510.
25. Pacagnella RDC, Martinez EZ, Vieira EM. Validade de construto de uma versão em português do Female Sexual Function Index. Cad Saude Publica. 2009; 25: 2333-2344.
26. World Health Organization Consultation on Obesity. Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation. Geneva: World Health Organization. 1999.
27. Tsigos C, Hainer V, Basdevant A. Management of obesity in adults: European clinical practice guidelines. Obesity Facts. 2008; 1: 106-116.
28. Associação Brasileira Para o Estudo da Obesidade e da Síndrome Metabólica. Diretrizes Brasileiras de Obesidade. 4th edition. São Paulo: Banda de Conteúdo. 2016.

29. Palma F, Volpe A, Villa P, Cagnacci A. Vaginal atrophy of women in postmenopause. Results from a multicentric observational study: the AGATA study. Maturitas. 2016; 83: 40-44.

30. Kingsberg SA, Wysocki S, Magnus L, Krychman ML. Vulvar and vaginal atrophy in postmenopausal women: findings from the REVIVE (Real Women's VIews of Treatment Options for Menopausal Vaginal Changes) survey. J Sex Med. 2013; 10: 1790-1799.

31. Oliveira J. Padrão hormonal feminino: menopausa e terapia de reposição. RBAC. 2016; 48: 198-210.

32. Goldstein I. Recognizing and treating urogenital atrophy in postmenopausal women. J Womens Health. 2010; 19: 425-432.

33. Tuntiviriyapun P, Panyakhamlerd K, Triratanachat S, et al. Newly developed vaginal atrophy symptoms II and vaginal pH: a better correlation in vaginal atrophy? Climacteric. 2015; 18: 246-251.

34. García-Closas M, Herrero R, Bratti C, et al. Epidemiologic determinants of vaginal pH. Am J Obstet Gynecol. 1999; 180: 1060-1066.

35. Harlow SD, Gass M, Hall JE, et al. Executive summary of the stages of reproductive aging workshop+ 10: addressing the unfinished agenda of staging reproductive aging. J Clin Endocrinol Metab. 2012; 97: 1159-1168.

36. Dennerstein L, Dudley EC, Hopper JL, Guthrie JR, Burger HG. A prospective population-based study of menopausal symptoms. Obstet Gynecol. 2000; 96: 351-358.

37. Blumel JE, Chedraui P, Baron G, et al. A large multinational study of vasomotor symptom prevalence, duration, and impact on quality of life in middle-aged women. Menopause. 2011; 18: 778-785.